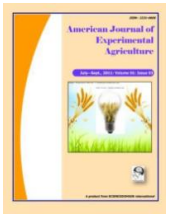

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Molecular Characterization of *Solanum* Species Using EST-SSRs and Analysis of Their Zinc and Iron Contents

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LAO and MDQ designed the study, wrote the protocol, and managed the literature searches. Authors AO, HAD and AA performed statistical analysis and wrote the first draft of the manuscript. Author EOB provided germplasm and other technical support. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objectives of study were to establish genetic diversity among the populations of three *Solanum* sp: *Solanum torvum*, *Solanum aethiopicum* and *Solanum anguivi* and identify species with the highest concentration of iron and zinc which can be recommended for improved maternal and child nutrition. It also aimed to identify polymorphic markers useful in *Solanum* species diversity studies or screening in Ghana.
Place and Duration of Study: CSIR-Crops Research Institute, Molecular Biology Lab, Kumasi. The study was carried between March 2011 and June, 2012.

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Methodology: Investigations were carried out on 30 accessions of three *Solanum* species assembled from five geographical regions of Ghana. EST-SSR and AAS were employed for the estimation of genetic diversity and assessment of mineral concentrations respectively. Data generated were analysed using POPGENE version 1_32, Genstat software version 9 and Microsoft excel software (Windows 2007).
Results: All the markers showed allelic polymorphism in all the accessions studied. Overall genetic diversity was considerably high (I =1.0032, He = 0.4942, Nei's He = 0.4859) and fixation index statistics (Fst) shows that 10% of the variation exists among the population. Clustering of the accessions did not exactly coincide to the populations of the different *Solanum* sp under study. Outcrossing rate (t = 0.8154) and Gene flow (Nm = 2.2071) of populations of the accessions were extremely high. Assessment of their mineral contents suggests that *S. torvum* and *S. aethiopicum* species are rich in Fe and Zn respectively.
Conclusion: EST-SSR markers and mineral analysis revealed genetic diversity among the different accessions. However the population studies suggest gradual homogeneity of these accessions due the high gene flow and outcrossing rate over time.

Keywords: Genetic diversity; solanum species; EST-SSR (expressed sequence tag-simple sequence repeat); mineral assessment and AAS (atomic absorption spectrophotometry).

1. INTRODUCTION

Solanaceae is a plant family comprising about 2300 species, nearly one-half of which belong to the genus *Solanum* [1]. The genomics in the genus *Solanum* are evolving at a moderate pace compared to other plant species [2]. *Leptostemonum*, subgenus of *Solanum*, comprise almost one third of the genus. They are distributed worldwide and include a number of economically important species of cultivated egg-plants which consist of two sub-groups namely *melongena* and *oliganthes* [1]. The former includes *S*. *melongena* L. (Brinjal egg-plant, aubergine) and *S*. *macrocarpon* (Gboma egg-plant), while the latter comprise *S. aethiopicum* L. [3,4]. *S. aethiopicum* and *S. macrocarpon* are known to be domesticated in Africa from their wild relatives namely *S. anguivi* and *S. dasyphyllum* respectively [5]. These eggplants are considered as very important vegetables in Ghana and West Africa [6,7,8,]. Another important wild relative is the *Solanum torvum* (Turkey berry) which can also be found in Mexico, Peru, Venezuela, and in the West Indies including Bermuda [9]. *Solanum* wild species or landraces are rich source of novel genes which can be introgressed into cultivated species [10]. Interestingly, *S. torvum* has been identified to carry resistance genes to serious diseases of eggplant, particularly bacterial and fungal wilts, and infections caused by nematodes [1,11,12,13]. *S. torvum* is used as rootstocks for controlling wilt disease in eggplant [14,15].

Fruits of *Solanum anguivi* can be utilized either in their fresh or dried state. It is believed to have medicinal properties particularly against high blood pressure and diabetes as well as being an appetizer. Nutritional analysis of *S. torvum* reveals that it has special properties of stimulating the production of blood cells and could be helpful in treating anaemia due to its high iron content. It contains about 30 mg of iron, 454mg of calcium and sizeable amounts of protein with other nutrients per 100 g edible portion [9]. Nutritionally the high iron content in *S. torvum* can significantly reduce the incidence of iron (Fe) deficiency [16,17,18] in most parts of the world especially when Fe rich genotypes are identified. Identification of mineral rich genotypes may require mineral characterisation and their diversity.

Photometry and Atomic Absorption Spectroscopy (AAS) has over the years been the most popular techniques used for qualitative and quantitative assessment of trace elements in foods, nanomaterials, biomaterials, forensics and industrial waste [19,20]. However, in recent times, emphasis is given to inductively coupled plasma-atomic emission spectroscopy (ICP-AES) due its high reproducibility and quantitative linear range compared to conventional AAS, and reduces molecular interferences due to a higher temperature (7000–8000 K) in the excitation source (plasma) [20]. Nevertheless, ICP-AES is more expensive than conventional AAS, and in complex samples, emission patterns can be difficult to interpret [21,22]. Moreover, ASS is the method of choice for most routine trace mineral element analyses and very effective for the determination of elements such as magnesium, zinc, and copper [19].

