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ORIGINAL ARTICLE

Genetic diversity in tomato accessions [*Solanum lycopersicum* (L.) H. Karst] from Nigeria employing morphological and SSR markers

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Abstract The level of genetic diversity among 40 accessions of tomato collected from Nigeria was assessed employing 10 quantitative traits and 13 EST-SSR markers. There were significant differences among accessions as revealed by the quantitative traits. Meanwhile, the level of polymorphism displayed by the EST-SSR markers was low with mean PIC of 0.13. The clusters formed by morphological traits were completely different from the ones established by EST-SSR markers, as supported by the weak correlation (r = 0.143) between morphological and molecular distances by Mantel test. PCA and bi-plot analyses indicate that fruit yield, average fruit weight, number of days to first flowering and first fruit set and plant height, number of branches and number of fruits per cluster had the highest discriminating potentials. The best accessions T5, T17 and T12 were vertex accessions in the fruit yield, plant height, number of fruits per cluster and number of main branches sector. Accessions T11, T32 and T38 were vertex accessions in the number of days to first flowering and number of days to fruit set sector, hence were late maturing accessions, poor in yield and can however be improved by crossing them with the accessions in the first sector.

Keywords Tomato · Genetic diversity · Morphological traits · Polymorphic · EST-SSR · Primers

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Introduction

One of the most important perennial vegetable in the world is tomato; it is generally cultivated as annual and consumed worldwide (Henareh et al. 2015); for various purposes which include cooking, paste and in making ketchup (Qumer et al. 2014). It is a member of Solanaceae popularly known as the nightshade family which consists of about 96 genera with about 3000 species across three subfamilies. The importance of Solanaceae lies in the caliber of plants included in the family; such as pepper, potato, tomato, petunia, tobacco and eggplant. Tomato has acquired different names from the time of Linnaeus up to what it is being called today (Foolad 2007).

Tomato does well in almost every part of Nigeria; however the best area of cultivation lies in the savannah agro-ecological zone due to the lower prevalence of pests and diseases of tomato (Ugonna et al. 2015). As at 2018, Nigeria was ranked the 13th largest tomato producing nation in the world with potential for improvement to become one of the top countries in the world in both production and export of tomato (FAOSTAT 2020). Unfortunately, the deficiencies in critical inputs, absence of improved technology, low yield and productivity, extreme post-harvest loses and absence of processing and marketing setups the country experiences up till now has kept its top position at bay. Another big challenge to tomato

productivity in Nigeria is the increased erosion of genetic resources which has led to narrow genetic variability among the cultivated species and their wild relatives. Enhanced genetic variability through widening of the genetic resources will effectively put the genetic weakness of the crop at bay (Silva et al. 2001).

Inter and intra genetic differences in population of tomato genotypes have been exploited employing techniques such as morphological markers, biochemical and molecular markers (Garcia et al. 2004). Morphological characterization of the species seems to be the most common tool employed for its improvement over the years since it allows selection to be based on excelled performance of individuals in terms of desirable traits. Nevertheless, selection of improved genotypes based on phenotype may be heavily influenced by environment thereby hindering the estimate of genetic diversity of the crop (Brunlop and Finckh 2010). Discriminatory power of morphological markers wanes with increased number of genotypes making morphological traits less efficient. On the other hand, molecular markers have not been found to be influenced by environmental factors, hence suitable in complementing morphological markers in diversity studies (Milevska et al. 2011). The success of molecular markers in cultivar identification has been previously reported (Lombard et al. 2001). Advantages of SSR markers over the traditional and biochemical methods lie in their immunity against environmental effects, extraordinary polymorphism level and their limitless obtainability. They have high discriminating capacities, they are fast to deploy, and they have multiple allelic potentials, co-dominant and can be deployed by Polymerase Chain Reaction (PCR). A combination of morphological markers and EST-SSR markers will be more effective in assessing the level of diversity of tomato having close genetic relatedness (Kwon et al. 2009; Herraiz et al. 2015).

The main objective of this study is to determine the genetic variability in forty accessions of tomato using morphological and EST-SSR markers, and to assess the extent of genetic diversity and character association among them.

Materials and methods

Materials

Forty accessions of tomato utilised in this study were collected from the Gene Bank of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan; seed outlets in Ibadan; Ikole Ekiti in Ekiti State and from local markets in Akungba-Akoko, Ondo State (Table 1). All the accessions were high yielding with determinate growth patterns and early maturing (between 60 and 120 days after sowing). The research was set up at the Research/Training Farm of Adekunle Ajasin University, Akungba-Akoko, Ondo State which is situated between Latitude 7° 28′ N and Longitude 5° 28′ E. The study area lies in tropical vegetation with annual rainfall between 1500 and 2000 mm and a mean temperature of about 30 °C.

