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Research Paper

Genetic and Mineral Diversity Among three *Solanum* Species using EST-SSR and AAS.

UNDER PEER REVIEW

ABSTRACT:

Aims: The objective of study is to establish genetic diversity among the populations of three *Solanum* sp: *Solanum torvum, Solanum aethiopicum* and *Solanum anguivi* as well as among the accessions of the populations under study. It is also to identify the species with the highest concentration of iron and zinc to be recommended for biofortification. Further more, this study seek 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.
Methodology: Investigations were carried out on 30 accessions of three *Solanum* species assembled from five geographical regions of Ghana to ascertain the genetic diversity of the accessions, differentiation of the populations of these species, assessment of their Fe and Zn concentration as well as their prospects for crop improvement and biofortification. EST-SSR and AAS were employed for the estimation of diversity and assessment of mineral respectively.
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 (F_{st}) shows that 10% of the variation exists among the population. Clustering of the accessions did not exactly coincide to the populations of the species under study. Outcrossing rate (t = 0.8154) and Gene flow (Nm = 2.2071) of populations of the accessions were extremely high. Assessment of their minerals suggests that *S. torvum* and *S. aethiopicum* species are the richest in Fe and Zn respectively.
Conclusion: 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.

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* (Sękara, 2007). The genomics in genus *Solanum* are evolving at a moderate pace compared to other plant species (Doganlar *et al*. 2002a). The genus solanum consist of subgenus, one of it being *Leptostemonum. Leptostemonum* which comprise almost one third of the genus and are distributed worldwide includes a number of economically important species of cultivated eggplants made of two sections namely *melongena* and *oliganthes* (Sękara, 2007). The former section includes *S*. *melongena* L. (brinjal eggplant, aubergine) and *S*. *macrocarpon* (Gboma eggplant), while the latter comprise *S. aethiopicum* L. (Levin *et al*. 2005, Levin *et al*. 2006). Lester (1998) recorded *S. aethiopicum* and *S. macrocarpon* were domesticated in Africa from their wild relatives *S. anguivi* and *S. dasyphyllum* respectively. *Solanum* wild species are a rich source of novel genes for introduction into cultivated species to enhance breeding, drought tolerance and important nutritional properties (Lucca *et al.* 2011). *S. anguivi* has a potential use as a genitor in breeding for disease resistance in *Solanum aethiopicum.* Suggestion has been made that traits such as plant habit, earliness, fruit quality and yield should be considered during selection and breeding (Bukenya-Ziraba, 2004). Fruits of *Solanum anguivi* are used fresh or dried and ground as medicine against high blood pressure, diabetes and also as an appetizer. On compounds the plants with their masses of red berries are appreciated for their ornamental value. As a semi-domesticated species it offers a great variation in the plants, flowers and fruits numbers, sizes, colours etc.

UNDER PEER REVIEW

Another important wild relative is the *Solanum torvum* (turkey berry) which has a native range from Mexico to Peru and Venezuela, and in the West Indies and Bermuda. Documentation on the 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. It also contains about 30mg of iron, 454mg of calcium and sizeable amounts of protein with other nutrients per 100g edible portion (Leelavathi *et al,* 2010). Interestingly, *S. torvum* has been identified to carry traits of resistance to most serious diseases of eggplant, particularly bacterial and fungal wilts, and nematodes (Collonnier *et al*, 2001; Dauney *et al*, 1991; Sakata *et al*., 1989, Stravato and Capelli, 2000). *S. torvum* is used as rootstocks for controlling wilt disease in tomato and eggplant (Peregrine and Ahmad, 1982; Singh. and Gopalakrishnan, 1997). In view of introgression of disease resistance into eggplant, attempts to cross sexually *S. torvum* with eggplant have resulted in only few viable seeds. However, embryo rescue was then used to recover those sexual hybrids, which were highly sterile (Bletsos, 2000; McCammon and Honma 1982). A survey conducted by Owusu Ansah *et al* (2001) indicated that the plant had huge potential in controlling certain diseases, including malaria and cough.

