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Characterization of fifteen microsatellite loci and genetic diversity analysis for the Ghanaian food security crop *Solenostemon rotundifolius* (Frafra potato)

Lichun Hua[1,4], Đenita Hadziabdic[1], Naalamle Amissah[2], Marcin Nowicki[1], Sarah L. Boggess[1], Margaret Staton[1], Nianjun Teng[3] and Robert N. Trigiano[1*]

[1]University of Tennessee Institute of Agriculture, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tennessee, USA.
[2]School of Agriculture, Department of Crop Science, University of Ghana, Accra, Ghana.
[3]College of Horticulture, Nanjing Agricultural University, Nanjing, People's Republic of China.
[4]Department of Plant Development and (Epi) Genetics, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands.

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Frafra potato (*Solenostemon rotundifolius*) is an important food crop indigenous to Western Africa and a member of the Labiaceae (mint) family. It is nutrient-rich and drought tolerant; however, despite its potential as a food security crop, it has not received the research support needed to promote its widespread adoption and safeguard its diversity. As a result, Frafra potato is becoming extinct and restricted to indigenous communities where it holds traditional importance. It is imperative that conservation methods are explored, but before then, the genetic diversity of the species should be assessed. The objectives of this study were to develop simple sequence repeats (SSRs) for Frafra potato from *de novo* sequencing of the genome, and to provide a preliminary assessment of genetic diversity of Frafra potato in Ghana. To this end, 57 accessions of Frafra potato obtained from three geographic regions of Ghana were characterized using 15 discovered microsatellites. Forty-six multilocus genotypes (MLGs) were identified among the 57 accessions. The genetic diversity among the MLGs was moderate-to-high. Preliminary evaluation of accessions from the three collection zones indicated the presence of population structure among Frafra potato individuals collected in Ghana, Africa.

Key words: Food security, Frafra potato, genetic diversity, microsatellites, simple sequence repeats, *Solenostemon rotundifolius*.

INTRODUCTION

Frafra potato (*Solenostemon rotundifolius* (Poir.) J.K. Morton, known in northern Ghana as Frafra potato or Persa, in South Africa as Zulu potato, and in India as Chinese potato, is an herbaceous plant that originates

*Corresponding author. E-mail: rtrigian@utk.edu. Tel: 865.386.1872. Fax: 865.974.4744.

from tropical Africa (Aculey et al., 2011). Currently, it is extensively cultivated in Mali, Ghana, Nigeria, and South Africa as well as in tropical Asia. *S. rotundifolius* (2n = 64) is a semi-succulent, aromatic herb between 40- 60 cm in height and is a member of the Lamiaceae or the mint family (Tindall, 1983). It produces edible, ovoid tubers up to 8 cm in length. The raw tubers are exceptionally nutritious, and 100 g of tissue contains 394 KJ (94 Kcal) of energy, 1.8 g protein, 0.2 g fat, 21.9 g carbohydrate, 17 mg calcium, 1.1 g fiber, and 6.0 mg iron (Anbuselvi, 2013). The tubers of Frafra potatoes are fermented to make alcoholic beverages (Opoku-Agyeman et al., 2007) or milled for flour, among other uses. However, Frafra potatoes' utility extends beyond that of food. Frafra potato tubers are popular for pharmaceutical and folk medicine applications. For example, the tubers are used to treat dysentery in rural Nigerian communities (Tindall, 1983). In addition to these uses, Frafra potato is also drought-tolerant (Scholten, 2007), probably because it efficiently processes and uses water (Nanema et al., 2009). Based on its superior advantages in nutritional composition, medicinal properties, and the ability to grow in tropical areas, Frafra potato is used as food for bridging the hunger gap (Sugri et al., 2013).

Despite the beneficial characteristics of Frafra potato, research into conservation and breeding of the species has been limited (Opoku-Agyeman et al., 2007; Kwarteng et al., 2018). Hence, it is imperative that the available FRgermplasm is collected and thereafter morphologically and genetically characterized to identify the best accessions for genetic crop improvement. To achieve this goal, specific microsatellite loci for Frafra potato were developed for assessing the genetic diversity among accessions from different regions in Ghana.

Microsatellite loci or simple sequences repeats (SSR) are repeated motifs of 2- to- 6 nucleotides that are found throughout the genome and occur mainly in non-coding regions, but are also present in coding segments (Ellegren, 2004). These markers have become relatively routine to discover at a very reasonable cost with the advent of high-throughput and advanced sequencing techniques for genomes. They are very efficient tools for assessing genetic diversity and population structure among the different cultivars and accessions grown by farmers as well as potentially useful accessions growing in the uncultivated environs. The aim of our study was to develop microsatellite loci specific to Frafra potato and preliminarily assess the population structure of the accessions via the genetic data gleaned from SSRs.

