Morphological and Molecular Characterization of Frafra Potato (*Solenostemon rotundifolius*) Accessions in Ghana

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ABSTRACT

Frafra potato [*Solenostemon rotundifolius* (Poir. J. K. Morton] is a minor tuber crop that has the potential to address food and nutrition security issues in the wake of climate change. The crop contains higher levels of iron compared to other tuber crops like cassava, yam and sweet potato. In addition, it is adapted to marginal soils and drought conditions. Unfortunately, it is becoming extinct as the focus has been on a few declining but widely cultivated crop species. In this study, fifteen microsatellites (SSR) primers were used to characterize 57 accessions collected from the Northern, Upper West and Upper East Regions of Ghana. These accessions were subsequently characterized using 15 qualitative and 15 quantitative morphological descriptors. Both morphological and molecular analyses grouped the accessions into two clusters. Accessions UE004, UE008 and QA99067 were found to be very promising and can be multiplied for farmers or used as parents in a hybridization programme for improvement of the crop.

Keywords: Accessions, characterization, simple sequence repeats, minor tuber, indigenous crop, food security.

Introduction

Frafra potato (*Solenostemon rotundifolius*) is a minor underutilized tuber crop with resilience to climate change and global warming. It originates from East and Central Africa and is currently grown in parts of Asia and Africa. In Ghana it is traditionally grown in the Northern, Upper East and Upper West regions (Sugri *et al.*, 2013) where it is seen as an important food security crop. The local name of the crop varies depending on the geographic location. It is referred to as 'Frafra' potato (Persa) in Ghana, 'Hausa' potato in Nigeria, 'Sudan' potato in Sudan, 'Zulu' potato in South Africa and 'Chinese' potato in parts of India (PROTA, 2004).

S. rotundifolius is a dicotyledonous root crop belonging to the mint family Lamiaceae or Labiatae and a subfamily Neteptoideae (Agyeno *et al.*, 2014), and has 2n = 62 chromosomes (PROTA, 2004). The leaves of Frafra potato have a characteristic, minty aromatic smell which is said to be the result of volatile oils present in the sacs of the leaves (Enyiukwu *et al.,* 2014). Its leaves are slightly thick and distinctively veined or thick with invisible veins (Nkansah, 2004). The leaves are arranged in a simple opposite fashion and are either mostly pigmented along the edge or the entire leaf, or scattered on the leaf surface. It has a succulent stem that is branched and either erect or decumbent. Its flowers come in hues of purple/pink (pale violet, bluish purple, white or pinkish) and are small, hermaphroditic and zygomorphous in nature. The flowers are borne on a distal inflorescence with thin false spikes that are approximately 15cm long (Enyiukwu *et al.*, 2014; Sugri *et al.*, 2013). The pedicel of the crop is about 1-2 mm long and has a campanulate calyx about 1-3 mm long (PROTA, 2004).

As a food crop, it is rich in all the nutritional elements expected in a staple crop. According to Nkansah (2004), the composition of a 100 g portion of the raw tuber of Frafra potato contains approximately 75.6 g of water, 394 kJ of energy, 1.4 g of protein, 0.2 g fat, 21.9 g of

carbohydrate, 1.1 g of fibre, 17 mg of Ca, 6.0 mg of Fe, 0.05 mg of thiamine, 1.0 mg of niacin, and 1 mg of ascorbic acid. The iron content of Frafra potato tubers is six times (6.0 mg/100 g) that of Irish potato (0.78 mg/ 100 g) and contains high amounts of reducing sugars (26 mg) in its tubers compared to other tuberous staples (Anbuselvi and Balamurugan, 2013a; Enyiukwu *et al.*, 2014; Kwarteng *et al.*, 2018). Its leaves also contain significant amounts of potassium (0.9 mg), phosphorus (24 mg) and 0.8 mg of sodium (Anbuselvi and Balamurugan, 2013b). It is known to have medicinal benefit due to the presence of a variety of secondary metabolites such as tannins, terpenoids, saponins, steroids, alkaloids and anthocyanins (Anbuselvi and Priya, 2013; Kwarteng *et al.*, 2018). The leaves of the crop are used in the treatment of dysentery, stomach pain, nausea, vomiting, diarrhoea, mouth and throat infections and as a purgative, a carminative and an anthelmintic (Anbuselvi and Priya, 2013; Archana *et al.*, 2015).

Despite these attributes, the availability of Frafra potato (FP) is impeded by a number of production constraints such as small tuber sizes, low yielding varieties, high postharvest losses, pests and diseases (Sugri *et al.*, 2013). Of these, small tuber sizes constitute the major constraint identified by Sugri *et al.* (2013) and hence the need for its improvement.

In order to address these production constraints, there is the need to identify promising Frafra potato accessions for use in crop improvement and cultivation. Characterization is the description of plant germplasm for identification and differentiation (de Vicente *et al.*, 2005) using physical, agronomic or morphological characteristics and molecular markers such as microsatellites to identify variation in plant species. Microsatellites, or simple sequence repeats (SSR) are repeated sequences of nucleotides distributed throughout the genome that occur mainly in non-coding regions (Ellegren, 2004). SSR markers have the advantage of being low-cost and highly polymorphic, making them an ideal tool for determining genetic diversity among different cultivars. It aids in the development of adequate information for species that are already existing and need improvement or are underutilized and require improvement (Bioversity International, 2007).

The aim of this study was to determine the variability and relatedness among Frafra potato accessions, and to identify potential accessions for cultivation and improvement.

Materials and Methods

Planting materials and DNA extraction

Fifty-seven (57) accessions of Frafra potato tubers from the Northern (15 accessions), Upper East (24 accessions) and Upper West (18 accessions) regions were obtained from the Savannah Agricultural Research Institute (SARI-CSIR) and the Plant Genetic Resource Research Institute (PGRRI) Bunso, Ghana. The tubers were planted out in the Department of Crop Science, University of Ghana. Leaf samples were collected and DNA extracted for molecular characterization using the Cetyl trimethylammonium bromide (CTAB) procedure (Winnepenninckx, 1998).

Experimental design, field layout and cultural practices

Field evaluations were carried out from September 2016 through March 2017 at the University of Ghana farms in the Coastal Savannah Zone of Ghana. The area has a mean annual rainfall of ~ 800 mm with a range of 500 mm to 1270 mm per annum. The field experiment was laid out in a randomized complete block design with three replications. Each experimental entry was represented by three mounds per replication, making a total of 171 mounds for the experiment. Mounds measured 1m x 1.5 m and were spaced 50 cm apart. Ten (10) sprouted tubers were transplanted onto the field at a planting distance of 20 cm apart per plot. Weeding was manually carried out as and when needed. To protect against insect pests, the field was sprayed with a systemic insecticide, Akape® 20SC (200 g/Litre imidacloprid) at a rate of 1ml per 2.2 litres of water two weeks after sowing and subsequently sprayed with Attack® (475 g/Litre pirimiphos-methyl +

25 g/Litre permethrin EC) at 2.5 ml per litre of water at six weeks after sowing.