Despite the significance of *melongena* sections (*S. melongena* L. and *S. macrocarpon*), in Ghana, *S. aethiopicum* and its wild *S. anguivi* as well as *S. torvum* are widely consume and plays a very important role in the diet of people. Not only is this crop consumed on almost daily basis by rural and urban families but it also represents the main source of income for many rural households in the forest zone of Ghana (Danquah-Jones 2000; Owusu-Ansah *et al*,2001).

The eggplant domesticated in African is one of the most important vegetable crops in Ghana and West Africa (Norman, 1992; Owusu-Ansah *et al.*, 2001; Bukenya-Ziraba et al (2004). In fact, the African eggplant was so much confused with the ordinary eggplant that this was considered by some a variety *violaceum* of *S. aethiopicum*. *Solanum aethiopicum* fruits are a fairly good source of calcium, phosphorus, iron, potassium and vitamin B group. *S. aethiopicum* and *S. macrocarpon* are of interest for genetic improvement of the *S. melongena,* considering the presence of several traits of agronomic interest in its germplasm (Alba *et al*. 2005). Gowda *et al.* (1990) used the interspecific hybridisation between *S. macrocarpon* and *S. melongena* to obtain hybrids resistant to shoot and fruit borer (*Leucinodes orbonalis*). Rizza *et al*. (2002) obtained dihaploid plants through anther culture of somatic hybrids between *S. melongena* and *S. aethiopicum* Gilo group which were characterized by complete resistance to fungal wilt caused by *Fusarium oxysporum* f. sp. *melongenae*.

Regardless of the importance of these *Solanum* species (*Solanum torvum, Solanum aethiopicum and Solanum anguivi*) in the diet of Ghanaians, very little work has been done to determine their genetic and population diversity and documentation of traits of importance for crop improvement. The objective of study is to establish genetic diversity among the populations of three *Solanum* sp: *Solanum torvum, Solanum aethiopicum* and *Solanum anguivi* as well as among the accessions of the populations under study. It is also to identify the species with the highest concentration of iron and zinc to be recommended for biofortification. Further more, this study seek to identify polymorphic markers useful in *Solanum* species diversity studies or screening in Ghana.

UNDER PEER REVIEW

2. MATERIAL AND METHODS

Plant Materials and Extraction of genomic DNA

30 accessions of *Solanum* sp. were collected from 5 geographical locations in Ghana. These accessions contained the 2 varieties of wild and domesticated *Solanum* sp. grown in Ghana thus *Solanum torvum, Solanum anguivi and Solanum aethiopicum.* For this study, the 30 assembled germplasm were grouped into 3 main populations according to their species. Table 1 gives the name, variety and the locality for the collection of the various species. 200mg of the young tender leaves were weighed into 2ml eppendorf tubes and grounded with liquid nitrogen to fine powder. The genomic DNA was extracted using the modified Egnin *et al* (1998). This modification was done with the introduction 7.5M Sodium acetate DNA and the addition of the RNAse step.

DNA Quantification and Gel electrophoresis

DNA quality was checked on 0.8% agarose in 1x TAE (Tris-acetic EDTA) buffer by gel electrophoresis with Ethidium bromide (10mg/ml). Electrophoresis of the DNA was carried at 100V for 40mins and then visualize 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 diluted to 10ng/µl for PCR amplifications.

Table 1

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	**Asante Akyem-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				

UNDER PEER REVIEW

PCR amplification and DNA assays.
DNA amplification was carried out with 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(10ul) contained 6.075µl of Nuclease free sterile water (DNA grade water),1µl of 10x PCR Buffer,0.9µl of $MgCl_2$ (25mM), 0.4µl dNTPs (10mM), primer 0.25µl (50µg/ml) of each forward and reverse, 0.125µl of SuperthermTaq Polymerase (1unit) and 1µl of 10ng DNA template. The PCR cycling conditions followed an initial denaturing step of 94^0C for 5mins a touchdown PCR protocol of 11 cycles of 94^0C for 30 secs, 60^0C for 30secs decreasing by 0.5 per cycle and 72^0C for 60 secs followed by 30 cycles of 94^0C for 30 secs, 55^0C for 30 secs and a final elongation step of 72^0C for 60 secs. In every experiment, a negative control was included were all the PCR components with the exception of the DNA template was added to detect contamination. 12 set of EST-SSR primers each forward and reverse was used in the experiment. Successful amplification products were examined by 6% polyacrylamide gel (Water, 10x TBE, 4% acryl amide (19:1), 10% APS and TEMED) electrophoresis in 1xTBE buffer and silver stained. A 100bp ladder (Gene Ruler ^{TM}, Fermentas) was used as a size marker. Table.2 gives a list of the primers used, their sequences and their allele size range (bp).