Propagation and nursery procedures

Forty germination baskets were labelled according to the different accessions and filled with well drained top soil. Each accession was sown in the already labelled baskets using broadcast method. The germination baskets were placed under shade in the nursery for proper germination and growth of seedlings. The seedlings were watered daily and number of days to germination was noted. The seedlings were transplanted to the field after nursing for 4 weeks.

Experimental design

The soil was ploughed and made ready for transplanting. The seedlings were planted in a Randomized Complete Block Design (RCBD) layout with three replicates; each replicate consisting of forty plots. Each plot had a dimension of 1 m × 1 m while the alley in between each plot was 0.5 m. The planting space was 0.3 m x 0.25 m. There were sixteen seedlings of each accession on individual plots. Each seedling was lightly watered immediately after transplanting. Cultural practices such as weeding, watering etc. were carried out subsequently after transplanting. No fertilizer application was done during the study.

Morphological traits

Five plants on each plot were randomly selected and tagged and their morphological data were taken every week for 4 weeks. The morphological data taken according to the Descriptors for tomato, IPGRI (2015) were plant height, number of main branches, days to first flowering, days to first fruit set, number of fruits per cluster, number of fruit per plant, average fruit weight, pericarp thickness, number of locules per fruit and fruit yield per plant.

Molecular characterization

The EST-SSR primers used for molecular characterisation are presented in Table 2. Seed samples were collected from the developing fruits of each accession for genomic DNA extraction. The fruits were carefully cut open with a sterilized razor blade and the seeds were squeezed out. The

Table 1 Forty accessions of tomato used for genetic variability study based on ten morphological traits and thirteen EST-SSR markers

S/N	Accession ID	Source of collection	Origin/primary source	Biological status	Code
1	NG/MR/JAN/10/001	NACGRAB	South-West, Nigeria	Landrace	T1
2	NHGB/09/113	NACGRAB	South-South, Nigeria	Landrace	T2
3	NGB01232	NACGRAB	Not available	Landrace	T3
4	NGB01363	NACGRAB	Not available	Landrace	T4
5	NGB01302	NACGRAB	Not available	Landrace	T5
6	NGBOB011301	NACGRAB	Not available	Landrace	T6
7	NG/AA/SEP/09/053	NACGRAB	South-West, Nigeria	Landrace	T7
8	L00169	NACGRAB	South-West, Nigeria	Wild cultivar	T8
9	NGB01254	NACGRAB	Not available	Landrace	T9
10	NGB01665	NACGRAB	Not available	Landrace	T10
11	NG/AA/SEP/09/042	NACGRAB	South-West, Nigeria	Landrace	T11
12	NGB01357	NACGRAB	Not available	Landrace	T12
13	NGB01358	NACGRAB	Not available	Landrace	T13
14	NGB01362	NACGRAB	Not available	Landrace	T14
15	NGB01359	NACGRAB	Not available	Landrace	T15
16	NG/SA/01/10/002	NACGRAB	Republic of Benin	Landrace	T16
17	NGB01371	NACGRAB	Not available	Landrace	T17
18	NG/SA/07/10/002	NACGRAB	Republic of Benin	Landrace	T18
19	L00170	NACGRAB	South-West, Nigeria	Wild cultivar	T19
20	AKUNGBA 1	Akungba-Akoko Market	South-West, Nigeria	Landrace	T20
21	GIRAFTO	Seed outlet, Ibadan	Unknown	Unknown	T21
22	IBADAN HAUSA 16	Seed outlet, Ibadan	North, Nigeria	Landrace	T22
23	ODIGBO HAUSA	Seed outlet, Ibadan	North, Nigeria	Landrace	T23
24	DERICA	Seed outlet, Ibadan	Kitano seeds, Netherlands	Hybrid variety	T24
25	YUTILI	Seed outlet, Ibadan	Unknown	Unknown	T25
26	NADIRA	Seed outlet, Ibadan	Technisem, West Africa	Hybrid variety	T26
27	KIARA	Seed outlet, Ibadan	Technisem, West Africa	Hybrid variety	T27
28	PANTHER17	Seed outlet, Ibadan	Technisem, West Africa	Hybrid variety	T28
29	COBRA26	Seed outlet, Ibadan	Technisem, West Africa	Hybrid variety	T29
30	T-8	Seed outlet, Ibadan	Unknown	Unknown	T30
31	TROPIMECH	Seed outlet, Ibadan	Technisem, West Africa	Improved variety	T31
32	ROMA VF	Seed outlet, Ibadan	Technisem, West Africa	Improved variety	T32
33	UC82B	Seed outlet, Ibadan	Technisem, West Africa	Unknown	T33
34	TIMA	Seed outlet, Ibadan	Unknown	Improved variety	T34
35	RIO GRANDE	Seed outlet, Ibadan	Technisem, West Africa	Improved variety	T35
36	ROMA SAVANA	Seed outlet, Ibadan	Technisem, West Africa	Improved variety	T36
37	LOCAL IKOLE	Ikole, Ekiti State	South-West, Nigeria	Landrace	T37
38	TEMARU 97	Seed outlet, Ibadan	Unknown	Unknown	T38
39	AKUNGBA 2	Akungba-Akoko Market	South-West, Nigeria	Landrace	T39
40	AKUNGBA 3	Akungba-Akoko Market	South-West, Nigeria	Landrace	T40