Morphological characteristics assessed

Morphological characterization was carried out using 15 qualitative and 15 quantitative descriptors, developed from descriptors used by Opoku-Agyemang *et al.* (2007) and Nanema *et al.* (2009). Table. 1 shows the descriptors used and their measurements. All qualitative characters were described using five randomly selected plants from each accession. Data on the five plants were collected using a scoring system, where the presence or the absence of a character is scored as 1, 2 or 3 depending on the number of variants observed in the accession. Where two different colours were involved, Mansell's colour chart was employed. Both continuous and discontinuous quantitative variables were scored.

Molecular characterization

Polymerase chain reaction (PCR) was performed using 15 oligonucleotide primers developed by Hua *et al.* (2018). Ten (10) µL reaction: 4 ng genomic DNA, 1×PCR Gold Buffer (Applied Biosystems, Carlsbad, California, USA), 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 primer pair (both forward and reverse) and sterile water. The Qiaxcel Advanced Electrophoresis system was used with an internal alignment marker 15/600bp and an internal size marker 25-500bp.

Data analysis

The data collected using these descriptors were subjected to univariate and multivariate analysis in R software version 3.3.3, GENSTAT 2012, and XLSTATS version 2017. The univariate analysis was done using results of the quantitative traits while the qualitative traits were tested using descriptive statistics. Multivariate analysis was also performed on both qualitative and quantitative traits using principal component analysis. Correlation in phenotypic traits was assessed using the quantitative traits. Cluster analysis was used to determine relatedness among the accessions.

Results

Variation in qualitative morphological traits

Variation in basal stem colouration was observed among the accessions, with 47% showing stem colourations while the remaining 53% did not show any basal stem colouration (Figure 1). Colouration of the first node was observed in 38 % of the accessions (Figure 2). The rest of the accessions had the normal green colouration of the first node.

Thirty-five of the accessions were short (15-29 cm), while the remainder were taller (> 20 cm) (Figure 3).

Diversity in leaf pigmentation was observed among the accessions, pigmentation along half the edge of the leaf towards the narrow upper region of the leaf, pigmentation on the leaf surfaces, pigmentation along the entire edge of the leaf and no pigmentation on the leaves (Figure 4). The last group was the most common. Foliage colour among the accessions also varied, with some accessions having light green (19.3%), green foliage (73.3%) and a mixture of green and light green foliage colouration (7.4%) (Figure 5).

Two major variants were observed in leaf waffleness (Figure 6). One variant with small and slightly thick leaves measuring about 4.5 cm in length was observed in 68 % of the accessions, and the remaining accessions had broad, thick leaves approximately 8 cm in length. Also, two types of leaf arrangements , dense and very dense, were observed (Figure 7).

There were two variants among the accessions regarding the type of inflorescence they produce: pale violet (51%) or bluish white (31.5%) (Figure 8). Ten of the accessions (17.5%) did not flower. Two tuber skin texture variants were present among the accessions (Figure 9). Thirty-six (36) accessions had smooth skin tubers, while the others (21) were rough skin. Diversity was observed also in the shapes of the tubers, in the form of three tuber shapes: oblong, ovoid and long (Figure 10).

Three tuber skin colours were observed in the collection studied (Figure 11). The most common was the brownish skin colour. Some accessions showed blackish skin and others had whitish skin. Variations observed in tuber size showed 44 accessions representing 77.2% of all the accessions studied had small tuber sizes, 10 accessions representing 17.5% showed medium tuber sizes and 3 of the accessions representing 5.3% had big tuber sizes. Figure 12 shows the distribution of tuber size among the accessions studied.

Table 1: Qualitative Descriptors used to characterize *S. rotundifolius* **accessions**

Trait	Description	Measurement
Basal colour of the stem	Variation in the colour of basal stem	1. Coloured 2. Not coloured
Colour of first node	Variation in the colour of the first node of some of the accessions	1. Coloured 2. Not coloured
Presence of pigmentation on leaves	The leaves of some accessions are coloured at different parts of the foliage.	1. Presence of pigmentation on top of the edge of leaves 2. Pigmentation on all leaves 3. Pigmentation on both edges of the leaf 4. No pigmentation on leaves
Foliage colour	Colour of the whole foliage in different shades of green	1. Light green 2. Dark green
Leaf waffleness	The leaves of various accessions are different in thickness and smoothness	1. Small sized and slightly thick and distinctively veined leaf about 4.5cm in length 2. Broad and very thick leaf of about 8cm in length with invisible veins
Leaf density	Arrangement of leaves and number of leaves on the stem	1. Dense 2. Highly dense
Flowering	Has the accession flowered or not	1. Flowered 2. No flowering
Type of flower	Different flower colouration in the accessions that flowered	1. Blue-pale 2. Purple 3. No flowering
Tuber texture	Variations in the tuber texture	1. Smooth 2. Rough
Tuber shape	Shape of harvested tubers	1. Oblong 2. Ovoid 3. Both 1 and 2
Tuber Skin colour	Colour of harvested tubers	1. Brownish 2. Blackish 3. Whitish
Presence of lateral tubers	Other tuber apart from the oblong, and Ovoid	1. Present 2. Absent
Tuber size	Size of harvested tubers	1. Small tubers (< 10 g) 2. Medium (> 10 g < 20 g) 3. Big (> 20 g)

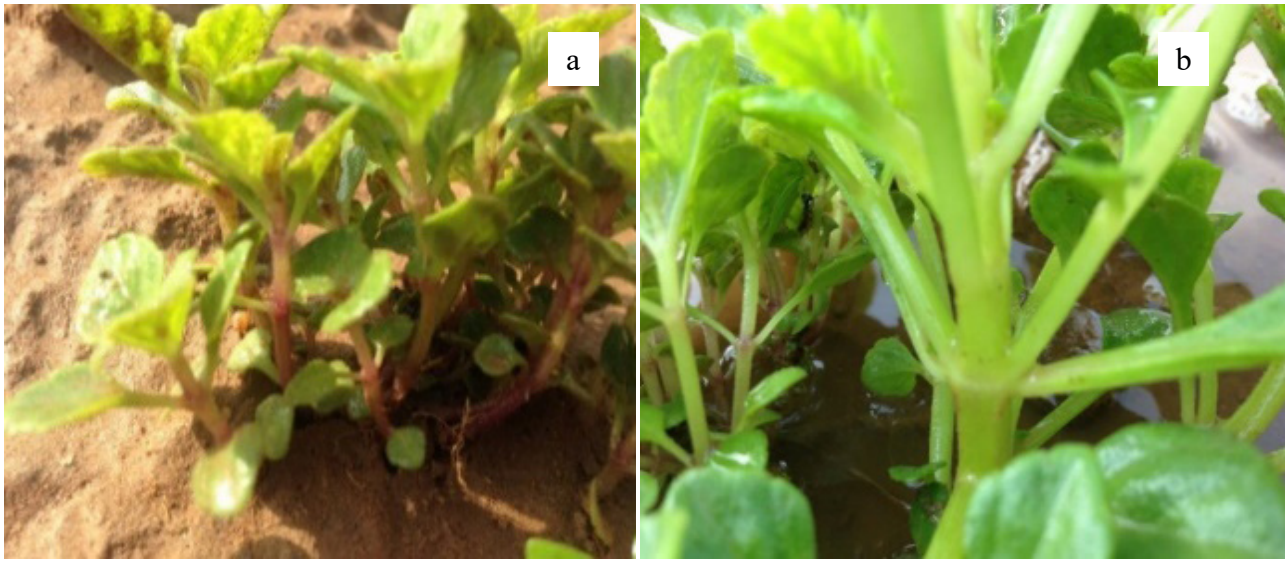

Fig. 1: Diversity in basal stem colour
(a= Coloured basal stem, b= Green Basal stem)