Silver Staining
The gels were placed on a shaker with a minimal shaking to allow solution to flow over gel swiftly. Fixation was done with 10% Glacial Acetic (100ml acetic acid, 900 ml water) for 15 mins. This was washed off with distilled water and 1.5% Nitric acid (15ml Nitric acid, 985ml water) solution was added for 5 mins. The silver stain solution (1.0g Silver nitrate, 1.5ml 37% formaldehyde, water up to 1000ml) proceeded after washing off the nitric acid solution for 20mins. Finally, the developer (30g Sodium carbonate, 1.5ml 37% formaldehyde and 0.25ml Sodium thiosulphate 10mg/ml, water up to 1000ml) was added and allowed to develop the photographic stains/ bands for visualization. This was stopped with 10% Glacial acetic acid and then stored in distilled water for photographic capturing and scoring.
Mineral Analysis
Oven dried milled *Solanum* samples of 0.5g were weighed into a digestion flask .15ml of a mixed reagent of perchloric acid and nitric acid was prepared in a ratio of 1:2 and 2ml of the prepared reagent was added to each sample and then digested.10ml of distilled water was added to the digested sample and then filtered into a 20ml volumetric flask up to the mark. The concentrations of the various samples were taken with the Atomic Absorption Spectrophotometer (AAS) after calibration with standard solution of the elements to be determined.
The iron and zinc concentrations readings from the Atomic Absorption Spectrophotometer (AAS) were recorded (Table 5)

Data collection and analysis
The amplified EST-SSR DNA bands representing different alleles were scored as different accessions on the co-dominant nature of EST-SSR markers. As a result, the 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. The F-statistics (*Fis, Fit and Fst*) (Wright, 1978) were also computed for polymorphic loci to test for the departure from Hardy–Weinberg equilibrium and to estimate genetic differentiation among *Solanum sp* 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 *Solanum sp* populations (Wright, 1978). All of the calculations were performed using POPGENE version 1_32 (Yeh *et al*., 1999).

A hierarchical analysis of molecular variance (AMOVA) of the *Solanum* sp populations was calculated to partition genetic diversity within and among regions. 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. In order to further appraise the genetic relationships of the *Solanum* sp populations as individual plants, a principle component analysis (PCA) was conducted based on the EST-SSR variation patterns (converted into the 1 and 0 matrix). The correlation matrix was selected to calculate coefficients of the first two principal components. Both hierarchal AMOVA and PCA were analysed using Genstat software.

UNDER PEER REVIEW

Furthermore, data collected on the minerals (Zn and Fe) of the accessions were also analysed using correlation to determine the degree of association between Zn and Fe of the accessions using Microsoft excel software.

3. RESULTS

Microsatellite Diversity Statistics for all Loci

A sum total of 64 alleles were generated for all loci with a mean of 5.333 per locus by the markers employed for the studies (Table 2). It was observed that all the EST-SSR markers showed allelic polymorphism (e.g. Figure 1) in all the assembled accessions studied. This implies that none of the locus was monomorphic. Locus EEMS 37 recorded the highest number of alleles as well as the highest level of genetic variability (Expected heterozygosity (*He*) = 0.843, Shannon's Information index (*I*) = 1.978). It also recorded a Nei's heterozygosity of 0.829 (not shown). On the other hand, four loci (EEMS 6, EEMS 13, EEMS 15 and EEMS 16) recorded the least number of alleles (3) with locus EEMS 6 having the least Shannon index (*I* = 0.4740).