seeds were transferred into the labelled Eppendorf tubes in an ice pack.

The DNA of each accession was extracted using extraction kit. The DNA isolation was done following the protocol as modified by the manufacturer of the kit (Zymo Research, The Epigenetics; United States of America). Thirteen EST-SSR markers published in Zhou et al. 2015 and supplied by Inqaba Biotech, South Africa were utilised in genotyping accessions. DNA amplification was done in a PCR machine; the cycling program for amplification consisted of 1 cycle of 4 min at 94 °C (initial denaturation) followed by 45 cycles of 15 s at 94 °C (denaturation)

Table 2 Thirteen EST-SSR primers used for screening forty accessions of tomato

Primer names	Sequence	Annealing temperature (°C)
EST-SSR1	ACCTACCTGTCTCCGCCTCT (F)	55
	TGACAAGGTAAAGCCAACCC (R)	
EST-SSR2	CTTATGTGAAAACACCTCGCTC (F)	54
	TTCAAAATTCCCCAAAGACG (R)	
EST-SSR7	GAAGAAGATGGTGGGGATGA (F)	54
	CTTGCAACAATCGTGAATGC (R)	
EST-SSR19	ACCTGCACACACCACACACT (F)	53
	GATCAAAGAAGCGGGATGAT (R)	
EST-SSR23	TAGACTGGGCCTGTGGTCTT (F)	52
	TGGTGAATCAATTTTGGGGT (R)	
EST-SSR25	ATTGGGGAATGGGTTTTCTC (F)	54
	AAACGAAGGCAACAACGAAG (R)	
EST-SSR26	TCAAATGGCTTCTCTCTTGTTCTTT (F)	54
	TTGTTGGAAACTCCTTTGGC (R)	
EST-SSR35	CATAAGAAGAAAGGTGTGAATGAGA (F)	52
	GTTGCTTTGTCTTTGTCGCC (R)	
EST-SSR42	CCAAAAGAAGTGGGTCCAAA (F)	54
	AAACTAGCGACAAATAAAAGCAGA (R)	
EST-SSR67	ATCTCGATTTGCTGCTCCAT (F)	54
	CAAGTTCACACCATTTTCTCTCC (R)	
EST-SSR71	GGACCAAGCGAAGTTGGATA (F)	54
	CGAGTGTTTCGCTTCTCCTC (R)	
EST-SSR77	GAGGACGACAACAACAACGA (F)	53
	GACATGCCACTTAGATCCACAA (R)	
EST-SSR83	TTAGGCAGCTTACGACTGGA (F)	51
	CCACAAATTCTTTTCCCCAA (R)	

F = forward primer; R = reverse primer

annealing of primers occurred at 55 °C and elongation of new strands occurred at 72 °C in a total volume of 10 µl containing 3 µl of DNA sample in an Eppendorf tube, 5 µl of 2× master mix, 1 µl of forward primer and 1 µl of reverse primer for each sample. The PCR yields (with 5 µl loading dye added) were confirmed in 0.5 g of agarose gel dissolved in 50 ml. 5× TBE buffer, melted in the microwave for 90 s and allowed to solidify in electrophoresis tank. The DNA bands were viewed on a photophorensis and scored as plus 1 for presence of polymorphism and 0 for absence.