Morphological diversity study has often been employed for genetic diversity studies in crop plants [23]. However variations in environmental conditions such as soil types and fertility levels, light, temperature and moisture regimes affect morphological characterization, particularly when experiments are repeated in time and/or space [24,25,26]. In addition, the genetic make-up of seed, field management practices has been reported to influence the morphology of a crop [27].

The weaknesses of morphological diversity or characterisation paved way for Molecular characterisation which offers the best estimate of genetic diversity since they are independent of the confounding effects of the environmental [28,29]. Among molecular markers, Simple Sequence Repeat (SSR) has proven to be effective due to its high polymorphic and co-dominance nature, reproducibility, affordability, low technical complexity [29] to mention but a few. It has also been widely employed for many *Solanum* sp diversity studies [30,31,32].

SSRs derived from ESTs or cytoplasmic DNA (cDNAs), are mostly genic SSRs and have the potential for application in gene function characterisation [33], association analysis for gene tagging [34,35,36], and quantitative trait loci (QTL) analysis [37,38,39]. EST-SSR designed for *Solanum* sp is currently employed for diversity study [30,40].

In Ghana, despite the role of eggplants on the livelihood of people [7], information on their mineral content and diversity has not been well documented especially, the *S. anguivi*, *S. aethiopicum* and *S. torvum* accessions. This study was therefore designed to establish genetic diversity among three populations of *Solanum* species: *Solanum torvum, Solanum aethiopicum* and *Solanum anguivi* employing EST-SSR primers. It also seeks to assess their mineral contents using the Atomic Absorption Spectrophotometry (AAS) and identify accessions possessing high amount of iron and zinc for enhanced child and maternal nutrition in Ghana and beyond [16,17,18]. The study may assist to identify polymorphic primers useful in *Solanum* species diversity studies or screening in Ghana.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extraction of Genomic DNA

Thirty (30) accessions of *Solanum* sp. were collected from 5 geographical locations in Ghana. The 30 assembled germplasm were grouped into three populations according to their species: *Solanum torvum, Solanum anguivi and Solanum aethiopicum* (Table 1). Two hundred milligram 200mg of each accession young tender leaves were weighed into 2ml eppendorf tubes and grounded with liquid nitrogen to fine powder. The genomic DNA was extracted using the Dellaporta protocol [41] with slight modifications. Thus the protocol was modified through the introduction of 7.5 M Sodium acetate DNA and the addition of the RNAse to digest the RNA present in the genomic DNA.

2.2 DNA Quantification and Gel Electrophoresis

DNA quality was checked on 0.8% agarose in 1x TAE (Tris-acetic EDTA) buffer by gel electrophoresis. Electrophoresis of the DNA was carried at 100V for 40mins and then visualized with a UV transilluminator. The concentration of the DNA was projected by the intensity and comparison to 1kb lambda DNA mass ladder (1kb, Invitrogen). The quantification of the DNA was evaluated by reading absorbance at 260nm and 280nm with the spectrophotometer. The DNA was then diluted to 10 ng/µl for PCR

2.3 PCR Amplification

PCR amplification of DNA was carried out using a 96 well plate AB Applied Biosystem Thermocycler. The PCR conditions were optimized for cycling number, concentrations of the primer, $MgCl_2$ and DNA template. The reaction mixture (10 ul) contained 6.075 µl of Nuclease free sterile water (DNA grade water),1 µl of 10x PCR Buffer,0.9 µl of $MgCl_2$ (25 mM), 0.4 µl dNTPs (10 mM), primer 0.25 µl (50 µg/ml) of each forward and reverse, 0.125 µl of Supertherm Taq Polymerase (1unit) and 1 µl of 10 ng DNA template. The PCR cycling conditions followed an initial denaturing step of 94°C for 5mins, a touchdown PCR protocol of 11 cycles of 94°C for 30 secs, 60°C for 30 secs decreasing by 0.5 per cycle and 72°C for 60 secs followed by 30 cycles of 94°C for 30 secs, 55°C for 30 secs and a final elongation step of 72°C for 60 secs. In each PCR, a negative control was included to detect contaminations. Twelve set of

EST-SSR primers (Table 2) were used in the experiment. PCR products were electropholysed on 6% polyacrylamide gel (Water, 10x TBE, 4% acrylamide (19:1), 10% APS and TEMED). A 100bp ladder (Gene Ruler ^{TM}, Fermentas) was used as a size marker. Amplified bands were visualized and scored.

2.4 Mineral Analysis

Determination of content of mineral elements using atomic absorption spectrophotometry (A.A.S.).