Fig. 2: Diversity in Colour of First node
(a= Coloured node, b= Normal green node colour)

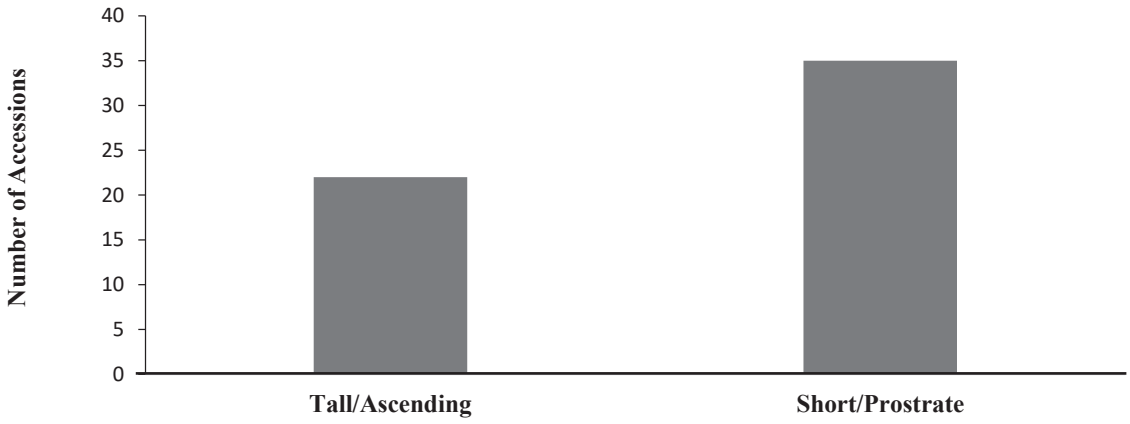

Fig. 3: Diversity in height and growth form among accessions

Fig. 4: Diversity in pigmentation on leaves
(a=Presence of pigmentation on half of the edge of leaf, b= Presence of pigmentation on Leaf (PPL) surface, c= PPL on entire edge of the leaf, d= No pigmentation on leaf)

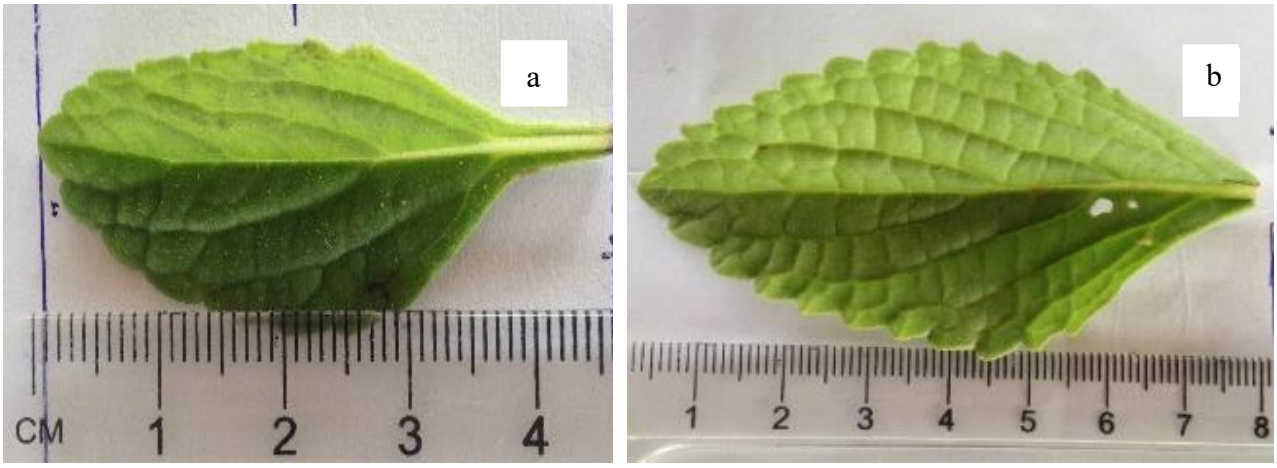

Fig. 6: Diversity in leaf waffleness
(a= Small sized and slightly thick leaf about 4.5 cm in length, b= Broad and very thick leaf of about 8 cm in length)

Fig. 7: Diversity in leaf density
(a= Dense, b= Highly dense)

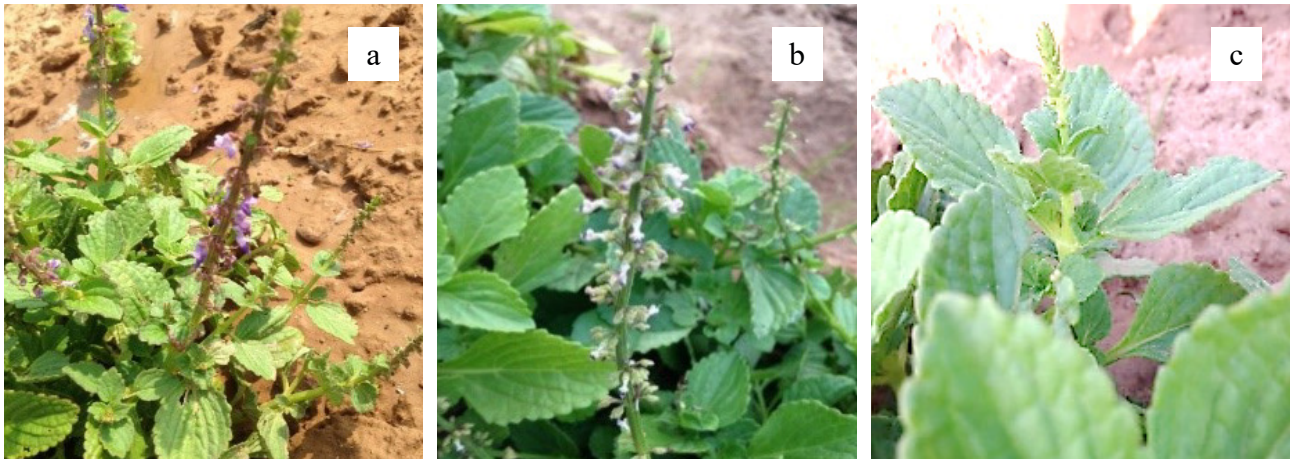

Fig. 8: Diversity in type of flower
(a= Pale violet, b= Bluish white, c= No flowering)

Fig. 9: Diversity in tuber skin texture
(a= Smooth Tuber Skin Texture (TTE), b= Rough TTE)