Data analysis

The morphological data collected were analysed using SPSS version 20. The data were subjected to Analysis of Variance (ANOVA) according to Singh and Chaudhary (1985). Duncan Multiple Range Test at $P \leq 0.01$ was used to separate the accession means. Genotypic and Phenotypic Variances (VG and VP) were estimated according to Prasad et al. (1981), Wricke and Weber (1986). The Phenotypic Coefficient of Variation (PCV %) and Genotypic Coefficient of Variation (GCV %) were estimated by the method of Burton (1952) and Johnson et al. (1955) and were classified according to Sivasubramanian and Menon (1973) as follows: 0–10% = low; 10–20% = moderate; > 20% = high. Broad sense Heritability (H^2B) was expressed as the percentage of the ratio of Genotypic variance (VG) to Phenotypic variance (VP) as described by Allard (1960) and was categorized according to Robinson et al. (1949) as follows: 0–30% = low; 30–60% = moderate; > 60% = high. Genetic Advance (GA) was estimated by the method given by Fehr et al. (1987); GA was also calculated as percentage of the mean (GAM) according to the formula of Johnson et al. (1955) and categorized as 0–10% = low; 10–20% = moderate; > 20% = high.

Genotypic and Phenotypic correlations were estimated with Plant Breeding Tools (PB-Tools 2014) version 1.4 (Biometrics and Breeding informatics, International Rice Research Institute). Phenotypic correlation coefficients were compared against t-table r (n − 2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their

significance (Fisher and Yates 1963). Where, r and n are correlation coefficients and number of observation respectively. The 't' table was entered with (n − 2) degree of freedom.

The molecular data were subjected to statistical analysis by employing Power Maker Version 3.5. The data on morphological and molecular traits were subjected to cluster analysis. Data on morphological traits were also subjected to Principal Component Analysis (PCA) and two dimensional ordinations (Bi-plot) of the genotypes were plotted. The relationship between molecular and morphological data was assessed with Mantel Test and correspondence analysis. Palaeontological Statistics Software Package for Data Analysis (PAST) version 4.01 (Hammer et al. 2001) was adopted for cluster analysis, PCA, Mantel test and correspondence analysis.

Results

Mean squares for all traits were highly significant ($P \leq 0.01$) among accessions. The most variable traits among accessions were: Harvested number of fruits per plant, average fruit weight, number of fruits per cluster, number of branches per plant and fruit yield per plant, while the least variable trait was number of days to first fruit set (Table 3). Accessions expressed a high level of variability for all traits. Plant height ranged from 13.69 cm in T9 to 47.12 cm in T40. Number of main branches ranged from 1.16 in T31 to 25.77 in T16. Number of days to first flowering ranged from 26.73 days in T40 to 54.87 days in T33. Number of days to first fruit set ranged from 34.95 days in T40 to 77.96 days in T33. Number of fruits per cluster ranged from 1.67 in T31 to 6.33 in T1. Harvested fruits per plant ranged from 3.33 in T31 to 120.00 in T40. Average fruit weight ranged from 1.54 g in T39 to 77.96 g in T33. Number of locus per fruit ranged from 2.00 in T8 to 4.10 in T37. Pericarp thickness ranged from 1.13 mm in T8 to 4.76 mm in T4. Fruit yield ranged from 80.61 g in T32 to 735.83 g in T33 (Table 4).

Phenotypic Variance (VP) was higher than genotypic variance (VG) in all traits. Also phenotypic Coefficient of Variation (PCV) was higher than Genotypic Coefficient of Variation (GCV) in all traits. The highest PCV (119.57%) was obtained in number of harvested fruits per plant, while the lowest (16.00%) was obtained in number of days to first fruit set. GCV was also highest (100.79%) in harvested fruits per plant and lowest (12.49%) in number of days to first fruit set. Heritability ranged from 53.95% in number of days to first flowering to 84.68% in number of branches per plant. Genetic advance as percent of mean (GAM) was highest (166.90%) in number of fruits per cluster and lowest (19.41%) in number of days to first flowering (Table 5).