Population Variability Similarity

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 exists 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 of populations of the study was extremely high (81.54%) with a gene flow of 2.2071. A dendogram based on Nei (1978) genetic Unweighed Pair Group Method with Arithmetic Mean (UPGMA) modified from neighbor of Philip version 3.5 was constructed (Fig. 2). *S. anguivi* and *S*. aethiopicum populations showed the greatest similarity and were separated from *S. torvum* which form different clusters at a genetic distance of 5.77594 (Table 4). These further elucidate genetic differentiation among populations.

Table 2

Locus	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

* na = Observed number of alleles ,* ne = Effective number of alleles [Kimura and Crow (1964)] * I = Shannon's Information index [Lewontin (1972), ASR = Allelic Size Range.

M 1 2 3 4 5 6 7 8 9 10111213141516171819202122232 4M 252627282930

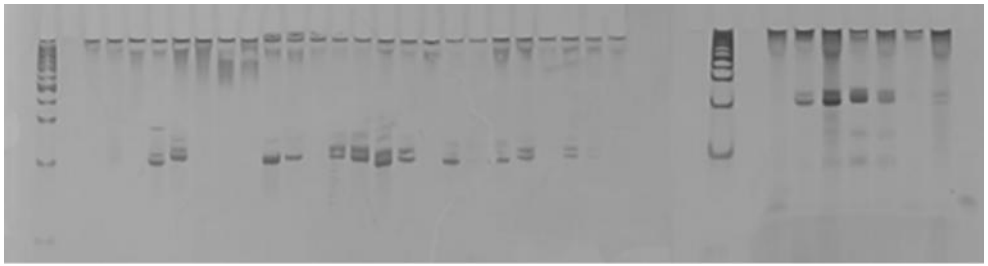

Figure 1:The banding pattern of primer EEMS 20 on accessions numbered 1-31: Hwidiem *S .torvum*= 1;Hwidiem *S. aethiopicum* = 2,Hwidiem *S.anguivi* = 3; Kenyase *S. aethiopicum* = 4; Kenyase *S.anguivi*= 5; Kenyase *S. torvum* = 6; Ntoturoso *S. torvum* = 7; Ntoturoso *S. aethiopicum* 1 = 8; Ntoturoso *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.

Table 3

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					
Over all 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.

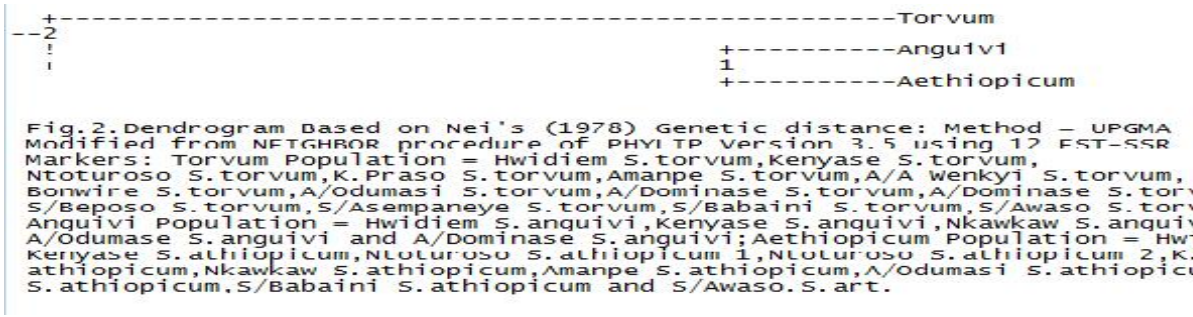

Fig.2.Dendrogram Based on Nei's (1978) Genetic distance: Method – UPGMA Modified from NEIGHBOR procedure of PHYLIP Version 3.5 using 12 EST-SSR Markers: Torvum Population = Hwidiem S.torvum,Kenyase S.torvum, Ntoturoso S.torvum,K.Praso S.torvum,Amanpe S.torvum,A/A Wenkyi S.torvum, Bonwire S.torvum,A/Odumasi S.torvum,A/Dominase S.torvum,A/Dominase S.torvum S/Beposo S.torvum,S/Asempaneye S.torvum,S/Babaini S.torvum,S/Awaso S.torvum; Anguivi Population = Hwidiem S.anguivi,Kenyase S.anguivi,Nkawkaw S.anguivi,Amanpe S.anguivi, A/Odumase S.anguivi and A/Dominase S.anguivi;Aethiopicum Population = Hwidiem S.athiopicum Kenyase S.athiopicum,Ntoturoso S.athiopicum 1,Ntoturoso S.athiopicum 2,K.Praso S. athiopicum,Nkawkaw S.athiopicum,Amanpe S.athiopicum,A/Odumasi S.athiopicum,A/Dominase S.athiopicum,S/Babaini S.athiopicum and S/Awaso.S.art.