Fig. 10: Diversity in tuber shape
(a= Oblong Tuber Shape (TS), b= Ovoid TS, c = Long TS)

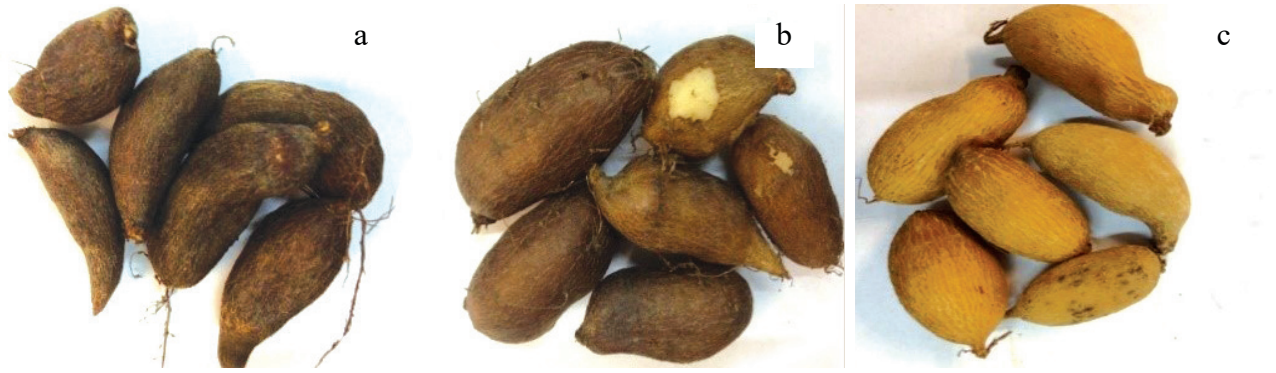

Fig. 11: Diversity in tuber skin colour
(a= Blackish tuber skin colour, b= Brownish tuber skin colour, c= Whitish tuber skin colour)

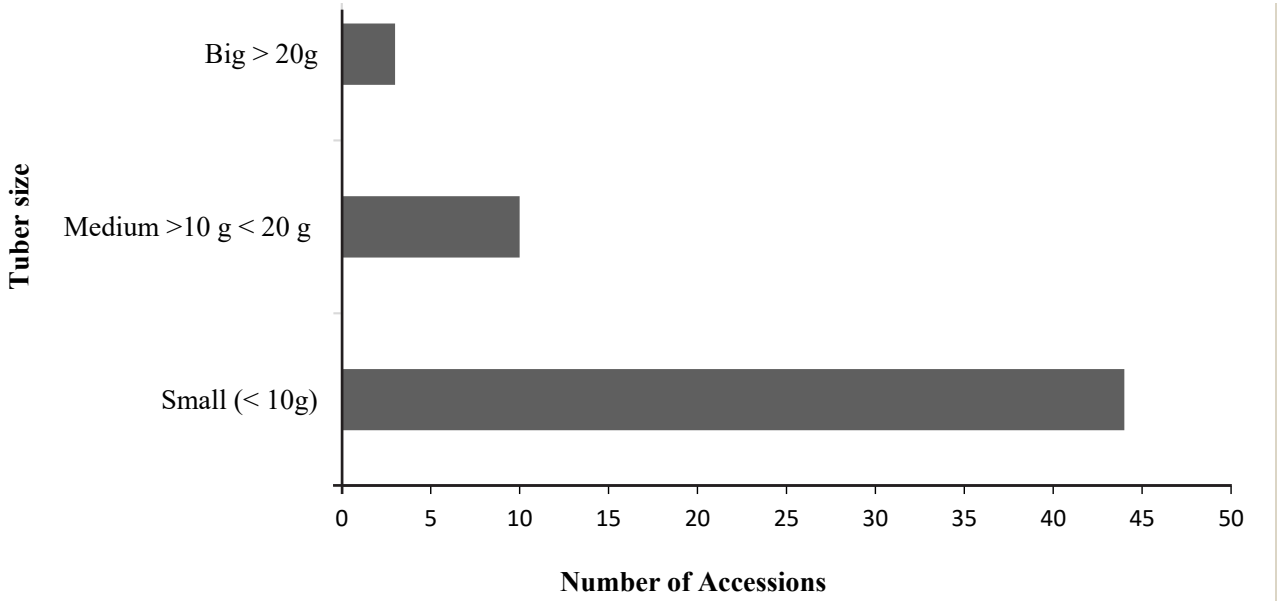

Fig. 12: Variation in tuber size among accessions

Relatedness of accessions based on qualitative traits

The sequential agglomerative hierarchical and nested (SAHN) cluster analysis using the Unweighted Pair Group Method on all qualitative descriptors grouped the accessions into three clusters (Figure 13). At a dissimilarity index of 86%, the first cluster included 10 accessions, the second cluster 36 accessions and the third cluster 11 accessions.

Associations among quantitative morphological traits

Days to flowering correlated negatively with leaf area, number of internodes, and the total number of tubers per plant, but this was not significant (Table 2). Days to flowering however correlated positively with all other descriptors. Days to maturity correlated negatively with plant height, length of the principal stem, number of internodes, and average weight of tubers, but correlated positively and significantly with Leaf area (r = 0.32, P < 0.05) and positively with the other descriptors. Plant

height correlated positively with leaf area and weight of total tubers per plant, positively and significantly with Length of the principal stem (r = 0.98, P < 0.001) and number of internodes (r = 0.71, P < 0.001), but negatively with the remaining descriptors. Leaf area had a negative correlation with days to flowering, a positive significant correlation with the total number of tubers per plant (r = 0.30, P < 0.05), and positive correlation with leaf area and weight of total tubers per plant.

Length of principal stem showed high positive and significant correlation with only number of internodes (r = 0.74, P < 0.001) and correlated positively with the weight of total tubers per plant. The number of internodes showed a positive correlation with the total number of tubers per plant, the weight of total tubers per plant, and the average weight of tubers.

The total number of tubers per plant showed a high positive correlation with the weight of total tubers per plant (r = 0.74, P < 0.01), but correlates negatively with average weight of tubers. The weight of total tubers per plant showed positive significant correlation with average weight of tubers.

Relatedness of accessions based on quantitative traits

The SAHN cluster analysis of the quantitative descriptors grouped the accessions into four clusters (Figure 14). At a dissimilarity index of 65 %, the first cluster included 18 accessions, the second 19 accessions, the third 18 accessions and the fourth two accessions (UE004 and QA99067).

Relative contribution of quantitative traits to diversity in accessions

The relative contribution of each quantitative morphological trait to the total variability observed among the Frafra potato accessions studied is shown in Table 3. The sum of the first five principal components accounted for about 75 % of the total quantitative variation observed among the accessions.

The first principal component accounted for about 24 % of the total quantitative variation, with NST, NBT, TTP, WTP and DBT as the key traits of importance. The traits HE, LPS and NIN were the key ones accounting for 19 % of the variability in the second principal component observed. The third principal component (14 %) had the diameter of small tubers (DST), the average weight of tubers (AWT) and diameter of medium tubers (DMT) showing the greatest influence.