MATERIALS AND METHODS

Plant materials and DNA extraction

Fifty-seven *S. rotundifolius* accessions were collected from farms in the upper east (24), upper west (18), and the northern regions (15) of Ghana, and grown at the Department of Crop Science, University of Ghana, Accra, Ghana. Genomic DNA was extracted from frozen leaves of the accessions using a CTAB extraction protocol (Cota-Sánchez et al., 2006) and quantified using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Microsatellite loci development

Genomic DNA from a single plant of *S. rotundifolius* '4E016', an upper east accession, was sequenced at the University of Texas, Austin Genomic Sequencing and Analysis facility using an Illumina MiSeq sequencer (Illumina Inc., CA, USA). Paired-end reads were trimmed with the Skewer program version 0.2.2 (Jiang et al., 2014). Sequencing adapters and low quality (mean quality <30), and short reads (<30 bases) were discarded. Read quality control was performed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). *De novo* assembly was performed using ABySS v1.9.0 (Simpson et al., 2009) with a k-mer size of 64. Sequence filtering for low complexity repeats was completed using the utility DustMasker (Morgulis et al., 2006) on the resulting unitigs. Perfect microsatellite loci were identified using an in-house developed perl script. The minimum and maximum motif frequency defined were 6- to- 20 bp di- or tri-nucleotide repeats and 4 to -20 bp tetra-nucleotide repeats. Also, forward and reverse primers flanking each marker were designed using Primer3 (Koressaar and Remm, 2007). Optimum primer size was 21 bp (range 18-27), annealing temperature was 60°C, and a GC content of 40- to -60%.

Microsatellite loci amplification

The PCR conditions described by Wadl et al. (2011) with some modifications were used to screen and characterize 50 microsatellite loci for *S. rotundifolius*. PCR was performed using 10 µl reaction containing the following in final concentrations: 4 ng genomic DNA, 1×PCR Gold Buffer, 2.5 mM $MgCl_2$ (Applied Biosystems, Carlsbad, California, USA), 0.25 mM dNTPs, 5% dimethyl sulfide (DMSO; Fisher Scientific, Pittsburgh, Pennsylvania, USA), 0.4 U AmpliTaq Gold Polymerase (Applied Biosystems), 0.25 µM of forward and reverse primer pairs (Integrated DNA Technologies, Coralville, Iowa, USA) and sterile, distilled water. The contents of the reaction mixtures were subjected to the same thermal cycling program found in Wadl et al. (2011). PCR products were separated using the QIAxcel Capillary Electrophoresis System (QIAGEN, Valencia, California, USA) and sized with a 25 to 500 bp size marker and an internal 15/600 alignment marker (Wang et al., 2009).

Data analyses

QIAxcel ScreenGel (Version 1.5.0) was used to determine the sizes of the PCR products. Flexibin was employed to convert raw allele length data into discrete allelic classes (Amos et al., 2006). The R packages Poppr (Kamvar et al., 2014, 2015) and hierfstat (Goudet, 2005) were utilized for primary data analyses including the number of alleles per locus, observed and expected heterozygosity, evenness, and allelic richness. The population structure across clone corrected dataset (n = 46) was evaluated using STRUCTURE v2.3.4 (Pritchard et al., 2000), and Principal Coordinate Analysis (PCoA) with GenAlEx v6.5 (Peakall and Smouse, 2012). Analysis of molecular variance (AMOVA) was conducted with Arlequin v3.5.1.2 (Excoffier and Lischer 2010). Although STRUCTURE and AMOVA analyses were in agreement with PCoA, they were completed with limited accessions of Frafra potato. Large-scale population analyses require larger sample sizes, hence we present preliminary STRUCTURE and AMOVA results.

Table 1. Characteristics of 15 microsatellite loci developed for 57 accessions of *S. rotundifolius* (Frafra potato) collected in Ghana, Africa.