UNDER PEER REVIEW

Table 4

Between	And	Length
2	Torvum	7.27970
2	1	5.77594
1	Anguivi	1.50376
1	Aethiopicum	1.50376

Cluster Analysis Based on EST-SSR.

The study also presents the molecular dendrogram of genetic relatedness among the individual accessions of the three populations under study as revealed by UPGMA cluster analysis based on the polymorphism of the 12 EST-SSR loci employing Jaccard Coeffciency using single link similarity matrix method (Fig. 3). The dendogram 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 cluster into two main clusters, i.e. Ntoturoso *S. aethiopicum 1* and the other 29 accessions which further separated into distinct subclusters of five. The accessions with the closest resemblance among the 29 accessions were revealed at the sixth cluster with a genetic similarity of 85% (Hwidiem *S. torvum,* A/A Wenkyi *S. torvum*, A/Dominase *S. anguivi* and A/ Odumasi *S. torvum*). The widest separation occurred between Hwidiem *S. torvum* and Ntoturoso *S. aethiopicum 1.* The clustering's did not exactly coincide with geographical origin as well as population or species groupings of the accessions. The scatter plot of the first and second principal components revealed a clear genetic variation and differentiation pattern among the individuals of the various populations. The large scattering of accessions of the various population species especially the *S. anguivi* and *S. aethiopicum* at the right hand side in the plot suggest their genetic differentiation and the clustering of the *S.torvum* individuals with few of the other two species at the left except K. Praso *S. torvum* suggest the genetic resemblance of most *S. torvum* accessions (Fig. 4).

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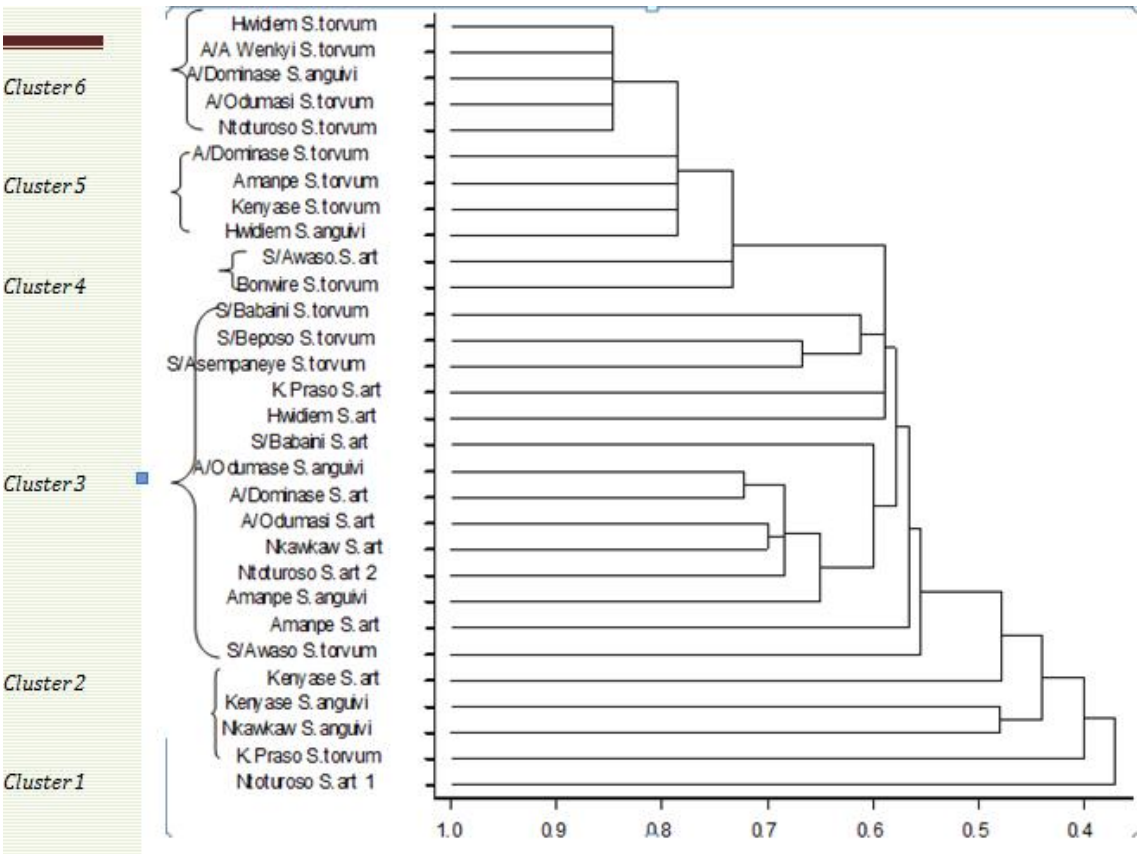