Sample dried seeds of each accession were dehulled (manually) and ground into fine powder. Five hundred milligram (0.5 g) of each sample (accession) was weighed into a Teflon beaker. Amounts of 5ml of HNO_3 (70% HNO_3) and 1ml of H_2O_2 (30% H_2O_2) were added. The Teflon beakers were covered and placed in a rotor for digestion. Each sample was digested in three replicates (Zuink and Planck, 1990). After the digestion, the inside walls of the Teflon beakers were washed with distilled water and made up to a final volume of 20 ml. The samples were then transferred from the Teflon beakers into test tubes. The digested samples were assayed for the presence of iron (Fe) and zinc (Zn) using Atomic absorption spectrophotometry in an acetylene-air flame employing the manufacturers recommended working conditions. Reference standards for the elements of interest, blanks and repeats of the samples were digested the same way as the actual samples. These served as internal positive controls.

2.5 Calculation of Concentrations of Specified Mineral Elements

For each of the two elements; Iron (Fe) and zinc (Zn), concentration in the samples was calculated using the formula:

Final Concentration (mg / kg) =

$$\frac{\text{Conc. (df) x Nominal volume}}{\text{Sample weight in grammes}}$$

Where,

Conc. (df) = AAS Conc. Reading, and
Nominal volume = Final volume after digestion

2.6 Data Collection

The amplified EST-SSR DNA bands representing different alleles were scored. Bands were recorded as homozygous genotypes (AA, BB, CC. . .) or heterozygous genotypes (AB, AC, BC. . .). Genetic parameters such as average observed allele number (*Na*), the percentage of polymorphic loci (*P*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Nei heterozygosity (*Nei He*), Shannon's diversity index (*I*) , and gene flow (*Nm*) were calculated to estimate the level of genetic diversity [42] . The F-statistics (F_{IS}, F_{IT} *and* F_{ST}) [42,43] were also computed for polymorphic loci to test for the departure from Hardy–Weinberg equilibrium and to estimate genetic differentiation among the *Solanum species* populations under study. The out-crossing rate [$t = (1-F_{IT})/(1 + F_{IT})$] was calculated based on the F_{IT} values to estimate indirectly the mating pattern of *the* populations [43].

2.7 Statistical Analysis

All the calculations were performed using POPGENE 32 version 1.32 [42,44]. A hierarchical analysis of molecular variance (AMOVA) of the *Solanum* species populations was calculated to partition genetic diversity within and among populations. For AMOVA, the genotype banding patterns were converted into a '1' (present) and '0' (absence) matrix and subjected to analysis, based on Jaccard coefficient using single linked similarity matrix method. Hierarchal AMOVA was analyzed using Genstat software version 9. Correlation between Zinc and iron were generated to determine the degree of association between Zn and Fe of the accessions using Microsoft Excel software (Windows 2007).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Diversity studies

Sixty four alleles were generated for all loci with a mean of 5.333 per locus (Table 2). It was observed that all the EST-SSR markers showed allelic polymorphism (Fig. 1). Primer EEMS 37 recorded the highest number of alleles and showed the highest level of genetic variability (Expected heterozygosity (*He*) = 0.843, Shannon's Information index (*I*) = 1.978). It also revealed Nei's heterozygosity of 0.829 (Table not shown). On the other hand, four primers (EEMS 6, EEMS 13, EEMS 15 and EEMS 16) recorded the least number of alleles (3) with locus EEMS 6 showing the least Shannon diversity index (*I* = 0.4740).

Table 1. Assembled accessions and source of collection

Accession	Region of collection	Lab code	Common name	Accession	Region of collection	Lab code	Common name
Hwidiem *S. torvum*	Brong Ahafo	HST 001	Turkey berry	AsanteAkyem- Wenkyi, *S. torvum*	Ashanti	AAWST 017	Turkey berry
Hwidiem *S. aethiopicum*	Brong Ahafo	HSA 002	African Garden eggs	Bonwire *S. torvum*	Ashanti	BWST018	Turkey berry
Hwidiem *S. anguivi*	Brong Ahafo	HSN 003	Uganda peas	Assin Odumase *S. aethiopicum*	Central	AOSA019	African Garden eggs
Kenyase *S. aethiopicum*	Brong Ahafo	KSA 004	African Garden eggs	Assin Odumase *S. torvum*	Central	AOST020	Turkey berry
Kenyase *S. anguivi*	Brong Ahafo	KSN 005	Uganda peas	Assin Odumase *S. anguivi*	Central	AOSN021	Uganda peas
Kenyase *S. torvum*	Brong Ahafo	KST 006	Turkey berry	Assin Dominase *S. aethiopicum*	Central	ADSA022	African Garden eggs
Ntoturoso *S. torvum*	Brong Ahafo	NST 007	Turkey berry	Assin Dominase *S. anguivi*	Central	ADSN023	Uganda peas
Ntoturoso *S. aethiopicum* 1	Brong Ahafo	NSA 008	African Garden eggs	Assin Dominase *S. torvum*	Central	ADST024	Turkey berry
Ntoturoso *S. aethiopicum* 2	Brong Ahafo	NSA 009	African Garden eggs	Sefwi Beposo *S. torvum*	Western	SBST025	Turkey berry
Kwahu Praso *S. torvum*	Eastern	KPST 010	Turkey berry	Sefwi Asempaneye *S. torvum*	Western	SAST026	Turkey berry
Kwahu Praso *S. aethiopicum*	Eastern	KPSA 011	African Garden eggs	Sefwi Babiani *S. aethiopicum*	Western	SBSA027	African Garden eggs
Nkawkaw *S. anguivi*	Eastern	NKSN012	Ugandan peas	Sefwi Babiani *S. torvum*	Western	SBST028	Turkey berry
Nkawkaw *S. aethiopicum*	Eastern	NKSA013	African Garden eggs	Sefwi Awaso *S. aethiopicum*	Western	SWSA029	African Garden eggs
Amanpe *S. anguivi*	Ashanti	AMSN014	Uganda peas	Sefwi Awaso *S. torvum*	Western	SWST030	Turkey berry
Amanpe *S. aethiopicum*	Ashanti	AMSA015	African Garden eggs				
Amanpe *S. torvum*	Ashanti	AMST016	Turkey berry				