GenBank accession number	Locus	Primer sequences (5'-3')	Repeat motif	Tm (°C)	Number of alleles	Size range (bp)	H_o	H_e	Evenness	Allelic richness
MH784481	Sr_001	F: CAGAAGGTGAGGGAGAAGTGG R: GAAAGAGGTAAGCAGTGCAGC	$(AT)_8$	60	5	86-117	0.04	0.65	0.85	3.74
MH784482	Sr_009	F: GTGAGTGAGAGACTTATTTGCCC R: AGCTTGACTGTACTCGACTCG	$(AT)_8$	60	3	227-233	0.11	0.42	0.65	2.85
MH784483	Sr_011	F: TCAACCCACATACAACTTGCG R: ACGGATCCTTGCTTGGATTGG	$(AG)_{15}$	61	5	110-141	0.21	0.54	0.66	3.59
MH784484	Sr_017	F: TAAACAGCTAGGCTCCAACCC R: CGTCCCTCCATTTCTGTATTGC	$(AG)_8$	60	5	186-208	1.00	0.53	0.85	2.69
MH784485	Sr_018	F: GTTGTGATGGCCGGATTGC R: ACATGAGAGATAGGCAACGAGC	$(GA)_7$	60	4	180-196	0.57	0.45	0.74	2.49
MH784486	Sr_020	F: TCTGAACCATTGATTAAGCGG R: TCTTCTCACCTGATTTCTCTCC	$(AT)_6$	57	9	132-161	0.64	0.77	0.76	4.43
MH784487	Sr_021	F: CCAACTCTATTCGTCGGCAGC R: AATCCATACCATACGCCAGGG	$(AAT)_{11}$	61	5	194-219	0.40	0.34	0.50	3.13
MH784488	Sr_023	F: GACATTTGGCGCTTTCTCACC R: TCCCAACAGTTTGATCCTCCC	$(CTG)_6$	60	4	350-368	1.00	0.68	0.86	3.60
MH784489	Sr_024	F: CTGCTGTAGCTTTCCCTTTGC R: AACTGGGCATGCTACTACTCG	$(TGT)_6$	60	5	162-177	1.00	0.65	0.78	3.82
MH784490	Sr_034	F: ATTGGCGGTTATTGGTTGACC R: CCCACCATACACATATATAATCGGC	$(TTA)_6$	59	4	250-261	0.20	0.42	0.59	3.20
MH784491	Sr_037	F: AATCCCATCAACAGCCTGAGG R: ATTGCTGGAAGAAATGCTGGC	$(TGC)_7$	60	6	157-171	1.00	0.74	0.84	4.66
MH784492	Sr_039	F: ATGAGGCAGAGAGAGGACTCC R: TGCACCACTGTCTTCTTCTCC	$(GAT)_6$	60	4	144-154	1.00	0.67	0.86	3.83

Table 1. Contd.

MH784493	Sr_044	F: TCGTGTAGCAATTTCTCACTGC R: GCCAGTGATTAGTTGCACTCG	$(TCT)_7$	60	5	241-253	0.02	0.72	0.88	3.78
MH784494	Sr_045	F: CATCGAATCTCAGATCGTGGC R: GTCGCCATCAAGTCTACTGCC	$(CGG)_6$	61	5	108-126	1.00	0.70	0.83	4.44
MH784495	Sr_046	F: TTCTCTTCCAATTCCTCCGCC R: TGCAGGGAAGGTTTAGGTTGG	$(CTT)_7$	60	6	115-129	0.57	0.67	0.77	4.20

Ho, observed heterozygosity; He, expected heterozygosity.

RESULTS

One gDNA sample ('4E016') was sequenced. Based on the 3,791,769 sequences (reads), 53,124 microsatellites were discovered. Among them, di-nucleotide repeats accounted for the highest (66.9%), whereas tri- or tetra-nucleotide repeats accounted for 11.5 and 21.5%, respectively. Primer pairs for 15,502 loci were designed based on these microsatellites and the percentages for di-, tri-, and tetra-nucleotide repeats were 70.9, 13.3, and 15.8%, respectively.

Fifty primer pairs were screened with six randomly chosen genomic DNA samples, and the fifteen primer pairs that amplified definitive polymorphic alleles without extraneous peaks were employed to analyze the 57 accessions. Clone correction with Poppr identified 11 clones of which five were from the upper east region, one from the upper west region, and five from the northern region. Clone-corrected data (n = 46) indicated the presence of seventy-five alleles among 15 polymorphic loci. The number of alleles per locus ranged between 3 (Sr_009) and 9 (Sr_020), with an average of 5 alleles per locus (Table 1). Observed heterozygosity of the 46 accessions was between 0.02 and 1.00, whereas the expected heterozygosity was 0.34 to 0.77. Compliance with the Hardy-Weinberg equilibrium was not evaluated because S. rotundifolius is reproduced clonally. Evenness and allelic richness were 0.76 and 3.36 across all loci, respectively, ranging from 0.50 (Sr_021) to 0.88 (Sr_044) for evenness and 2.49 (Sr_018) and 4.66 (Sr_037) (Table 1). Analysis of microsatellite data from the three collection zones in Ghana with STRUCTURE and principal coordinate analysis (PCoA) indicated the possibility of three different clusters. However, a more extensive collection of accessions is required to confirm these findings (Figure 1).