Figure 15 shows a biplot using the first and the second principal components. Principal component one separated the accessions into two major groups of 25 and 32 showing that the accessions within a group are closely related than accessions separated into the other group. Principal component two divided the accessions into two major groups of 26 and 31 as well. The plotting of both PC1 and PC2 grouped the accessions into four groups with closely related accessions clustering in one group and diverse accessions forming other groups.

Relatedness of accessions based on combined quantitative and qualitative traits

The SAHN cluster analysis using the combination of quantitative and qualitative descriptors grouped the accessions into three clusters (Figure 16). At a 95 % dissimilarity index, cluster one included 23 accessions, cluster two 32 accessions and the third, tow accessions two accessions.

Relatedness of accessions based on SSR results

The results of the SAHN cluster analysis using the 15 simple sequence repeat markers is shown in the dendogram in Figure 17. The accessions were grouped into ten main clusters, namely cluster 1 to cluster 10. The majority of accessions were grouped in cluster 1 (25 accessions). Cluster 2 had two accessions which are UE018 and UW015, cluster 3, 11 accessions, cluster 4 seven accessions, clusters 5, 8, 9 and 10 had one accession each: UE005, UW022, WHITE and QA99016 respectively. Meanwhile, cluster 6 had six accessions and cluster 7 had two accessions (UE014B and QA99067) clustering as one group.

Genetic parameter summary

Primers Sr021 and Sr034 showed the highest allele frequency of 0.82 and 0.83 respectively (Table 4). On average, all the primers showed 0.54 allele frequency. Primer Sr020 showed the highest allele number, while on average, all the primers showed 5 alleles. While the diversity of the genes among the accessions was 0.57, primer Sr020 showed the highest diversity of 0.78. On average, all the primers showed 0.58 heterozygosity. Primers Sr023, Sr024, Sr037, Sr039 and Sr045 showed the highest heterozygosity of 1 or 100 %. The most polymorphic primer was Sr020 while the least polymorphic was Sr034. All the primers showed 0.52 polymorphism information content, on the average.

Table 2: Correlation coefficient (*r*) among quantitative descriptors

	DFL	DMA	HE	LA	LPS	NIN	TTP	WTP
DMA	0.16 ns							
HE	0.11 ns	0.12*						
LA	-0.01 ns	0.32*	0.16 ns					
LPS	0.15 ns	-0.12 ns	0.98***	0.16 ns				
NIN	-0.002 ns	-0.19 ns	0.71***	0.24 ns	0.74***			
TTP	-0.01 ns	0.20 ns	-0.05 ns	0.29*	-0.03 ns	0.01 ns		
WTP	0.13 ns	0.24 ns	0.01 ns	0.22 ns	0.01 ns	-0.01 ns	0.74***	
AWT	0.04 ns	-0.01 ns	-0.06 ns	0.20 ns	-0.05 ns	0.02 ns	-0.01 ns	0.41**

*P< 0.05, **P<0.01, ***P<0.001, ns, not significant. Days to flowering (DFL), Days to maturity (DMA), Height (HE), Leaf area (LA), Length of principal stem (LPS), Number of internodes (NIN), Total number of tubers per plant (TTP), Weight of total tubers per plant (WTP), Average weight of tubers (AWT)

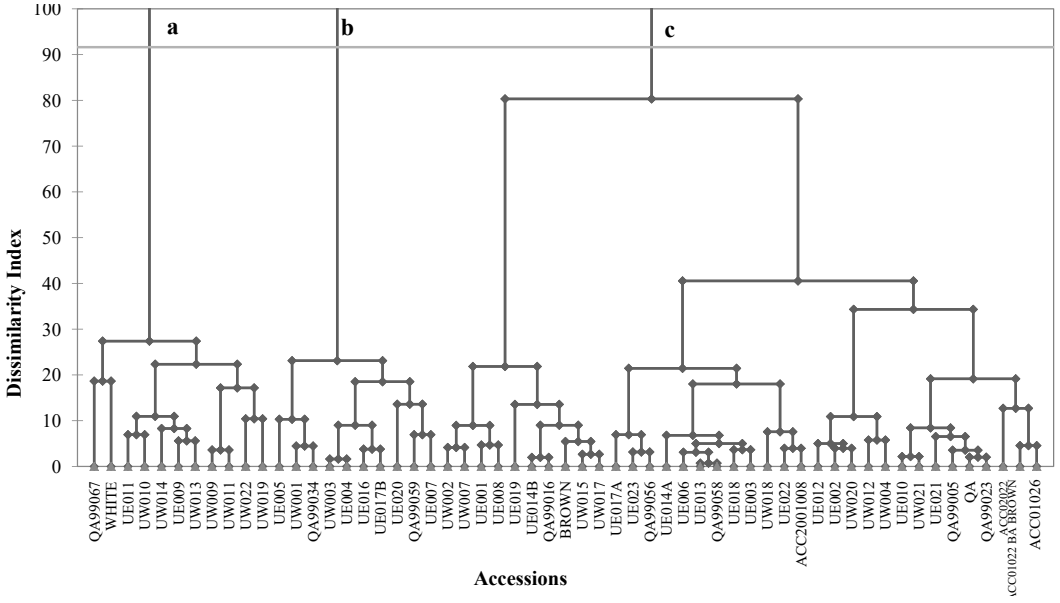

Fig. 13: Dendrogram of Frafra potato accessions based on qualitative descriptors

Table 3: Principal component analysis of quantitative morphological traits

	Principal component axes (Loadings)				
Trait	PC1	PC2	PC3	PC4	PC5
DFL	0.228	0.058	-0.035	**0.690**	-0.133
HE	-0.319	**0.881**	-0.134	-0.044	-0.051
LPS	-0.311	**0.894**	-0.130	-0.037	-0.060
NIN	-0.247	**0.831**	-0.039	-0.050	-0.070
LA	0.331	0.436	0.188	-0.074	**0.672**
DMA	0.390	-0.034	-0.045	0.368	**0.686**
NST	**0.806**	0.235	-0.422	-0.010	-0.065
NMT	0.122	-0.353	-0.333	**-0.645**	0.109
NBT	**0.503**	-0.021	0.465	-0.212	-0.131
TTP	**0.818**	0.227	-0.438	-0.036	-0.059
WTP	**0.857**	0.255	-0.041	-0.175	-0.161
DST	0.358	0.213	**0.628**	-0.237	0.025
DMT	0.030	0.105	**0.668**	0.470	-0.085
DBT	**-0.713**	0.055	0.098	-0.350	0.271
AWT	0.349	0.115	**0.675**	-0.344	-0.098
Variation (%)	**24.359**	**18.867**	**13.861**	**10.894**	**7.360**
Cumulative variation (%)	**24.359**	**43.226**	**57.088**	**67.982**	**75.341**