Figure 3

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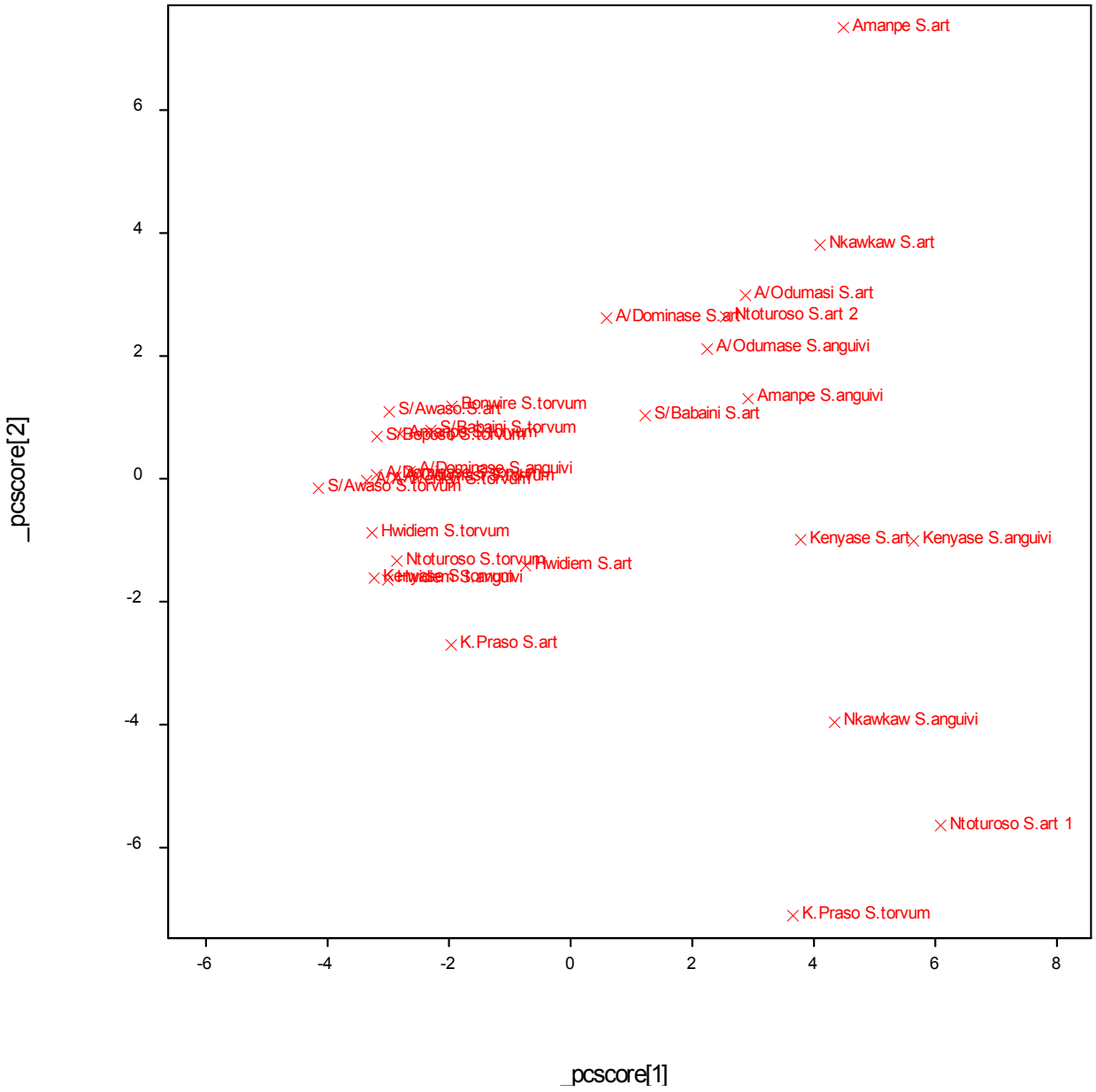

