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Molecular Mapping and Genetic Diversity Studies in Sweet Potato

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ABSTRACT

The sweet potato [*Ipomoea batatas* (L.) Lam] is a native species of South America belongs to the genus in the family Convolvulaceae. Sweet potato is the seventh most important crop in the world and a major source of food and nutrition in developing countries. World sweet potato production is around 106 million tons in an area of about 8.1 million ha. Asia is the world's largest sweet potato producing region with the maximum production of 88.5 million tons in 4.4 million ha. China is the world's leading sweet potato producer with 81 million tons and contributes 76% to the world's production followed by Uganda with 2 million tons. So far three genetic linkage maps developed in sweet potato using different mapping population. Molecular markers were used to map the genes of root knot nematode resistance, carbohydrate metabolic genes, feathery mottle virus resistance, virus disease and carotene genes were studied using different markers. Similarly, genetics diversity work done in sweet potato worldwide with different source of germplasm and different marker system like morphological, RAPD, ISSR, SSR, DAF, AFLP, SAMPL for identifying duplicates and developing core collection in the germplasm.

Keywords: Sweet potato, markers, mapping population, linkage map, QTL, virus resistance
Abbreviations: **AFLP**, amplified fragment length polymorphic DNA; **AMOVA**, analysis of molecular variance; **CIP**, International Potato Centre; **LG**, linkage group; **LSU**, Louisiana State University; **MAS**, marker-assisted selection; **QTL**, quantitative trait loci; **RAPD**, random amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **RKN**, root knot nematode; **SCAR**, sequence characterized amplified region; **SSR**, simple sequence repeat; **STS**, sequence-tagged site

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INTRODUCTION

The sweet potato [*Ipomoea batatas* (L.) Lam] is a native species of South America, with the Caribbean region and they are extending from Guatemala to Peru, including Colombia and Ecuador, considered as its center of origin (Austin 1987). Sweet potato is the seventh most important crop in the world and a major source of food and nutrition in developing countries (CIP 1996). Asia is the world's largest sweet potato producing region in which China contributes 76% of its world production (81 MT). India is the seventh sweet potato producing country in Asia which is nearly one million tons, followed by Philippines (5 MT) and Bangladesh (3 MT). India and Taiwan, it is the one of the important stable food crop (FAO 2012). Recent studies on diversity assessment using molecular markers found the highest diversity in Central America and supported the hypothesis that this region is the primary center of diversity and most likely the center of origin of sweet potato (Huang and Sun 2000; Zhang *et al*. 2000). Sweet potato is the most

Received: 1 November, 2011. Accepted: 1 November, 2012.

Invited Review

important food crop in the genus Ipomea which is the largest genus in the family *Convolvulaceae*, and series Batatas consisting of about 650 species. It is a hexaploid plant with 2n=6x=90 chromosomes and self-incompatible species.

In India, it is cultivated mainly in Orissa, Uttar Pradesh, West Bengal, Bihar and North Eastern states with an area of 0.12 Million hectares and productivity of 9 t ha^{-1}. So far, more than 27 varieties released, out of which 16 varieties released from CTCRI, Trivandrum. Sweet potato germplasm contains 1100 accessions which include 693 indigenous and 407 exotic collections from other countries including CIP, Lima, Peru. The main objective of the germplasm project is to collect genetic variability available from both indigenous as well as from other countries (exotic) and conserved to characterize and evaluation for proper utilization. The main objective of the sweet potato improvement programme is developing white and orange fleshed sweet potatoes (WFSP/OFSP) with high dry matter content (CTCRI annual report 2010-11).

Plant molecular biology offers a great potential for plant breeding as it promises to provide several tools to reduce the time taken to produce crop varieties with desirable characters. With the use of molecular techniques it would now be possible to hasten the transfer of desirable genes among varieties and to introgress novel genes from related wild species. Polygenic characters which were previously very difficult to analyse using classical genetic analysis, would now be easily tagged using molecular markers. Techniques which are particularly promising in assisting selection for desirable characters involves the use of several types of molecular markers such as restriction fragment length polymorphism (RFLP) (Botstein *et al*. 1980), random-amplified polymorphic DNA (RAPD) (Williams *et al*. 1990) sequence characterized amplified regions (SCAR) (Williams *et al*. 1991), sequence-tagged site (STS) (Fukuoka *et al*. 1994), inter-simple sequence repeat (ISSR) (Zietkiewicz *et al*. 1994), amplified fragment length polymorphic DNA (AFLP) (Vos *et al*. 1995) and simple sequence repeat (SSR) (Hearne *et al*. 1992). The utility of molecular markers in crop breeding is reviewed by Maheswaran (1997), Mohan *et al*. (1997) Gupta and Roy (2002), Collard *et al*. (2005) and Milee *et al*. (2008).