Note: **DFL** = Days to flowering, **HE** = Height, **LPS** = Length of principal stem, **NIN** = Number of internodes, **LA** = Leaf area, **DMA** = Days to maturity, **NST** = Number of small tubers, **NMT** = Number of medium tubers**, NBT** = Number of big tubers, **TTP** = Total number tubers per plant, **WTP** = Weight of total tubers per plant, **AWT =** Average weight of tubers, **DST** = Diameter of small tubers, **DMT** = Diameter of medium tubers, **DBT** = Diameter of big tubers, **V** = Percentage variability, and **CV** = Cumulative variability

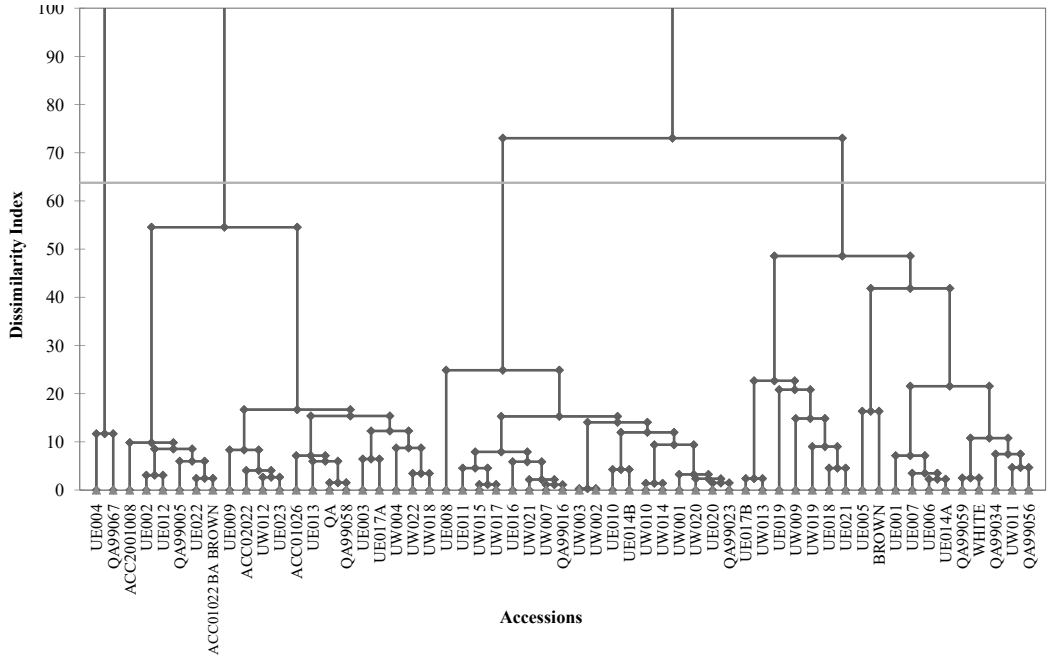

Fig. 14: Dendrogram of Frafra potato accessions based on quantitative descriptors

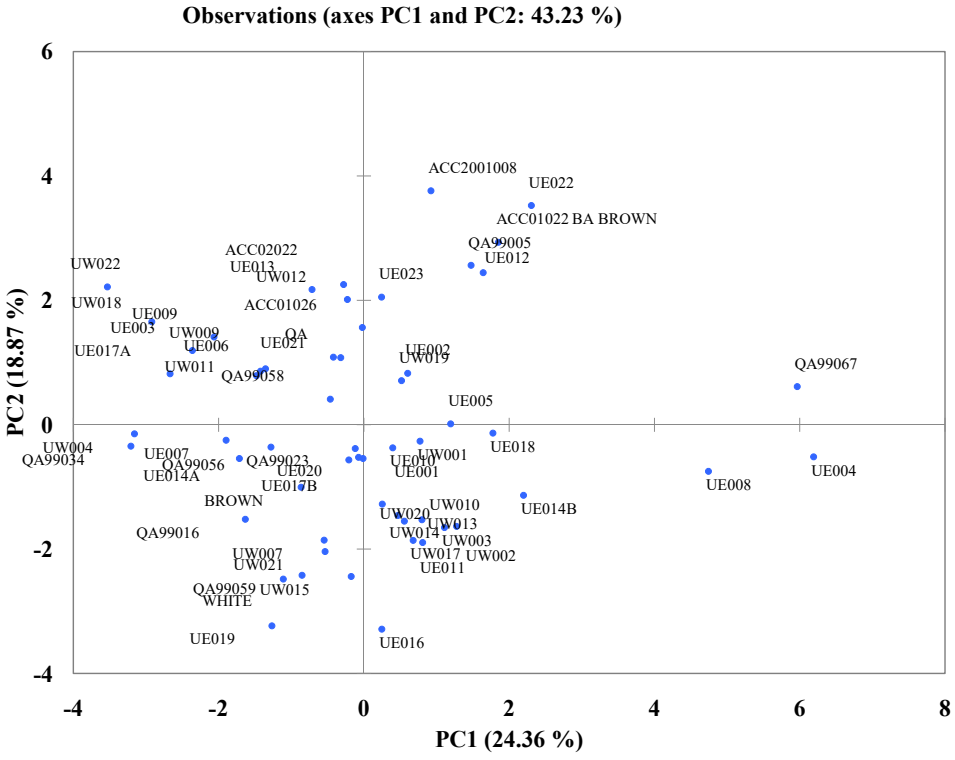

Fig. 15: Interspecific differences in Frafra potato accessions as given by PC1 and PC2.

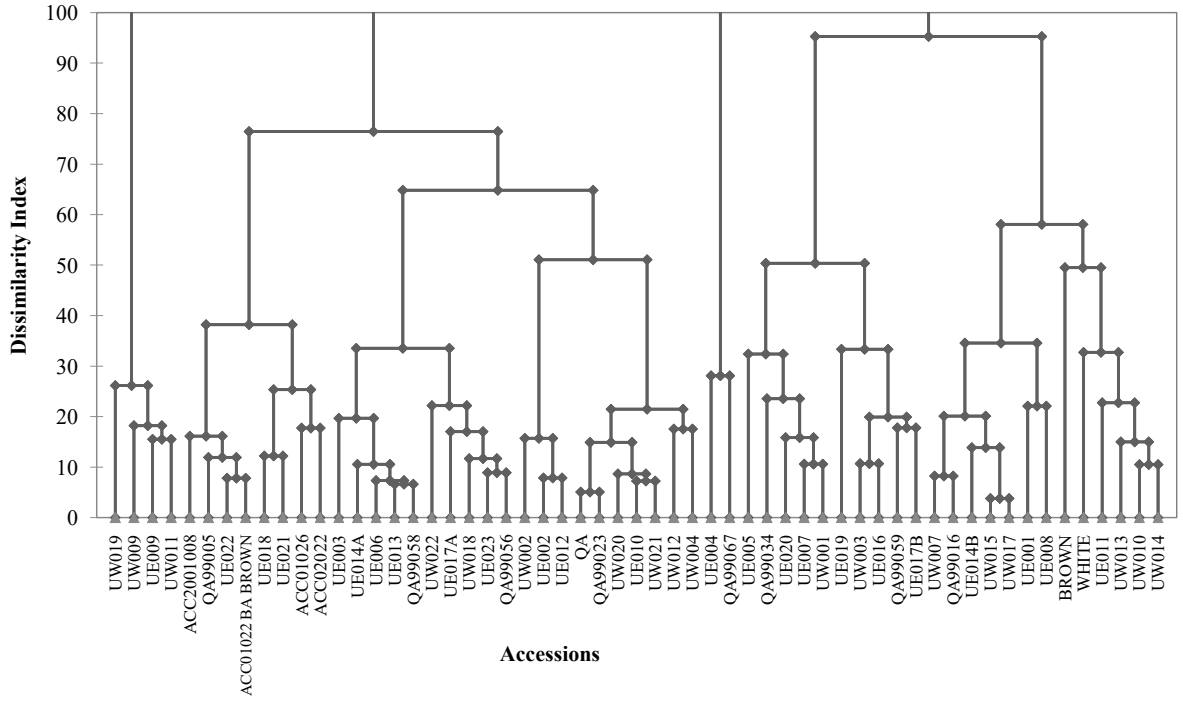

Fig. 16: Dendrogram of Frafra potato accessions based on qualitative and quantitative descriptors