Figure 4: Scatter plot of the first and second principle components based on variation of 12 EST- SSR loci for 30 individuals (3 populations) of *Solanum* sp

UNDER PEER REVIEW

Variation based on Minerals.

The result on minerals displays the concentrations of Fe and Zn of the various accessions grouped in their respective species as detected by AAS (Table 5). The result clearly shows that the concentrations of both minerals for each accession are distinct and that no accession recorded the highest concentration for the two elements analyzed . However, one accession (Assin Odumase *S. anguivi*) recorded the least amount of both Fe (7.26 mg / Kg) and Zn (9.16 mg / Kg). It was observed that the accessions with the highest concentration of Fe and Zn was found among the *S. torvum* species (Assin Odumase *S. torvum*: 261.48 mg / kg) and the *S. aethiopicum* species (Sefwi Awaso *S. aethiopicum*; 29.16 mg / kg) respectively. The table also shows statistically weak or insignificant degree of association between the Fe and Zn for each species as well as their overall. This further confirms genetic diversity among the *Solanum* sp accessions or population under study

Table 5

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	-0.4455
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	
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	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	
Amanpe S. anguivi	127.56	15.08	0.041778
Assin Odumase S. anguivi	7.26	9.16	
Assin Dominase S. anguivi	45.36	28.52	
Mean	40.70	18.83	
Hwidiem S. art	155.96	18.52	
Kenyase S. art	114.24	17.16	
Ntoturoso S. art 1	114.28	23.6	
Ntoturoso S. art 2	68.32	21.64	
Nkawkaw S. art	14.24	20	
Amanpe S. art	40.28	18.36	
Assin Odumase S. art	23.38	22.8	-0.67893
Assin Dominase S. art	165.16	14.2	
Sefwi Awaso S. art	29.84	29.16	
Sefwi Babiani S. art	223	14.56	
Mean	100.69	19.79	
Overall Correlation			-0.41491

UNDER PEER REVIEW

Values above were computed at a significant level of $P = 0.05$.

4. DISCUSSION

Loci and Population Variation via Microsatellite

The 100% allelic polymorphism among the individuals of the species generated by the use of the EEMS loci or 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 EST-SSR markers is also consistent with similar finding by Aniko (2008) who worked on the genetic mapping and phylogeny studies of eggplant and where EEMS 48, one of above efficient markers produced the highest number of alleles. This suggests that in selecting primers for biodiversity evaluation for eggplant or species closely related to those under study, 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* spp and their populations possessed relatively high genetic diversity (*He* = 0.494, *Nei's He* = 0.486, *I* = 1.003), although with considerable variation among populations. This result is consistent to similar work by Demir *et al* (2010), Saraye and Ranghoo-Sanmukhiya (2008), Tumbilen *et al* (2011) and Ali (2011) which employed either or at least two of the following: SSR markers, AFLP makers, RAPD and morphological descriptors for characterisation of eggplant. Genetic variation (*I*) observed among the individual accession (Table 1) where either comparably close or far higher than among three populations with the exception of few loci (EEMS 6, EEMS 16 and EEMS 46). According to Muller *et al* (2001), this can be attributed to a high genetic overlap as a result of probable common ancestry. Further more, the low values recorded as F_{is} (0.4188) and F_{it} (0.4779) as well as high values recorded by *t* (0.8154) and *Nm* (2.2071) from the EST-SSR analysis suggest that the *Solanum* sp under study were more mongrelize or open pollinated with wide pollen-mediated gene flow though they can pollinate themselves due to their hermaphroditic nature.