Molecular markers have several advantages over the phenotype based markers that were previously available to plant breeders. They offer greater scope for improving the efficiency of plant breeding by carrying out selection indirectly on the trait of interest by using a set of tightly linked markers to the trait of interest. The markers at DNA level are used to construct the linkage maps, locate the genetic loci on specific chromosomes, characterize the germplasm for its genetic diversity and finally to exercise marker assisted selection (MAS).

SWEET POTATO GENETIC MAP

The first sweet potato genetic map was published based on 632 AFLPs from Tanzania and 435 markers from Bikilmaliya, a biparental mapping population, and these two sister maps, that collectively contributed to main map, covered 3655.6 cM and 3011.5 cM, respectively (Kriegner *et al*. 2003). Collectively, both biparental maps produced 170 linkage groups (LGs). Genetic linkage analysis is quite a challenge as sweet potato is a complex ploidy. In this study, the first framework map was elaborated using single-dose markers that segregated into a 3:1 or a 1:1 ratio. The remaining interspread duplex and double-simplex markers that resulted from sweet potato hexaploidy were used to detect homologous groups within the parental maps and corresponding LGs among those maps. The type of polyploidy for the entire genome was examined using the ratio of linkage in the coupling phase to linkage in the repulsion phase and the ratio of non-simplex to simplex markers. In this study, the marker segregation pattern supported the concept of polysomic inheritance and preferential pairing.

Among the two genetic maps that are ready for release is a map developed at West Virginia State University, which were enriched using SSR/AFLP loci. AFLPs are developed from HindIII, and Mse1 combinations at an interval of 1 cM every 5 cM to aid in distinguishing and identifying new traits and in seeking functional information. A genetic map is being developed with AFLP/SSR- amplified polymorphisms for hexaploid sweet potato using a segregating population derived from a biparental cross between the cultivars 'Excel' and 'SC 1149'. The original population size is 180, of which 70 F_1s are from Excel and 110 F_1s are from SC1149-19. A random pick of 50 F_1s of Excel and 50 F_1s of SC1149-19 is selected for microsatellite amplifications. Sixty SSR primers identified as polymorphic between parents were used to amplify 100 seedlings. The number of loci amplified ranged from one to eight (Nimmakayala *et al*. 2004). The segregation patterns were classified following Wu *et al*. (1992) and Kriegner *et al*. (2003) into simplex, duplex, triplex, or quadruplex markers, regardless of whether sweet potato status as an auto-, allo-, or segmental polyploid. Cervantes-Flores *et al*. (2008) presented another genetic map; this map is based on a mapping population consisting of 250 individuals of a cross between Beauregard (a leading orange fleshed USA sweet potato cultivar) and Tanzania (a cream-fleshed African sweet potato landrace). The mapping population was phenotyped for several traits of economic importance such as resistance to Root Knot Nematode and Sweet Potato Feathery Mottle Virus and dry matter content. A total of 1751 (Beauregard) and 1944 (Tanzania) AFLP markers, of which 1511 and 1303 were single-dose markers, were scored. Framework maps consisting of 86 and 90 linkage groups for Beauregard and Tanzania, respectively, were developed using JoinMap and MAPMAKER programme. A total of 947 single-dose markers were placed in the final framework linkage map for 'Tanzania'. The linkage map size was estimated as 5792 cM, with an average distance between markers of 4.5 cM. A total of 726 single dose markers were placed in the final framework map for 'Beauregard'. The linkage map length was estimated as 5276 cM, with an average distance between markers of 4.8 cM. Further, this study also included preliminary analysis with the framework maps to detect quantitative trait loci (QTLs) associated with the traits studied.

Zhang *et al*. (2004) mapped a total of 25 markers developed using genes involved in carbohydrate metabolism. Of these, 22 genes were mapped, under single-dose conditions, on a previously constructed framework linkage map of sweet potato built with AFLP markers. Thirteen loci, distributed in 13 different Linkage Groups, were mapped in the female parent, whereas 9 loci were mapped on 8 Linkage Groups in the male parent. The co-location of these candidate gene markers with QTLs for starch and drymatter accumulation is being verified. The development of these tools will improve the hypothesis testing toward the development of referential frameworks and will be useful for rapid identification of beneficial allele combinations.