Table 2. List of primers and their genetic variability estimates

Primer	Repeat motif	na *	Forwad primer (5'-3')	Reserve primer (5'-3')	ASR	*ne**	*He**	*I**
EEMS 16	(AC)7	3.000	CAATTTTTCGGTTCACTAATCAAG	CTTCAAGGAAAAAGGAGGCC	135-141	1.575	0.371	0.603
EEMS 7	(T)13	3.000	CCATGCCAGAATGGAAACTT	AACGAAAACACGATCAACCC	250-260	2.135	0.540	0.911
EEMS 10	(A)20	4.000	TCAAGCAGAACGAAGATGGA	GTAGGGGACGTGGATTCAGA	266-290	1.527	0.351	0.708
EEMS 15	(C)12	3.000	GGGACAAATCTGACCTTTGG	CTGGTGGCAAATTCTTCGAT	270-294	2.273	0.570	0.950
EEMS 28	(TAA)21	5.000	GACGATGACGACGACGATAA	TGGACTCACAACTCAGCCAG	180-236	3.416	0.719	1.335
EEMS 46	(ACCAGC)6	4.000	ACCAAACGTGCATGAAACAA	GGAAATGTTGGTGGAATTGG	245-265	1.227	0.188	0.412
EEMS 50	(TA)9(GA)8	5.000	AAATCCCGGCCATTCTGTGTA	ACATCGTTCCGCCTCTATTG	218-226	2.135	0.540	1.012
EEMS 37	(TCC)5	10.000	CCCTTCCTACCCACACTTCA	GTTTTGCACCTTTCCATCGT	114-123	5.844	0.842	1.978
EEMS 6	(T)14	3.000	TCATGCGAAGATTAATTAAATGTGA	GAGTGGATGATCAAGAATGGC	268-274	1.315	0.244	0.485
EEMS 20	(AT)8	9.000	AACATCAGCCAGGGTGTTTC	TGCTGAAAATTACAAGCAAA	221-227	4.615	0.797	1.846
EEMS 48	(TAA)20(CGA)8	8.000	CAATGCAAACAATTATCATTTCG	TCGATGTTGTCGTCGTT	223-241	1.676	0.410	0.980
EEMS 49	(TA)12(GA)7	7.000	TGAAATTGATCAATACCTATAAATTTAG	GAAAGCCAGGATAGCATTCG	145-153	1.539	0.356	0.817
Total		64				29.277	5.928	12.036
Mean		5.333				2.440	0.494	1.003
SD		2.535				1.452	0.211	0.494

The study reveals genetic variability among individuals in each population [*I* = 1.0032, *He* = 0.4942, *Nei's He* 0.4859 and *Ho* = 0.2417 (Table 3)]. Nevertheless, genetic variability was not uniformly distributed across the population. The highest genetic differentiation existed among the *S. aethiopicum* population with the least among the *S. torvum* population. The overall value of F_{ST} reveals that approximately 10% of the total genetic diversity exists among the population. F_{IS} and F_{IT} of less than 0.5 in the *Solanum sp* populations indicates some degree of heterozygosity within the populations studied and a significant departure of allelic frequencies from the Hardy–Weinberg equilibrium. The overall outcrossing rate was extremely high (81.54%) with a gene flow of 2.2071. A dendogram (Fig. 2) based on genetic Unweighted Pair Group Method with Arithmetic Mean (UPGMA) [45] showed that *S. anguivi* and *S. aethiopicum* populations had the greatest similarity and were separated from *S. torvum* which form different clusters at a genetic distance of 5.77594 (Table 4).

3.2 Cluster Analysis

UPGMA cluster analysis based on the 12 EST-SSR loci employing Jaccard Coefficient single link similarity matrix method (Fig. 3) demonstrated genetic differentiation by revealing six distinct clusters from a genetic similarity distance of 37.3% up to 85%. However at a genetic distance of 37.3%, all accessions of the various populations could be grouped into two main clusters, i.e. Ntoturoso *S. aethiopicum1* and the other 29 accessions which further separated into five distinct sub clusters. The accessions with the closest resemblance were revealed at the sixth cluster at a genetic similarity of 85% (Hwidiem *S. torvum,* A/A Wenkyi *S. torvum*, A/Dominase *S. anguivi*, A/ Odumasi *S. torvum* and ntoturoso *S. torvum*). The widest separation occurred between Hwidiem *S. torvum* and Ntoturoso *S. aethiopicum* 1. The clustering did not exactly coincide with geographical origin as well as population or species groupings of the accessions. This shows a clear genetic variation and differentiation pattern among the individuals of the various species.