DISCUSSION

In this study, the first set of microsatellites from S. rotundifolius has been developed, and these loci provide a set of markers to evaluate the genetic diversity of cultivated Frafra potato. The genetic diversity among the sampled Frafra potato accessions was moderate - to- high, and in our sample of 57 accessions, there were only 80% MLGs indicating clonal reproduction among these accessions. Balloux et al. (2003) indicated that asexual or clonally reproducing populations of plants sustain higher genetic diversity at loci compared to sexually reproducing plants. However, the report also stated that clonal plants typically have fewer genotypes which appear to be true for Frafra potato. Preliminary results of this research suggest the presence of population structure among the accessions from the three regions. However, more accessions must be included in the data set before this finding can be confirmed. It can also be inferred that trade of plants among local farmers contributes to levels of genetic diversity found here. Furthermore, this research data is restricted to the limited number of genotypes collected in three regions of Ghana and does not provide a complete picture of the current status of this indigenous crop that is vulnerable to potential abiotic and biotic stresses.

Conclusion

The microsatellite markers developed for this study were successfully deployed to assess the genetic diversity of Frafra potato in Ghana. The present study will facilitate further genetic and more complete population studies of this critical African food security crop across a more

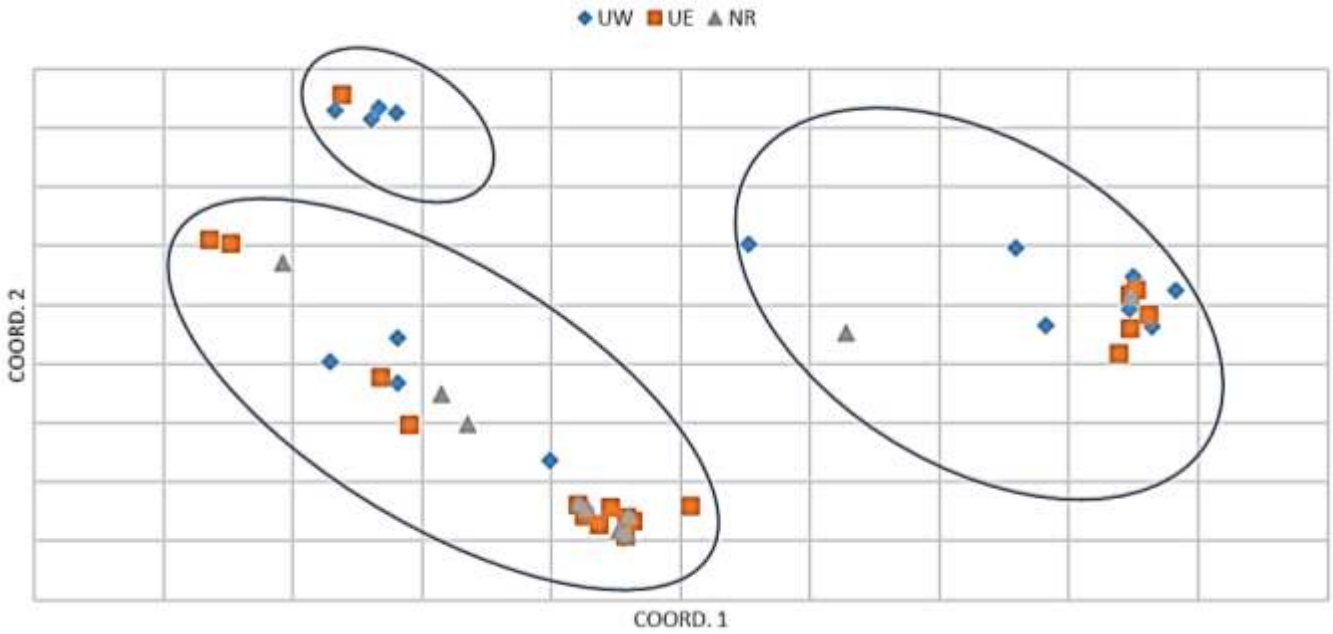

Figure 1. Principal coordinate analysis (PCoA) of *S. rotundifolius* (Frafra potato) accessions from Ghana, Africa. Analysis of clone corrected data revealed three groups among the collection zones. UW = Upper West; UE = Upper East and NR = Northern Region of Ghana.

extensive geographical area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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