MAPPING GENES OF AGRONOMIC IMPORTANCE

Root knot nematode

Amplified Fragment Length Polymorphism (AFLP) marker profiles for individuals in two F_1 populations of sweet potato were used in association studies to identify AFLP markers suitable for identification of plants possessing a resistant reaction to southern root-knot nematode (RKN). Population one consisted of 48 half-sib genotypes developed at the Louisiana State University (LSU) and the second population consisted of 54 full-sibs developed by the East African and International Potato Center (CIP) sweet potato breeding programs. Results for plant nematode resistance indicate a bimodal distribution among the genotypes for the LSU population and a normal distribution for the CIP population. Using analysis of molecular variance (AMOVA) and two multivariate analysis techniques *i.e.*

logistic regression and discriminate analysis, 5 and 4 AFLP markers that had a strong and significant association with respect to the resistance trait were selected for the LSU and CIP populations, respectively. A comparative analysis of the power of discriminate analysis models for southern RKN resistance class prediction achieved 88.78% (LSU) and 88.04% (CIP) classification efficiencies (Mcharo *et al*. 2005).

Sweet potato feathery mottle virus

Sweet potato virus disease (SPVD), a result of the co-infection of whitefly transmitted *Sweet potato chlorotic stunt virus* (SPCSV) (genus Crinivirus, family Closteroviridae) and the aphid-transmitted *Sweet potato feathery mottle virus* (SPFMV) (genus Potyvirus, family Potyviridae), is the most destructive disease of sweet potato in East Africa. A study was conducted to establish if genotypes identified as resistant or susceptible to SPVD in Kenya could be distinguished using molecular markers. A total of 47 unrelated sweet potato genotypes were selected from germplasm collections and classified into two phenotypic groups as resistant or susceptible to SPVD. Genotype selection was based on disease severity or days to symptom development in plants following graft inoculation. Amplified fragment length polymorphism (AFLP) marker profiles were generated for each individual and used in association studies to identify markers suitable for classifying the two pre-defined phenotypic groups. Analysis of molecular variance showed significant variation between the two groups using 206 polymorphic AFLP markers. Discriminate analysis and logistic regression statistical methods were used to select informative markers, and to develop models that would classify the two phenotypic groups. A training set of 30 genotypes consisting of 15 resistant and 15 susceptible were used to develop classification models. The remaining 17 genotypes were used as a test set. Four markers, which gave 100% correct classification of the training set and 94% correct classification of the test set, were selected by both statistical methods (Miano *et al*. 2008).

Sweet potato virus disease

Mcharo and LaBonte D (2005) studied marker linked resistance to SPCSV and SPFMV virus using 87 F_1s from cultivars of Tanzania and Wagabolige using RFLP and RAPD primers. Among the progenies 45 of the clones were resistant to SPCSV and 37 were resistant to SPFMV. The RFLP marker e41m33.a contributed the greatest variation in SPCSV resistance and RAPD marker S13.1130 accounted for most of the variation inSPFMV resistance. These diseases are characterized by chlorosis, small, deformed leaves, and severe stunting and can reduce yields of infected plants by over 90%. SSR markers were used to characterize Kenyan sweet potato genotypes for resistance to the SPVD and high dry matter content. Eighty nine genotypes with a mean symptom severity score of between 1 and 1.5 were selected following graft inoculation with SPVD-infected scions and characterized using 6 SSR primers. The 6 SSR primer pairs had average polymorphic information content (PIC) of 0.47. The average number of alleles within the 89 genotypes across the 6 loci was 13.52. Cluster analyses revealed a 50% variation among the 89 genotypes. The dendrogram did not reveal any unique clustering of the genotypes according to dry matter content and reaction to SPVD. The genetic differences among the SPVD resistant genotypes and those with high dry matter revealed by the distinct groups suggest a significant genetic variability and the presence of different sources of resistance to SPVD and high dry matter (Karuri *et al*. 2009).

Sweet potato β-carotene gene

DNA were isolated from 250 hybrid seedlings developed from S1 (white fleshed cultivar) x ST 14 (orange fleshed cultivar). Among the 150 progenies were used for mapping β-carotene gene with five ISSR markers. Based on the single marker analysis (SMA), one marker ISSR 818 3/1 associated with β-carotene identified (Vimala and Mohan 2010).