Plant height was highly significant and positively correlated with fruit yield per plant (r = 0.38**) at genotypic level only. Number of branches was positively correlated with fruit yield per plant (r = 0.35** and 0.31**) at both genotypic and phenotypic levels. Number of days to first flowering negatively correlated with fruit yield per plant (r = − 0.33*) only at genotypic level. Days to first fruit set was highly significant and negatively correlated with fruit

Table 3 Analysis of variance (ANOVA) for forty accessions of tomato on ten quantitative traits

Source of variation	Degree of Freedom	PH (cm)	NB	NDF	NDFFS	NFC
Accessions	39	141.11**	119.88**	115.29**	128.63**	5.40**
Replication	2	689.09**	10.12**	398.07**	330.76**	3.33**
Error	78	29.56	6.82	25.54	22.60	0.36
CV (%)		22.24	32.64	11.85	10.0	41.38
GM		24.45	8.00	42.65	47.59	1.45

Source of variation	HFP	AFW (g)	NLF	PT (mm)	FYP (g)
Accessions	2259.53**	891.72**	0.74**	2.47**	72,711.2**
Replication	193.58**	144.34**	0.11**	0.43**	2083.3**
Error	270.11	119.69	0.13	0.19	10,001.32
CV (%)	64.33	43.85	13.81	13.33	30.97
GM	25.55	24.95	2.61	3.27	322.95

CV coefficient of variation; *GM* grand mean. *PH* plant height; number of branches; *NDF* number of days to first flowering; *NDFFS* number of days to first fruit set; *NFC* number of fruits per cluster; *HFP* harvested fruits per plant; *AFW* average fruit weight; *NLF* number of locus per fruit; *PT* pericarp thickness; *FYP* fruit yield per plant

**Highly significant

Table 4 Mean values and standard error of forty accessions of tomato on ten quantitative traits