3.3 Variation Based on Minerals

The result on mineral analysis for the concentrations of Fe and Zn showed that no accession recorded the highest concentrations of both minerals (Table 5). However, one accession (Assin Odumase *S. anguivi*) recorded the least concentration for both Fe (7.26 mg/Kg) and Zn (9.16 mg/Kg). Averagely the *S. torvum* and *S. aethiopicum* population had the highest concentration of Fe and Zn respectively. The highest concentration of Fe was recorded by Assin Odumase *S. torvum* (261.48 mg/kg) and the highest zinc concentration was recorded by Sefwi Awaso *S. aethiopicum* (29.16 mg/kg). Statistically insignificant degree of association was observed between Fe and Zn for each species (Table 5).

4. DISCUSSION

4.1 Diversity Studies

The 100% allelic polymorphism among the individuals of the species generated by the use of the EEMS primers indicates that all the loci are very informative especially EEMS 37, EEMS 20 and EEMS 48 which produced ten, nine and eight alleles respectively with the former producing the highest diversity (*I* = 1.978). The informative nature of the EST-SSR markers is also consistent with similar finding by Aniko et.al. [26] who employed the same primers utilised in this study for genetic mapping and phylogenetic studies of eggplant with EEMS 48 producing the highest number of alleles. This suggests that in selecting primers for biodiversity evaluation for eggplant or species closely related, the three primers mentioned above can be given priority. The present results based on the polymorphic pattern of 12 selected EST-SSR loci confirmed that *Solanum* sp or populations under studied possessed relatively high genetic diversity (*He* = 0.494, *Nei's He* = 0.486, *I* = 1.003). These agrees with results by other workers [26,27,35,46] who employed either SSR, AFLP and/or, RAPD markers as well as morphological descriptors for characterization of eggplant. Except a few loci (EEMS 6, EEMS 16 and EEMS 46), the genetic variation (*I*) observed among the individual accessions of the various species (Table 1) where either comparably close or higher than genetic variation (*I*) obtained for the three populations. According to Muller *et al* (2001) [47], this can be attributed to a high genetic overlap as a result of probable common ancestry. Furthermore, the low values of F_{IS} (0.4188) and F_{IT} (0.4779) as well as high values of *t* (0.8154) and *Nm* (2.2071) from the EST-SSR analysis suggest that the *Solanum* sp under study were more cross pollinated with wide pollen-mediated gene flow although they can undergo self-pollination due to their hermaphroditic nature.