Sweet potato yield-related traits

Chang *et al*. (2009) studied the QTL associated with top weight, root weight, root number, root shape, root skin colour and flesh color using ISSR markers. Two mapping populations of nearly 120 F_1 plants were derived from a reciprocal cross between 'Nancy Hall' (NH) and 'Tainung 27' (TN27). Two partial linkage maps in the sweet potato which use simplex and double-simplex ISSR markers were constructed. The length of each linkage group spanned a very wide range of 10.7~149.1 centiMorgans (cM). These maps consist of 37 NH and 47 TN27 markers with map lengths of 479.8 and 853.5 cM, respectively. Twenty-two of these QTL-mapped markers were unique to the specific traits, and two were linked to two traits in each linkage map. These markers appear to be applicable to crop improvement. The details of various mapping populations used in genome and gene mapping in sweet potato are furnished in **Table 1**.

GENETIC DIVERSITY STUDIES

Morphological and biochemical diversity

Huaman *et al*. (1999) characterized 1939 Peruvian collections out of 5000 sweet potato accessions available at the International Potato Center (CIP) based on morphological traits and electrophoretic banding patterns of total proteins and esterase. A total of 21 morphological descriptors were used to support a clustering pattern based on UPGMA.

Randomly amplified polymorphic DNA (RAPD)

Fifteen 10-mer primers, in combination with the Stoffel fragment, were used to detect RAPD among 26 accessions of sweet potato from Oceania, Peru, the Philippines, and the United States and between 8 *Ipomoea* species from section Batatas. Phenotic and principal coordinate analysis of the 56 polymorphisms detected within the hexaploid *I. batatas* clearly delineated the South Pacific and the Peruvian sweet potato lines. The two US cultivars clustered with the Oceanic materials. Cladistic and phenetic analysis of 8 *Ipomoea* species supports previously published phylogenies based on morphological and RFLP data. Among the species examined, *I. tabascana*, *I. trifida* and the tetraploid forms of *I. batatas* from Mexico and Ecuador, including *I. batatas* var. apiculata, are the taxa most closely related to the cultivated hexaploid *I. batatas*. These findings support the utility of RAPD markers for evaluating genetic diversity in sweet potato and for establishing taxonomic and evolutionary relationships in *Ipomoea* (Jarret and Austin 1994).

Connolly et al. (1994) studied genetic fingerprints of six clonal cultivars and to estimate genetic distances between these cultivars. The level of polymorphism within the species was extremely high. From the 36 primers used, 170 fragments were amplified, of which 132 (77.6%) were polymorphic. Out of 36 primers, 26 RAPD primers enabled the discrimination of all six genotypes. Villordon and LaBonte (1995) studied the genetic variation among the Jewel sweet potato clones using 38 RAPD primers. The 'Jewel' clones polymorphic bands ranged from 7.1 to 35.7% in five of eight clone groups and yield differences ranged from 27 to 46% within the Jewel clones. The results suggest the usefulness of arbitrarily-primed markers in detecting intraclonal variation in sweet potato DNA polymorphisms and indicate an underlying genetic cause for phenotypic variability in the crop.

Harvey *et al*. (1997) studied nine New Zealand kumara cultivars including three identified as 'ancient' or 'pre-European', two modern, and four reputedly derived from 19th century introductions using RAPD primers. The cul-

Table 1 Various mapping populations used in genome and gene mapping in sweet potato.

Mapping population	Purpose	Reference
Tanzania/Bikilmaliya	Genome mapping	Kriegner *et al*. 2003
Excel/SC1149	Genome mapping	Nimmakayala *et al*. 2004
Beauregard/Tanzania	Genome mapping using carbohydrate metabolic genes	Zhang *et al*. 2004
48 half sib-USA 54 full sib-CIP, Peru	Gene mapping for root knot nematode	Mcharo *et al*. 2005
Tanzania x Wagabolige cultivar- 87 F_1s	SPCSV, SPFMV virus mapping population	Mcharo *et al*. 2005
Beauregard/Tanzania	Genome mapping	Cervantes-Flores *et al*. 2008
47 sweet potato lines - resistant and susceptible to virus diseases	Association mapping of sweet potato feathery mottle virus resistance	Miano *et al*. 2008
89 sweet potato lines - resistant and susceptible to virus diseases	Association mapping of sweet potato virus disease resistance and dry matter	Karuri *et al*. 2009
120 F_1s of NH x TN 27 and reciprocals	Yield related QTLs	Chang *et al*. 2009
S1(white fleshed) x ST 14 (orange fleshed) - 250 F_1 progenies	Gene mapping for β-carotene gene	Vimala and Mohan 2010
E Shu 3 Hao x Guang 2k-30	EST based SSR marker validation	Wang *et al*. 2011

tivars derived from the 19th century introduction clustered in one group, a group which also included one modern cultivar. Two ancient cultivars were closely related to each other, but distant from the other ancient cultivar and the other groups, a result which is consistent with two separate, possibly pre-European lines. The theoretical historical origin of each cultivar was supported.