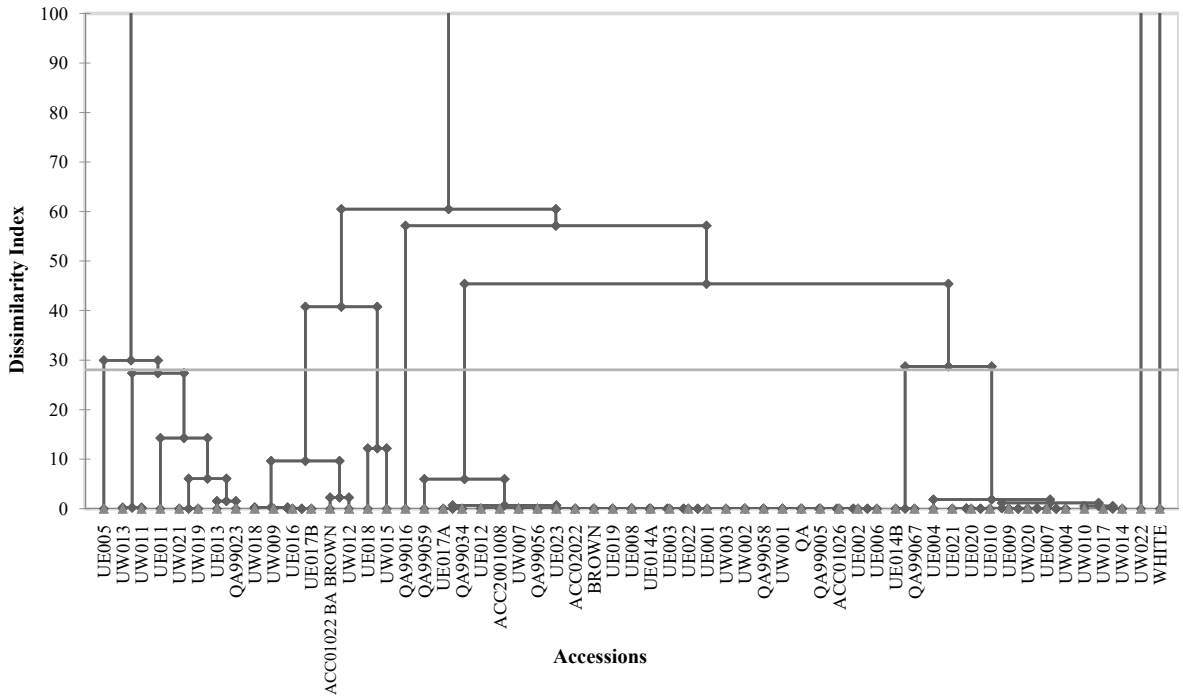

Fig. 17: Dendrogram of Frafra potato accessions based on SSR allelic data for fifteen primer

Discussion

The qualitative morphological traits showed an extensive diversity among the accessions studied. Colouration in Lamiaceae is reported to be due to the presence of anthocyanins which are mainly cyanidin saccharides and tannins (Opoku-Agyemang *et al.*, 2007). Nanema *et al.* (2009) observed similar variants in a diversity study using 155 Frafra potato accessions. The presence of pigmentation on the first node was observed in majority of the accessions used in the study. This observation was contrary to Opoku-Agyemang *et al.* (2007) who found pigmentation on the first node in a few of the same accessions in a previous study. The different results between the two studies could be attributed to differential expression of the anthocyanin gene or genotype-by-environment interactions resulting from the different study environments.

The presence of anthocyanins was also expressed in a form of pigmentation on the leaves of the accessions with the majority of the accessions not showing pigmentation, just as reported by Agyeno *et al.* (2014). Foliage colouration is a very important trait which conservationists and plant breeders cannot afford to neglect, as it could be linked to chlorophyll content (Liu *et al.*, 2014), an important trait indicating a plant's photosynthetic ability, which has a direct influence on crop yield. Nanema *et al.* (2009) and Opoku- Agyemang *et al.* (2007) observed similar traits at 53 % and 55 % respectively for the green foliage colour, indicating that the commonest, which could be the wild type foliage colour of the crop, is green.

Leaf waffleness and shape is an important trait in plants because it relates positively to chloroplast and stomata content (Kirhorekumar *et al.,* 2006). Nkansah (2004) reported two kinds of leaf forms and shape; these are predominantly slightly thick and distinctively veined or very thick with invisible veins. The current study observed that the majority of the accessions showed slightly thick and distinctively veined leaf types. This is an important trait that needs consideration for the breeding of Frafra potato since leaf waffleness and size influences carbon

dioxide intercept and water use efficiency for growth and yield (Edison *et al*., 2006). Two kinds of leaf arrangement were observed. Most of the accessions had erect stems with dense simple opposite leaf arrangements, which agrees with a report by PROTA (2004). The rest of the accessions showed prostrating stems with very dense leaf arrangement. Leaf arrangement is known to influence light intercept and as a result affect photosynthetic action for growth and yield (Falster and Westoby, 2003). Flowering was variable among the accessions studied. Most of the accessions showed pale violet flowering which agreed with the literature (Taparga, 2001; PROTA, 2004).

Tuber characteristics are major traits used in the characterization of tuber crops for production and breeding purposes. Three main tuber skin colours were observed among the accessions; these were blackish, brownish and whitish, which correspond to nagra, rubra and alba as reported by NRI (1987). The majority of the accessions showed brownish tuber skin colour and this trait divided them into three morphotypes. Tanzubil *et al*. (2005) reported three main morphotypes using the above observed tuber skin colours. Prematilake (2005) and Jayakody *et al*. (2005), concluded that there were two morphotypes of Frafra potato in Asia based on the tuber skin colours seen in the current research. This is a good breeding trait for beauty and consumer acceptability. Based on tuber characteristics such as skin texture and shape, the accessions were characterized into two morphotypes. Tuber texture is reported to be advantageous for tuber storability (Cornelius, 1998). Two main shapes of Frafra potato tubers which are oblong and ovoid have been reported (Nkansah, 2004). The majority of the accessions produced oblong tubers. However, a long tuber shape was found mixed among the oblong and ovoid tuber shapes.