TRT	PH (cm)	NB	NDF	NDFFS	NFC	HFP	AFW (g)	NLF	PT (mm)	FYP (g)
T1	20.3 ± 3.8^{abcdef}	20.6 ± 3.6^{k}	37.1 ± 1.9^{abcdefg}	41.0 ± 2.4^{abcd}	6.8 ± 0.3^{p}	106.2 ± 9.0^{fg}	3.1 ± 0.9^{ab}	2.0 ± 0.0^{ab}	1.3 ± 0.1^{a}	324.0 ± 72.3^{cdefghij}
T2	24.5 ± 3.1^{abcdefg}	11.2 ± 0.6^{hi}	42.4 ± 6.4^{bcdefghijk}	45.8 ± 6.2^{abcdefghi}	4.6 ± 0.8^{ghijkl}	22.6 ± 2.3^{abc}	20.7 ± 3.5^{abcdefg}	3.6 ± 0.5^{jkl}	2.9 ± 0.1^{cdef}	485.5 ± 120.2^{hijklm}
T3	26.7 ± 2.9^{abcdefg}	6.9 ± 1.9^{bcdefgh}	41.7 ± 3.6^{bcdefghij}	45.6 ± 3.5^{abcdefghi}	6.0 ± 0.4^{mnop}	13.6 ± 1.7^{ab}	14.7 ± 0.8^{abcde}	2.3 ± 0.1^{abcdef}	3.2 ± 0.3^{cdefgh}	203.8 ± 34.6^{abcdef}
T4	31.2 ± 3.6d^{efg}	13.3 ± 0.0^{i}	34.5 ± 3.9^{abc}	38.8 ± 3.5^{abc}	5.7 ± 0.2^{lmnop}	17.1 ± 1.4^{ab}	18.4 ± 3.7^{abcdefg}	2.2 ± 0.2^{abcde}	3.2 ± 0.0^{cdefghi}	310.9 ± 52.6^{bcdefghij}
T5	32.3 ± 0.7^{efg}	10.7 ± 1.3^{ghi}	39.1 ± 1.4^{bcdefghi}	43.0 ± 2.0^{abcdef}	5.6 ± 0.2^{klmnop}	24.9 ± 2.2^{abc}	25.0 ± 3.1^{bcdefgh}	3.2 ± 0.0^{hijk}	3.7 ± 0.2^{fghijklm}	611.4 ± 28.9^{mn}
T6	23.8 ± 3.0^{abcdefg}	5.8 ± 0.6^{abcdefg}	42.3 ± 6.2^{bcdefghijk}	53.4 ± 0.7^{fghijk}	3.5 ± 0.0^{cdefgh}	14.7 ± 0.8^{ab}	26.9 ± 1.3^{cdefgh}	2.1 ± 0.0^{abc}	4.0 ± 0.0^{ghijklmn}	395.2 ± 6.9^{fghijkl}
T7	33.6 ± 0.1^{fg}	10.0 ± 1.6^{fghi}	37.5 ± 0.5^{abcdefgh}	40.9 ± 0.5^{abcd}	5.7 ± 0.1^{lmnop}	27.0 ± 3.5^{abc}	17.3 ± 1.2^{abcdef}	2.9 ± 0.2^{defghi}	3.5 ± 0.1^{efghijklm}	460.4 ± 26.8^{ghijklm}
T8	14.1 ± 0.8^{a}	22.2 ± 1.7^{kl}	42.7 ± 2.4^{bcdefghijk}	46.4 ± 2.4^{bcdefghi}	5.6 ± 0.0^{lmnop}	63.7 ± 11.2^{de}	3.5 ± 0.7^{ab}	2.0 ± 0.0^{a}	1.1 ± 0.1^{a}	208.0 ± 18.5^{abcdef}
T9	13.6 ± 0.7^{a}	3.1 ± 0.9^{abc}	42.1 ± 6.4^{bcdefghijk}	52.7 ± 0.9^{fghijk}	3.3 ± 0.1^{bcdef}	7.8 ± 1.4^{ab}	18.6 ± 1.9^{abcdefg}	2.2 ± 0.0^{abcd}	2.7 ± 0.0^{cde}	141.0 ± 10.5^{abcd}
T10	21.3 ± 2.2^{abcdef}	7.9 ± 0.4^{cdefgh}	39.9 ± 5.3^{bcdefghij}	48.7 ± 1.5^{cdefghijk}	3.4 ± 0.0^{bcdefg}	16.6 ± 1.4^{ab}	17.4 ± 0.6^{abcdef}	2.8 ± 0.0^{defghi}	3.2 ± 0.1^{cdefgh}	288.2 ± 16.5^{bcdefgh}
T11	18.2 ± 4.1^{abcd}	4.2 ± 0.2^{abcd}	48.7 ± 3.1^{fghijkl}	52.9 ± 3.2^{fghijk}	4.3 ± 0.2^{fghijk}	13.2 ± 1.8^{ab}	12.0 ± 1.7^{abcd}	2.0 ± 0.0^{abc}	2.4 ± 0.2^{bc}	154.4 ± 12.9^{3abcde}
T12	32.6 ± 5.3^{efg}	14.2 ± 1.2^{ij}	35.4 ± 1.7^{abcd}	38.8 ± 1.5^{abc}	5.7 ± 0.3^{lmnop}	35.5 ± 9.3^{abcd}	16.7 ± 2.0^{abcdef}	3.1 ± 0.1^{ghijk}	3.1 ± 0.1^{cdefg}	563.8 ± 115.0^{lmn}