The island of New Guinea is considered a secondary center on diversity for sweet potato, because of its range of isolated ecological niches and large number of cultivars found within a small area. Information of genetic diversity in Papua New Guinea (PNG) sweetpotato is essential for rationalizing the global sweetpotato germplasm collection. Using RAPD primers, Zhang *et al*. (1998) compared the genetic variation and genetic diversity in 18 PNG cultivars versus 18 cultivars from South America. The analysis of molecular variance revealed large genetic diversity in both groups of cultivars. The PNG cultivars are also less divergent than their South American ancestors as the mean genetic distance in PNG group is significantly smaller than that of South American group. This study shows that PNG cultivars, after many years of isolated evolution in an unique agro-ecological environment are substantially divergent from their ancestors in South America. The genetic diversity level in PNG cultivars is significantly lower than that in South American cultivars. Sagredo *et al*. (1998) used random amplified polymorphic DNA (RAPD) markers to understand the genetic diversity of 28 Chilean cultivars. This study was very informative for understanding historical records of sweet potato breeding and cultivar collection.

Available evidence shows that sweet potato originated from either Central or South American lowlands with subsequent dispersal to North America, Europe, Africa, Asia and the pacific islands. A total of 71 polymorphic RAPD molecular markers were used to assess the genetic relationships amongst 74 sweet potato varieties originating from a total of 23 sweet potato producing countries within six geographical regions (Gichuki *et al.* 2003). Multidimensional scaling (MDS) revealed that the South American and the Central American/Caribbean genotypes formed two separate clusters. East African varieties, which have unique characteristics from other traditional varieties, were distinct from other traditional varieties from South America and Oceania. These results support the reported hypothesis of the origin and dispersal of the sweet potato and indicate that the primary centre of diversity probably has two distinct gene pools. It is proposed that the dispersal of the sweet potato from its origin may have mainly involved varieties from Central America/Caribbean as opposed to varieties from South America. There is an indication that new gene pools may be evolving in Africa and Asia due to hybridization and adaptation to the local environments.

Valadares *et al*. (2011) studied the genetic diversity among Tocantins germplasm material using 14 RAPD primers. Cluster analysis confirmed the wide diversity among the genotypes and four genotypes highly dissimilar in all characteristics were selected for future breeding programs.

Inter simple sequence repeats

Huang and Sun (2000) used inter simple sequence repeats (ISSR) for restriction site variation in four non-coding regions of chloroplast DNA and scored 2071 bands in 40 accessions of *Ipomoea*. This study included *I. trifida*, *I. ramosissima*, *I. umbraticola*, and *I. triloba*. The study concluded that *I. triloba* could be an ancestor of *I. batatas*. Ma *et al*. (2009) studied the Chinese germplasm material for selecting high carotene lines for sweet potato breeding programme with agronomic traits and markers (RAPD, ISSR). Fifteen sweet potato clones and there crossed seeds were evaluated, great variation for carotene content and storage root yield was observed among the different parental material. Among the ISSR and RAPD markers, 89.6% variation shown by ISSR and 74.4% variation by RAPD primers for the high carotene parents. The results from the molecular markers and agronomic traits analysis can supply a valuable theory reference for the selection of parents for breeding of new varieties with high carotene content.

He *et al*. (2007) studied the genetic diversity of one hundred landraces from six geographical regions of China and eight cultivars were assessed using inter-simple sequence repeat (ISSR) markers. Fourteen ISSR primers, revealed 239 polymorphic bands with an average of 17 polymorphic bands per primer. The UPGMA dendrogram showed the same genetic relationship among the cultivars in agreement with their known origin and the very wide genetic diversity of the Chinese landraces. The cluster analysis classified the materials into two groups: a major and a minor group.