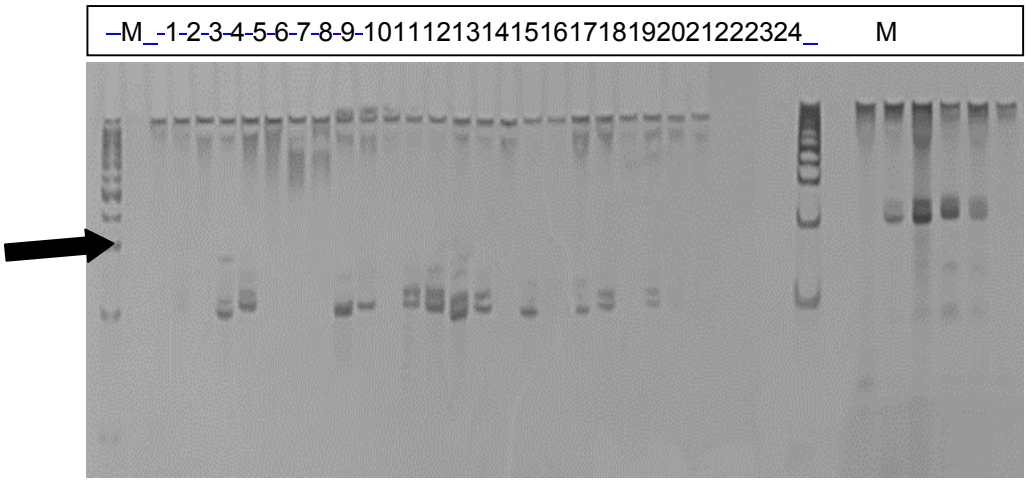

Fig. 1. The banding pattern of primer EEMS 20 on accessions numbered 1-30: Hwidiem *S. torvum*= 1; Hwidiem *S. aethiopicum* = 2, Hwidiem *S. anguivi* = 3; Kenyase *S. aethiopicum* = 4; Kenyase *S. anguivi*= 5; Kenyase *S. torvum* = 6; Ntoturoso *S. torvum* = 7; Ntoturoso *S. aethiopicum* 1 = 8; Ntoturoso *S. aethiopicum* 2= 9; K. Praso *S. torvum* =10; *K. Praso S. aethiopicum* = 11; Nkawkaw *S. anguivi*= 12; Nkawkaw *S. aethiopicum*= 13;Amanpe *S. anguivi* = 14; Amanpe *S. aethiopicum* = 15; Amanpe *S. torvum* = 16; A/AWenkyi *S. torvum* = 17; Bonwire *S. torvum* = 18; A/ Odumase *S. aethiopicum*= 19; A/Odumase *S. torvum*= 20; A/Odumase *S. anguivi* = 21; A/Dominase *S. aethiopicum* =22; A/Dominase *S. anguivi* = 23; A/Dominase *S. torvum* = 24; S/Beposo *S. torvum* = 25; S/ Asempaneye *S. torvum* = 26; S/Babiani *S. aethiopicum*= 27; S/Babiani *S. torvum* = 28; S/Awaso *S. aethiopicum*= 29;S/Awaso *S. torvum*=30, M= Marker, 200 bp

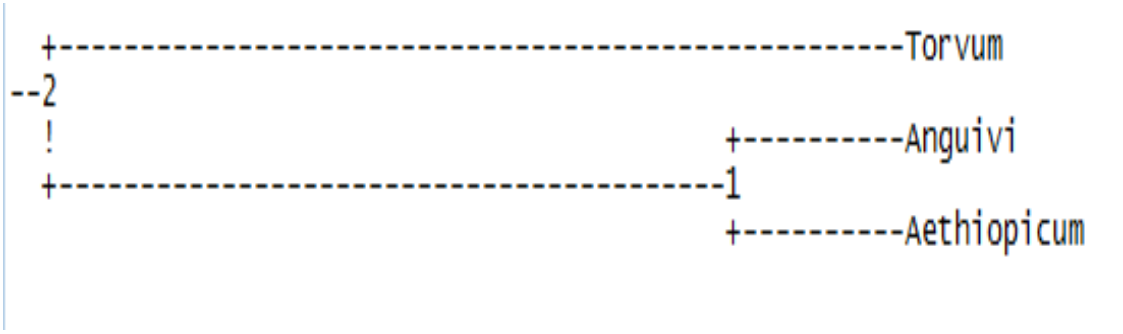

Fig. 2. Dendrogram based on Nei's (1978) genetic distance: method-UPGMA modified from NEIGHBOR procedure of PHYLIP Version 3.5 using 12 EST-SSR markers

Torvum population=*Hwidiem S. torvum, Kenyase S. torvum, Ntoturoso S. torvum, K. Praso S. torvum, Amanpe S. torvum, A/A Wenkyi S. torvum, Bonwire S. torvum, A/Odumasi S. torvum, A/Dominase S. torvum, S/Beposo S. torvum, S/Asempaneye S. torvum, S/Babiani S. torvum, S/Awaso S. torvum*

Anguivi population=*Hwidiem S. anguivi, Kenyase S/anguivi, Nkawkaw S. anguivi, Amanpe S.anguivi, A/Odumase S.anguivi , A/Dominase S.anguivi*

Aethiopicum population=*Hwidiem S. aethiopicum, Kenyase S .aethiopicum, Ntoturoso S. aethiopicum, K. Praso S. aethiopicum, Nkawkaw S. aethiopicum, Amanpe S. aethiopicum, A/Dominase S. aethiopicum, S/Babiani S. aethiopicum, S/Awaso S. aethiopicum*

Table 3. Genetic variability estimates of the genotypes

Population region	N_a	N_e	PL	*P* %	H_o	H_e	Nei's H_e	*I*	F_{is}	F_{it}	F_{st}	*t*	*Nm*
Solanum torvum	3.5000	1.7284	10	83.33	0.1154	0.6692	0.3180	0.6369					
Solanum anguivi	3.2500	2.3840	9	75.00	0.3194	0.4949	0.4537	0.8444					
Solanum aethiopicum	4.3333	2.5263	12	100.00	0.3485	0.5841	0.5575	1.0582					
Overall mean	5.3333	2.4398	12	100.00	0.2417	0.4942	0.4859	1.0032	0.4188	0.4779	0.1017	0.8154	2.2071

Na= average number of alleles; Ne= average number of effective alleles (Kimura and Crow 1964); PL= number of polymorphic Loci; P= percentage of polymorphic loci; Ho, observed heterozygosity (Levene, 1949); He= expected heterozygosity(Levene, 1949); Nei's He = Nei's expected heterozygosity (Nei, 1973); I, Shannon diversity index (Lewontin 1972); Fis, Fit and Fst, estimates of F-statistics of regional populations (Hartl and Clark 1989); t, out crossing rate = (1- Fit)/ (1 + Fit) (Cao et al., 2006); Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fs