T13	20.7 ± 3.4a^{bcdef}	9.5 ± 1.8^{efghi}	40.1 ± 1.0^{bcdefghij}	44.0 ± 0.9^{abcdefgh}	4.8 ± 0.4^{hijklm}	21.9 ± 5.6^{abc}	17.9 ± 0.6^{abcdefg}	3.3 ± 0.0^{ijk}	2.7 ± 0.0^{cde}	387.8 ± 86.9^{fghijkl}
T14	25.7 ± 3.4^{abcdefg}	7.7 ± 0.5^{cdefgh}	47.8 ± 0.7^{efghijkl}	52.4 ± 1.0^{efghijk}	5.4 ± 0.0^{klmno}	41.0 ± 2.8^{bcd}	3.7 ± 1.6^{ab}	2.0 ± 0.0^{a}	1.3 ± 0.1^{a}	144.4 ± 53.7^{abcd}
T15	29.9 ± 4.2^{cdefg}	18.3 ± 0.1^{jk}	32.8 ± 1.7^{ab}	36.0 ± 1.6^{ab}	5.4 ± 1.0^{klmno}	62.8 ± 11.1^{de}	6.3 ± 0.6^{abc}	2.7 ± 0.2^{abcdefghi}	1.7 ± 0.1^{ab}	391.1 ± 62.4^{fghijkl}
T16	29.7 ± 3.6^{cdefg}	25.7 ± 0.9^{l}	40.8 ± 4.2^{bcdefghij}	45.1 ± 4.3^{abcdefghi}	5.3 ± 0.3^{klmno}	19.4 ± 2.1^{abc}	16.4 ± 4.4^{abcdef}	2.7 ± 0.2^{bcdefghi}	2.6 ± 0.3^{cd}	300.7 ± 48.2^{bcdefghi}
T17	37.4 ± 8.7^{gh}	11.0 ± 6.0^{ghi}	36.2 ± 4.0^{abcde}	40.3 ± 4.1^{abcd}	6.3 ± 0.4^{op}	26.1 ± 3.8^{abc}	19.0 ± 1.1^{abcdefg}	3.2 ± 0.2^{hijk}	3.2 ± 0.1^{cdefghi}	489.7 ± 47.4^{ijklm}
T18	30.3 ± 2.4^{cdefg}	10.6 ± 0.2^{ghi}	34.3 ± 0.9^{abc}	37.8 ± 0.8^{abc}	6.0 ± 0.1^{mnop}	26.6 ± 2.7^{abc}	19.9 ± 1.2^{abcdefg}	2.6 ± 0.1^{abcdefghi}	3.1 ± 0.0^{cdefg}	526.3 ± 31.0^{klm}
T19	29.5 ± 4.1^{bcdefg}	9.8 ± 2.26^{fghi}	37.8 ± 1.4^{abcdefgh}	41.2 ± 1.9^{abcde}	6.2 ± 0.6^{op}	51.6 ± 6.9^{cd}	5.4 ± 0.7^{abc}	2.3 ± 0.2^{abcdef}	1.6 ± 0.0^{a}	270.7 ± 24.7^{abcdefg}
T20	30.17 ± 7.04^{cdefg}	20.6 ± 1.7^{k}	36.7 ± 3.8^{abcdef}	41.0 ± 4.4^{abcd}	6.2 ± 0.7^{nop}	35.2 ± 8.6^{abcd}	16.8 ± 1.9^{abcdef}	2.4 ± 0.1^{abcdefg}	3.6 ± 0.1^{efghijklm}	563.2 ± 101.4^{lmn}
T21	18.0 ± 3.2^{abcd}	4.5 ± 1.0^{abcde}	47.5 ± 2.5^{defghijkl}	55.2 ± 1.7^{hijk}	3.3 ± 0.0^{bcdefg}	7.6 ± 2.4^{ab}	46.2 ± 2.9^{hi}	2.9 ± 0.2^{efghij}	4.1 ± 0.1^{hijklmn}	340.6 ± 90.0^{defghijk}
T22	24.4 ± 3.6^{abcdefg}	3.4 ± 0.9^{abc}	43.6 ± 2.9^{bcdefghijkl}	47.7 ± 2.9^{cdefghij}	4.9 ± 0.1^{ijklmn}	11.1 ± 0.7^{ab}	13.1 ± 2.8^{abcd}	2.2 ± 0.0^{abcde}	3.2 ± 0.3^{cdefghi}	141.9 ± 22.8^{abcd}
T23	22.7 ± 0.9^{abcdef}	9.2 ± 0.2^{defghi}	42.6 ± 0.5^{bcdefghijk}	46.0 ± 0.7^{abcdefghi}	4.5 ± 0.0^{fghijkl}	18.7 ± 1.2^{ab}	18.3 ± 0.8^{abcdefg}	2.0 ± 0.0^{abc}	2.4 ± 0.2^{bc}	342.2 ± 9.3^{defghijk}
T24	23.1 ± 2.0^{abcdef}	6.3 ± 1.0^{abcdefgh}	41.0 ± 5.7^{bcdefghij}	45.1 ± 6.0^{abcdefghi}	3.0 ± 0.5^{bcde}	13.8 ± 1.3^{ab}	36.7 ± 2.3^{efghi}	2.4 ± 0.1^{abcdefgh}	3.4 ± 0.1^{defghij}	506.4 ± 51.0^{jklm}