DNA amplification fingerprinting

He *et al*. (1995) used DNA amplification fingerprinting (DAF) on 73 plant introductions of sweet potato drawn from the USA and New Guinea along with tetraploid *I. batatas* and *I. triloba*. In this study, US cultivars formed a single cluster, indicating less diversity, while accessions from New Guinea showed wide variation. To extend further their study Prakash *et al*. (1996) used DAF on 30 cultivars that also included Regal and Excel, lines that are developed using a population- based breeding approach. Regal and Excel have shown greater divergence from other heirlooms. In this study, a total of 144 bands were used to support a phenogram depicting molecular relationships among cultivars. Wang *et al*. (1998) used DAF on 42 sweet potato accessions from Guangdong and Fujian provinces of China and from Japan to verify pedigree records. This study concluded that the DAF could resolve the domestication history of sweet potato germplasm.

Amplified fragment length polymorphism

From CIP germplasm, 69 sweet potato cultivars from four geographical regions of Latin America were fingerprinted using AFLP markers (Zhang *et al*. 2000). The highest genetic diversity was found in Central America, whereas the lowest was in Peru-Ecuador. These results support the hypothesis that Central America is the primary center of diversity and most likely the center of origin of sweet potato and Peru-Ecuador should be considered as a secondary center of diversity. Fajardo *et al*. (2002) used AFLP markers for studying genetic diversity in 141 accessions derived from botanical seed in different Papua New Guinea areas. Two hundred polymorphism markers were identified and utilized in the analysis. The molecular analysis revealed relatively limited genetic diversity within and between sites.

Comparative analyses of genetic diversity and phylogenetic relationships of sweet potato and its wild relatives in *Ipomoea* series Batatas were conducted using amplified fragment length polymorphism (AFLP) and sequence data from the internal transcribed spacer (ITS) region of the ribosomal DNA (Huang *et al*. 2002). Low ITS divergence among 13 species of ser. Batatas resulted in poorly resolved relationships. Of the species examined, *I. trifida* was found to be the most closely related to *I. batatas*, while *I. ramosissima* and *I. umbraticola* were the most distantly related to *I. batatas*.

Although originally domesticated in tropical America, the sweet potato has a long history of cultivation in the Pacific region. While the post-Columbus dispersal of sweet potato to Asia and the Pacific is well documented, the hypothesis that there was a prehistoric transfer of sweet potato by Peruvian or Polynesian voyagers from Peru to Oceania. Zhang *et al*. (2004) studied the 80 accessions from Pacific region and Latin America for its genetic diversity using AFLP markers. Multidimensional scaling (MDS) and analysis of molecular variance (AMOVA) revealed a large genetic variation in the Oceania gene pool, far greater than that in Peru-Ecuador. The Mexican cultivars were grouped together with those of Oceania. In contrast, there is little association between the Peru-Ecuador germplasm and that of Oceania. These results suggest that Peru-Ecuador may not be the source of the Oceania germplasm.

Bruckner *et al*. (2005) presented comprehensive amplified fragment length polymorphism (AFLP)-based genetic diversity study on 775 accessions from the Plant Genetic Resources Conservation Unit USDA-ARS in Griffin and the International Potato Center (CIP) in Lima, Peru. The data of 183 polymorphic bands were subjected to ANOVA and principal coordinate analysis to conclude that several clusters existed in the collection.

Simple sequence repeats

A microsatellite-enriched library has been developed for the purpose of capturing repeat motifs containing genomic fragments. The capturing procedure involves the hybridization of genomic DNA fragments to biotin-labeled synthetic SSR oligonucleotides of various repeat types. Fragments are captured using streptavidin paramagnetic bead affinity selection methods. The resultant fragments from final PCR were cloned, and of these, 96 randomly selected clones were sequenced. These sequences were trimmed by removing the vector and adaptor sequences, and finally 20 sequences containing long repeat motifs of GA, CA, AGA, and ACA with sufficient flanking sequence were selected for primer design. Primers were designed to amplify repeat units of 20 sequences and tested for polymorphisms in a set of reference genotypes (Nimmakayala *et al*. 2004). These microsatellite markers will be useful for assessing sweet potato molecular diversity and/or saturate the genetic map of sweet potato. Buteler *et al*. (1999) reported 63 microsatellite loci of which only 9 were resolvable. In this study, out of nine amplified microsatellites, five loci segregated in Mendelian fashion. However, this is the first attempt ever made at generating this important class of markers. Zhang *et al*. (2000) developed 12 SSR markers from dinucleotide tandem repeats screening. Among 12 SSR primers, 6 primers polymorphic were screened for 113 varieties of CIP and revealed a total of 70 alleles with allele size ranged from 102-173 bp.