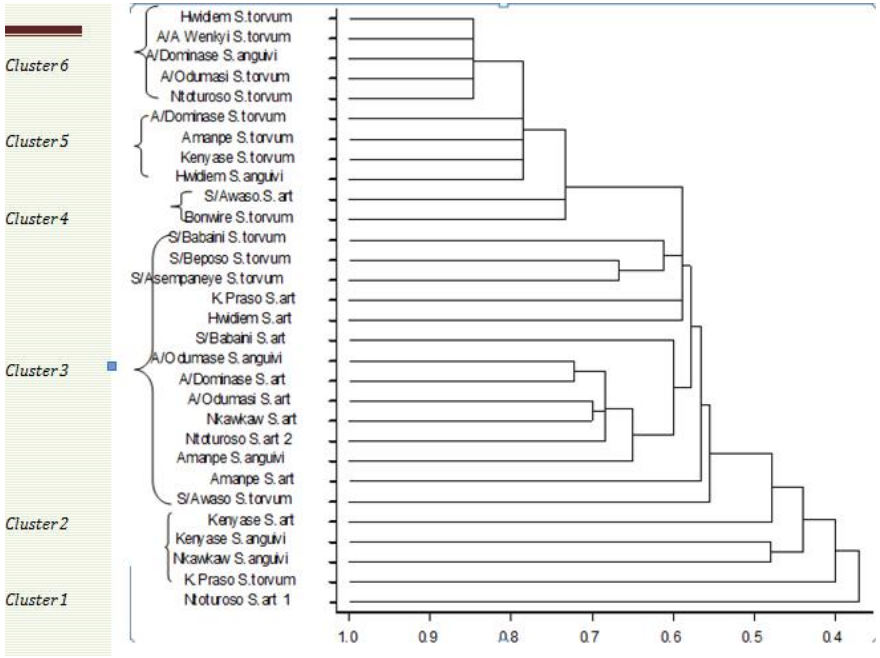

Fig. 3. Dendrogram showing the relationships among the various accessions

Table 4. Genetic distance between populations

Between	And	Length
2	*S. torvum*	7.27970
2	1	5.77594
1	*S. anguivi*	1.50376
1	*S. aethiopicum*	1.50376

Table 5. Mineral content of accessions and simple linear correlation coefficients between the iron and zinc variables studied considering the values expressed in dry matter basis for the 30 accessions of *Solanum* sp. (n=10 for each accessions; *: P<0.05)

Designation	Mineral		Correlation
	Fe (mg/Kg)	Zn (mg/Kg)	
Hwidiem *S. torvum*	68.12	18.76	
Kenyase *S. torvum*	44.24	21.56	
Ntoturoso *S. torvum*	256.36	13	
Kwahu Praso *S. torvum*	126.8	13.28	
Amanpe *S. torvum*	173.88	14.08	
Bonwire *S. torvum*	8.64	12.64	
Asante Akyem Wenchi *S. torvum*	106.92	12.88	
Assin Odumase *S. torvum*	261.48	14.72	
Assin Dominase *S. torvum*	89.16	17.88	-0.4455
Sefwi Beposo *S. torvum*	215.44	10.72	
Sefwi Awaso *S. torvum*	91.8	14.56	
Sefwi Asempaneye *S. torvum*	59.68	14	
Sefwi Babiani *S. torvum*	130.96	14.48	
Mean of *torvum*	125.65	14.78	
Hwidiem *S. anguivi*	30.36	27.12	
Kenyase *S. anguivi*	17.84	18.76	
Nkwakaw *S. anguivi*	15.8	14.32	0.041778
Amanpe *S. anguivi*	127.56	15.08	
Assin Odumase *S. anguivi*	7.26	9.16	
Assin Dominase *S. anguivi*	45.36	28.52	
Mean of *anguivi*	40.70	18.83	
Hwidiem *S. aethiopicum*	155.96	18.52	
Kenyase *S. aethiopicum*	114.24	17.16	
Ntoturoso *S. aethiopicum* 1	114.28	23.6	
Ntoturoso *S. aethiopicum* 2	68.32	21.64	
Nkawkaw *S. aethiopicum*	14.24	20	
Amanpe *S. aethiopicum*	40.28	18.36	-0.67893
Assin Odumase *S. aethiopicum*	23.38	22.8	
Assin Dominase *S. aethiopicum*	165.16	14.2	
Sefwi Awaso *S. aethiopicum*	29.84	29.16	
Sefwi Babiani *S. aethiopicum*	223	14.56	
Mean of *aethiopicum*	100.69	19.79	
Overall Correlation			-0.41491

The population dendogram estimated from Nei's unbiased measures of genetic identity and genetic distance also confirm the genetic variation among the populations with the population of *S. torvum* and *S. aethiopicum* being widely separated from each other. This is more evident in Hwidiem *S. torvum* and Ntoturoso *S. aethiopicum* 1 which were two accessions with widest separation (Fig 3). The close relatedness of *S. aethiopicum* and *S. anguivi* from the result

clearly demonstrate that the two populations have a common ancestry as *S. anguivi* is the wild type of egg plant from which *S. aethiopicum* was domesticated [5]. This is consistent with report by Lester [5] that *S. aethiopicum* and *S. macrocarpon* were domesticated in Africa from their wild relatives *S. anguivi* and *S. dasyphyllum* respectively. Furthermore, UPGMA dendrogram by Sakata and Lester [48] also indicated *S. anguivi* and *S. aethiopicum* as duplicates proving their close relatedness. This further explains the reason for wide diversity between *S. aethiopicum* and *S. torvum*. In principle, the wide variation between *S. torvum* and the two other populations offer a broad genetic base with the potential for wider adaptation in a range of agroecosystems. This also indicates the genetic potential of the populations for breeding purposes [42].