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* been widely separated from each other. 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 from which *S. aethiopicum* was domesticated. This finding is consistent with the result obtained by Lester (1998) which states that *S. aethiopicum* and *S. macrocarpon* were domesticated in Africa from their wild relatives *S. anguivi* and *S. dasyphyllum* respectively. Further more, dendrogram of *Solanum* species by UPGMA based on the genetic distance of Nei and Li by Sakata and Lester (1997) represented *S. anguivi* and *S. aethiopicum* as duplicates proving their close relatedness. This explains why there is 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 and hence potential for their adaptation to a wide range of agroecosystems, indicating the usefulness of each population especially *S. torvum* which is more diverse from the others as a valuable genetic resources for selection and development of superior synthetic varieties with high level heterosis.

Variation via Cluster Analysis

From the dendogram, cluster five and six constitutes mainly *S. torvum* accessions with the exception of A/ Dominase *S. anguivi* and Hwidiem *S. anguivi* in cluster 5 and 6 respectively. The inclusion of the *S. anguivi* accessions among the *S. torvum* can be justify by the high rate of outcrossing and gene flow (*t* = 81.54%; *Nm* = 2.2071). This can also be confirmed by the clustering of most *S. torvum* accessions and the two *S. anguivi* accessions mentioned above at the left hand of the PCA. It can therefore be suggested that about 85% of the *S. torvum* genome has been introgressed into such *S. anguivi* accessions making them lose their unique genetic characteristics. This further implies that the *S. torvum* accessions in cluster

UNDER PEER REVIEW

5 and 6 which are about 15% dissimilar of each other and has the least population diversity (*I* =0.6369) can be assembled and conserved for breeding purpose to avoid contamination of this pure stocks in the future. Nevertheless, the remaining clusters constitute a muddle of the three populations. It was haphazardly clustered without conceding to their species or geographical regions where they were collected, a situation also accounted for by the extremely high gene flow and the low values of F_{is} and F_{it} resulting in probable heterozygosity of those accessions. In principle, considerable gene flow is an evolutionary force that tends to maintain genetic homogeneity among populations (Slatkin, 1987) and, in contrast, limited gene flow may promote substantial genetic differentiation among populations (Cao *et al*, 2006). Hence, the assortment of the three populations in most of the cluster suggests gradual homogeneity of the accessions with time due to gene flow.

Variation among accessions via mineral

The distinct concentration of the minerals analysed among the accessions also display variation among the accessions. Averagely the *S. torvum* species showed a higher concentration of Fe (Mean = 125.65 mg / kg) and *S. aethiopicum* species also exhibited averagely the highest concentration of Zn (Mean = 19.79) as well as a considerable amount of Fe (Mean = 100.69). Interestingly, the *S. aethiopicum* being the domesticated form of *S. anguivi*, contained averagely, a significant concentration of both Fe and Zn than *S. anguivi* demonstrating a sense of high heterosis than its parent. Nevertheless, since no single accession contained the highest concentration of the two minerals analysed as evident by their poor correlation, it therefore implies that the two minerals cannot be selected together at the same time and have to be selected individually for any biofortification programme. On the other hand, a hybrid between the two Fe and Zn rich accessions can be developed through crossing.

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 distinct *S. torvum* species suggested above.

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UNDER PEER REVIEW

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

'Author A and Author B designed the study, wrote the protocol and performed the experiment, Author C and Author D performed the statistical analysis of the study and wrote the first draft of the manuscript. 'Author E and F managed the literature searches of the study. 'All authors read and approved the final manuscript.

CONSENT (WHERE EVER APPLICABLE)

Consent not applicable.

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