Tuber size distribution obtained was similar to that reported by Opoku-Agyemang *et al*. (2007). As this stands out as the major constraint of consumer acceptability of the crop (Sugri *et al*., 2013), accessions with big tubers in this study could be the starting material for developing tubers for consumer acceptability.

The quantitative data showed that flowering was observed at a minimum of 52 days and a maximum of 92 days after planting. Days to maturity was observed at a minimum of 92 days and a maximum of 136 days. Ouédraogo *et al*. (2007) reported longer days to maturity, between 120 and 180 days, compared to the current study. Meanwhile, Tarpaga (2001) observed similar days to maturity of 106 days. The mean diameter of the biggest and the smallest tubers was 26.14 mm and 11.09 mm respectively. The mean diameter of medium-sized tubers was 17.10 mm, ranging from 11.45 mm to 20.55 mm, and this was variable among the accession. Earlier research by Nanema *et al*. (2009) observed similar tuber diameter ranges (small = 10.30 mm, medium ones = 18.80 mm, big tubers = 28.50 mm). The low level of variability in the tuber size of *Solenostemon rotundifolius* observed in a previous study by Abraham and Radhakrishnan (2005) corroborates findings of the current study. The height of the plant and length of the principal stem both recorded 35 cm. The shortest plant was 15 cm and the tallest 35 cm. NRI (1987) reported height ranges of 20 cm to 30 cm. The result is similar to the reported height range for the crop. Meanwhile, Nkansah (2004) reported a height range of 40 cm to 60 cm. The variation observed could relate to the time of planting and the availability of resources (water and nutrient) since the crop is photosensitive. The accessions had a minimum of 5 internodes and a maximum of 13. The maximum and minimum leaf area observed was 87.5 cm^2 and 164 cm^2 respectively, although a larger leaf area is desirable for maximum light interception for photosynthesis (Charles-Edwards, 1982).

The minimum and the maximum number of tubers per plant observed was 17 and 126 respectively. Earlier research reported a similar number of tubers per plant of 100 to 150 tubers (Tarpaga, 2001). Weight of tubers per plant was very variable [minimum (13.7 g) and maximum (323.8 g)]. Such high variability is indicative of wide variation in the yield of the accessions studied. Earlier research by Tarpaga (2001) reported a lower number of tubers per plant (32 – 65 tubers), (16 – 36 tubers) and weight of tubers per plant (54 – 126 g) and (9 – 20 g) respectively. Meanwhile, Ouedraogo *et al*. (2007)

observed the tubers per plant ranging between 100-150 tubers. The average weight of tubers was given as 18.4 g minimum and 76 g maximum among the accessions. This shows a high level of variability in tuber yield of the crop.

Plant height correlated positively and significantly with days to maturity, implying that increases in plant height lengthen the days to maturity. Nanema *et al*. (2009) observed a similar correlation between height and days to maturity. Leaf area correlates positively with days to maturity, indicating the variation of these traits in the same direction. Length of the principal stem had a strong positive correlation with height, so it could be inferred that the length of the principal stem is the key determinant of the height of the accessions studied. Positive significant correlation was observed between number of internodes, leaf area and length of principal stem. A positive significant correlation was seen between the number of tubers per plant and leaf area, implying that leaf area affects the number of tubers per plant in the same direction. Nanema *et al*. (2009) had similar results. Weight of total tubers correlated positively with the number of tubers per plant, indicating that weight of tubers per plant increases as the number of tubers increases or decreases. Nanema *et al*. (2009) observed correlations between the weight of tubers per plant and number of tubers per plant. Total weight of tubers per plant correlated positively with the other descriptors analyzed for correlation. Total weight of tubers per plant also had a positive significant correlation with the average weight of tubers per plant.

The first principal component which represented about 24.36 % of the total variation seen in the accessions using quantitative descriptors had the number of big tubers, number of small tubers, total number of tubers per plants and the total weight of tubers correlating strongly with the first principal component. This implies that the first principal component increases or is dominated by these descriptors. The second principal component (18.87 %) increases with increasing height, length of principal stem and number of internodes. The third principal component increases with increasing diameters of small and medium size tubers, and the average weight of tubers. The fourth principal component increases with increasing days to flowering and decreasing number of medium tubers. The fifth principal component increases with increasing leaf area and days to maturity. UE004, UE008 and QA99067 recorded high values for PC1 and UW002, UW018, UE009, UE003 and UE017A recorded high values for PC2 based on the PCA plot.

Dendrogram analysis of the quantitative data classified the accessions into three morphotypes at a dissimilarity index of 65 %. Clusters one and two are closely related to each other and cluster three consisted of only two accessions thus corroborating the tree plot from the qualitative data. A tree plot generated from the combination of both quantitative and qualitative descriptors distinguished the accessions into three clusters at a 95 % dissimilarity index. Cluster three which consists of UE004 and QA99067, though it is bifolius and dissimilar from all the accessions, is later seen to show similarity with cluster 2. Hence the tree plot derived from the combined qualitative and quantitative data suggests the existence of three morphotypes, which could later be narrowed to two and subsequently a single population. It is therefore not wrong to assume that the collected accessions could be showing phenotypic differences that they acquired as a result of mutations at certain points to aid adaptation to the varying environments in which they are grown.

Molecular data were used to generate a dendrogram. The percentage similarity observed among the accessions ranges from 30 % to 100 %. There were 10 clusters at a 30 % dissimilarity index which was further distinguished into three clusters at a 60 % dissimilarity index. At a 95 % dissimilarity coefficient, the SSR results characterized the accessions into two clusters. The second cluster at 95 % consisted of only two accessions which were different from all the other accessions studied. These two accessions are WHITE and UW002 which were standing alone until a 95 % dissimilarity coefficient. These two accessions were the only ones that had white tuber skin colour, a possible indication that the difference observed in the molecular results, or the clustering of both of these accessions in one group, is due to an alteration in the genome, specifically the genes that code for tuber

skin colour. These could be due to a nonsense mutation caused by extreme temperatures which converted the codon for tuber skin colour into a stop codon. Consequently, these two accessions do not translate the amino acid for tuber skin colour, leading to a situation known as albinism (white tuber skin colour), which might explain the similarity in the genome. The results from the molecular data indicate that the accessions studied could be derivatives of a single population and any visible difference could be attributed to a response to the environment and/or mutations.

Conclusion

The study revealed that considerable morphological variability exists within the 57 Frafra potato accessions studied. Morphological descriptors characterized the accessions into three morphotypes. Based on these quantitative descriptors, UE004, UE008 and QA99067 are the suggested promising accessions to be considered for improvement in tuber size and yield. The molecular characterization using SSR marker analysis consisting of fifteen primer pairs revealed diversity in the genome of the 57 Frafra potato accessions. The SSR results revealed 58 % heterozygosity among the accessions studied. The results of the molecular study showed relatedness in the genome of the accessions and hence placed the accessions into two groups which were further merged into a single group. Hence the molecular results suggest that the accessions came from a single population.

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