4.2 Variation via Cluster Analysis

The dendogram reveals that cluster five and six constitutes mainly *S. torvum* accessions with the exception of A/ Dominase *S. anguivi* and Hwidiem *S. anguivi* in cluster five and six respectively. Cluster five and six were captured at a genetic distance of 0.85 (85%) and 0.77 (77%) respectively. The inclusion of the *S. anguivi* accessions among the *S. torvum* may be attributed to the high outcrossing rate and gene flow (t = 81.54%; Nm = 2.2071). It can be inferred from the results of the study that about 85% of the *S. torvum* genetic characteristics has been introgressed into such *S. anguivi* accessions making them lose their unique genetic characteristics (Fig. 3). This also implies that the *S. torvum* accessions in cluster five and six should be assembled and conserved for breeding purpose to avoid contamination. Nevertheless, the remaining clusters constitute a mixture of the three populations which grouped not based on geographical origin or species as indicated by the low values of F_{IS} and F_{IT} resulting in probable heterozygosity of those accessions [42].

4.3 Variation among Accessions via Mineral Content

The distinct concentration of the Fe and Zn analyzed among the accessions also display variation among the accessions. Diversity in Fe and Zn concentrations in the accessions of the present study agrees with similar findings by several early workers [49,50]. This suggests that micronutrient enrichment traits also exist in the egg plants as evident in other crops such as rice and common beans (*Phaseolus vulgaris L.)* [51,52]

Concentrations obtained for iron is higher than recorded by Chinese red long-grain varieties (64 mg/kg) [53,16] but lower than values obtained by Doku [19] for Awerema brown rice (386.6±0.46 mg/kg), an *Oryza glaberrima* accession in Ghana. However, since eggplants do not undergo polishing before usage compared to brown rice, the eggplant accession with the highest concentration still holds a great prospect in human nutrition. With the exception of accessions with the least iron concentration for the three species of the present study, (Bonwire S. *torvum*, Assin Odumase *S. anguivi* and Nkawkaw S. *aethiopicum*) concentration of iron recorded for the rest of accessions can supply adequate iron to the human body when ingested taking into account the recommended dietary allowance of iron for males and females being 10 g and 15 g respectively [52,54]. These however suggest the usefulness of Assin Odumase *S. torvum*, the accession rich in iron, for crop improvement.

The recommended dietary allowance of zinc is 15 mg and 10 mg for male and females respectively. With the exception of the accession with the least value (9.16 mg / kg) of Zinc, frequent ingestion of the accessions under study with other zinc rich food would help improve the Zn needs of the human body especially Sefwi Awaso *S. aethiopicum*. However, range of Zn concentrations recorded for accessions of the present studies are lower compared to the Zn range content (14-59 mg/kg) recorded by several authors: Frei and Becker, 2005; Kennedy and Burlingame, 2003 as well as Gregorio, 2002 [16,55,56] for different rice varieties.

Nevertheless, the range of Zn concentrations recorded for the present study are higher compared to range of concentrations recorded by other eggplant and closely related vegetables mineral study. Arivalagan et al. [49] for instance recorded 0.73 mg/kg – 2.33 mg/kg for 32 eggplants as the Zn concentrations range and Kamga et al. [50] also recorded 7.63±0.005 µg/g – 102.57±2.589 µg/g as the Zn concentration range for 5 indigenous African vegetables.

The study did not reveal any accession among the three egg plant species possessing high concentration of both iron and zinc neither any accessions with high correlation for Fe and Zn.

Therefore the outstanding concentrations recorded by Assin Odumase *S. torvum* and Sefwi Awaso *S. aethiopicum* for iron and zinc respectively suggest their usefulness in a breeding program whose objective is to improve iron and zinc concentration. Thus the two species can be utilized in a hybridisation program to develop an eggplant rich in iron and zinc.

5. CONCLUSION

In conclusion, the EST-SSR markers and mineral analysis revealed genetic diversity among the individual accessions of the populations studied without duplications. However the population studies suggest the gradual homogeneity of these accessions due the high gene flow and outcrossing rate over time. It is therefore recommended that the accessions should be collected and conserved to avoid further contamination especially with the almost distinct *S. torvum* species. Molecular diversity of the species under study or closely related ones can easily be carried out with EST-SSR primer EEMS 37. Assin Odumase *S. torvum* and Sefwi Awaso *S. aethiopicum* can be recommended as a good source for improved child and maternal health in Ghana because of their high content of iron and zinc respectively.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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