Eight SSR markers were used for genetic diversity study among Chinese, Japanese and Taiwan sweet potato cultivars, hybrids, polycross and landraces (Hwang *et al*. 2002). The total polymorphism identified was 85% and polycross-derived cultivars possessed high levels of genetic diversity and originated from various genetic resources, and suggested the usefulness of polycross breeding strategy in spite of frequent cross-incompatibility. Yanez and Oriando (2002) developed 15 pairs of SSR primers and eight of them showed polymorphism in eleven sweet potato accessions from different places of the world.

Hu *et al*. (2004) screened 1425 *I. trifida* sequences available from Gene bank to identify 61 microsatellite-containing sequences. Of these 61 sequences, they used 12 microsatellites to amplify sweet potato cultivars and wild species. A high degree of transportability was reported among species. To date, a total of 4829 sequences are available for *I. batatas* that might result in potential SSR resources. Hu *et al*. (2004) developed polymorphic microsatellite markers using small-insert enriched library (32 SSR), microsatellite-enriched library (47 SSR) and mining of EST databases (151 SSR). 27 library based and 120 EST based SSR primers were showed polymorphism among different accessions. Veasey *et al*. (2008) studied the genetic diversity among 78 Brazilian landraces collected from 19 local communities using eight SSR primers. Each primer pair generated three to ten clearly scorable polymorphic fragments. Based on the study, variability (58.2%) was distributed within the households and 18.1% between the households within the communities. Yada *et al*. (2010), studied the genetic relationships among 192 superior, high yielding, and disease-resistant sweet potato accessions from the Ugandan germplasm collection were analyzed using 10 fluorescent labeled SSR markers. The number of polymorphic alleles detected per locus ranged from two to six with a mean of four, a confirmation of the effectiveness of microsatellite primers. Cluster analysis divided the accessions into four major groups with no relationship to the district of origin. Two sets of duplicates were identified through SSR genotyping.

Genetic diversity of 89 sweet potato genotypes was evaluated using morphological and molecular markers. Twenty three unique alleles, ranging from 3 to 6/locus were detected using six SSR markers. Cluster analysis showed a Jaccard co-efficient ranging from 0.5 to 1.0 indicating high genetic diversity. Comparison between morphological and molecular data using the mantel test revealed a low correlation ($r = -0.05$) between the two data sets. Despite the poor correlation both techniques showed a high degree of variation among the genotypes suggesting great genetic diversity in Kenyan sweet potato genotypes that can be utilized in breeding programs (Karuri *et al*. 2010). Schalfeitner *et al*. (2010) developed microsatellite markers using pyro- and Sanger sequenences. Identified 1661 gene based microsatellite sequences, of which 223 were selected for polymorphism identification and 195 were found polymorphic among 6 sweet potato and 2 trifida accessions. Wang *et al*. (2011) investigated 1, 81,615 ESTs for the identification and development of SSR markers and identified total of 8,294 SSRs from 7,163 SSR-containing unique ESTs. Based on these SSR-containing sequences, 1,060 pairs of high-quality SSR primers were designed and used for validation of the amplification and assessment of the polymorphism between two parents of one mapping population ('E Shu 3 Hao' and 'Guang') and eight accessions of cultivated sweet potatoes. The results showed that 816 primer pairs could yield reproducible and strong amplification products, of which 195 (23.9%) and 342 (41.9%) primer pairs exhibited polymorphism between 'E Shu 3 Hao' and 'Guang

Table 2 Different molecular markers used for sweet potato diversity studies.

Population used	Reference
Morphology and isozymes	
1939 accessions - CIP, Peru	Huaman *et al*. 1999
Randomly Amplified Polymorphic DNA (RAPD)	
26 accessions – Oceania, Peru, Philippines, US	Jarret and Austin 1994
8 *Ipomoea* species	
6 cultivars - 36 primers	Connolly *et al*. 1994
9 cultivars - New Zealand	Harvey *et al*. 1997
18 cultivars - Papua New Guinea	Zhang *et al*. 1998
18 cultivars - South America	
18 cultivars - Chile	Sagredo *et al*. 1998
74 varieties - 23 countries	Gichuki *et al*. 2003
Brazilian cultivars	Valadares *et al*. 2011
Inter-Simple Sequence Repeat (ISSR)	
I. trifida, I. ramosissima, I. umbraticola, I. triloba, I. triloba, *I. batatas*	Huang and Sun 2000
100 landraces and 8 cultivar from China	He *et al*. 2007
15 high carotene lines and crosses	Ma *et al*. 2009
DNA Amplification Fingerprinting (DAF)	
73 accessions - USA, New Guinea., Tetraploid- *I. batatas* (*I. triloba*)	He *et al*. 1995
30 cultivars	Prakash *et al*. 1996
42 accessions - Guangdong, Fuji and Japan	Wang *et al*. 1998
Amplified 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 and Latin America	Zhang *et al*. 2004
775 accessions - USA	Bruckner *et al*. 2005
Simple Sequence Repeat (SSR)	
Ipomoea cultivars – China, Japan, Taiwan	Hwang *et al*. 2002
Cultivars and wild species	Hu *et al*. 2004
50 cultivars - Brazil	Veasey *et al*. 2008
89 cultivars - Kenya	Karuri *et al*. 2010
92 cultivars –East Africa	Tumuwegamine *et al*. 2011
Selective Amplification of Microsatellite Polymorphic Loci (SAMPL)	
22 elite cultivars - Taiwan	Tseng *et al*. 2001

2k-30' and among the 8 cultivated sweet potatoes, respectively.

Tumwegamire (2011) studied the genetic diversity among 85 East African farmer varieties and 7 non-African germplasm were analyzed using 26 SSR primers. A total of 158 alleles were scored with an average of 6.1 alleles per SSR loci. There was significant difference in East African and non-African germplasm, but there is no difference among white fleshed and orange fleshed cultivars (Tumwegamire *et al*. 2011).

Selective amplification of microsatellite polymorphic loci (SAMPL)

SAMPL markers were used to analyze the genetic relationship between 22 elite cultivars of sweet potato used in polycross breeding in Taiwan (Tseng *et al*. 2001). Among the 12 SAMPL primer pairs tested, 7 amplified 19 loci and total 55 alleles were amplified. The SAMPL data suggest that Taiwan landraces are distantly related to Chinese and Japanese cultivars. Employment of SAMPL markers is efficient compared to other molecular methods like RAPD and SSR.

Sequence-specific amplified polymorphism

Berenyi *et al*. (2002) developed a new class of markers from RNaseH-LTR regions of the Ty-copia retrotransposon that have shown 97 to 99% polymorphism in comparison with 70 to 90% in AFLPs and 88% in RAPDs, thus demonstrating their efficiency.

Cleaved amplified polymorphic sequences

Zhang *et al*. (2003) used cleaved amplified polymorphic sequences (CAPSs) and SNPs for the molecular characterization of the genes involved in starch metabolism, and an understanding of these gene-specific functions, phenotypic consequences, and interactions with the environment are important for improving the starch yield of sweet potato. Using a CAPS technique, generated a group of functional molecular markers using newly developed expressed sequence tags (ESTs) as well as available nucleotide sequences of sweet potato in public databases.

Tanaka *et al*. (2010) designed markers, 13 CAPS-base primer pairs from the exon sequences of 11 sweet potato genes to amplify fragments containing an intron. By digesting the amplified products with 8 restriction enzymes having different recognition sites, a total of 27 polymorphic marker fragments were obtained. Genotyping of 60 Japanese sweet potato cultivars using these markers suggested that the markers can effectively distinguish cultivars. Among the genes used for primer design, the gene encoding the dihydroflavonol 4-reductase (DFR) showed the largest degree of polymorphism. Various works conducted to study the extent and nature of genetic diversity using molecular markers in sweet potato was given in the **Table 2**.

FUTURE PERSPECTIVES

Genetic improvement and understanding genetic nature of the Sweet potato crop is difficult due to hexoploidy, heterozygous and heterogeneous nature of the crop. But recent time due to development of molecular markers especially SSR markers leads to better understanding of the genetic nature of the crops is easy. In sweet potato recent time more of SSR markers were developed using genomic, short sequence information and EST database, due to that more than 1000 SSR primers are available. These developed SSR markers will provide a valuable resource for genetic diversity, evolution, linkage mapping, comparative genomics, gene-based association studies, and marker-assisted selection in sweet potato genetic study. Since these markers were

developed based on conserved expressed sequences, it will be valuable for functional analysis of candidate genes.

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