T25	20.1 ± 3.4^{abcdef}	3.3 ± 0.4^{abc}	49.6 ± 2.7^{hijk}l	54.4 ± 2.4^{ghijk}	3.8 ± 0.4^{defghi}	8.4 ± 0.9^{ab}	40.3 ± 2.7^{ghi}	2.0 ± 0.0^{abc}	4.3 ± 0.0^{jklmn}	337.2 ± 22.6^{defghijk}
T26	22.2 ± 1.5^{abcdef}	1.3 ± 0.0^{a}	43.2 ± 2.3^{bcdefghijkl}	47.6 ± 2.3^{cdefghij}	4.5 ± 0.3^{fghijkl}	6.8 ± 0.4^{a}	52.0 ± 10.6^{ij}	3.1 ± 0.0^{ghijk}	3.5 ± 0.2^{defghijkl}	346.6 ± 51.5^{efghijk}
T27	17.4 ± 2.4^{abcd}	2.3 ± 0.1^{ab}	54.1 ± 1.5^{kl}	58.7 ± 1.3^{jk}	3.7 ± 0.3^{defghi}	6.0 ± 0.5^{a}	26.6 ± 1.1^{cdefgh}	2.5 ± 0.1^{abcdefgh}	3.2 ± 0.1^{cdefgh}	160.1 ± 20.3^{abcde}
T28	22.9 ± 5.3^{abcdef}	1.4 ± 0.1^{a}	45.8 ± 3.3^{cdefghijkl}	50.1 ± 3.1^{defghijk}	3.0 ± 0.0^{bcde}	3.8 ± 0.5^{a}	67.1 ± 12.2^{jk}	3.7 ± 0.4^{kl}	3.8 ± 0.5^{ghijklm}	244.4 ± 15.7^{abcdef}
T29	17.9 ± 1.1^{abcd}	1.6 ± 0.2^{ab}	50.5 ± 1.1^{ijkl}	54.8 ± 2.1^{hijk}	4.0 ± 0.1^{efghij}	7.5 ± 1.2^{ab}	38.7 ± 4.2^{fghi}	2.3 ± 0.1^{abcdef}	4.3 ± 0.0^{klmn}	282.5 ± 19.9^{bcdefg}
T30	16.6 ± 2.6^{abc}	3.0 ± 0.4^{abc}	50.6 ± 2.5^{ijkl}	56.2 ± 3.0^{ijk}	2.2 ± 0.2^{ab}	6.8 ± 0.9^{a}	33.2 ± 9.6^{defghi}	2.7 ± 0.1^{abcdefghi}	4.4 ± 0.1^{mn}	209.2 ± 39.9^{abcdef}
T31	22.1 ± 1.9^{abcdef}	1.1 ± 0.1^{a}	50.4 ± 5.4^{ijkl}	53.9 ± 5.3^{fghijk}	1.6 ± 0.3^{a}	3.3 ± 0.3^{a}	36.4 ± 1.7^{efghi}	2.4 ± 0.1^{abcdefg}	3.9 ± 0.3^{ghijklmn}	121.1 ± 10.9^{ab}
T32	18.0 ± 0.9^{cdefg}	1.5 ± 0.1^{a}	49.1 ± 0.9^{ghijkl}	53.3 ± 1.3^{fghijk}	2.1 ± 0.1^{ab}	3.7 ± 0.2^{a}	21.7 ± 3.2^{abcdefg}	2.2 ± 0.2^{abcd}	4.2 ± 0.7^{jklmn}	80.6 ± 6.6^{a}
T33	15.4 ± 2.8^{ab}	1.9 ± 0.4^{ab}	54.8 ± 5.1^{l}	59.1 ± 5.1^{k}	4.5 ± 0.2^{fghijkl}	13.5 ± 5.2^{ab}	77.9 ± 30.7^{k}	2.8 ± 0.1^{cdefghi}	3.7 ± 0.4^{fghijklm}	735.8 ± 166.0^{n}
T34	25.0 ± 5.8^{abcdefg}	3.6 ± 1.0^{abc}	44.2 ± 1.4^{bcdefghijkl}	48.4 ± 2.2^{cdefghijk}	3.5 ± 0.4^{cdefgh}	8.6 ± 1.3^{ab}	38.7 ± 4.3^{fghi}	2.6 ± 0.2^{abcdefghi}	4.7 ± 0.4^{n}	332.9 ± 60.7^{cdefghijk}
T35	20.7 ± 3.9^{abcdef}	2.3 ± 0.7^{ab}	48.0 ± 4.5^{efghijkl}	54.9 ± 5.3^{hijk}	2.4 ± 0.1^{abcd}	4.8 ± 1.1^{a}	32.5 ± 6.7^{defghi}	2.3 ± 0.0^{abcdefi}	4.1 ± 0.1^{ijklmn}	141.8 ± 0.1^{abcd}
T36	23.4 ± 7.9^{abcdef}	3.6 ± 0.9^{abc}	46.4 ± 3.4^{cdefghijkl}	50.5 ± 3.2^{defghijk}	3.8 ± 0.4^{defghi}	10.4 ± 0.0^{ab}	43.9 ± 1.0^{hi}	2.1 ± 0.0^{abcd}	4.3 ± 0.1^{jklmn}	458.1 ± 11.5^{ghijklm}
T37	19.4 ± 3.4^{abcde}	3.9 ± 0.7^{abc}	45.3 ± 2.7^{cdefghijkl}	50.2 ± 3.4^{defghijk}	2.8 ± 0.2^{abcde}	8.9 ± 1.1^{ab}	31.3 ± 3.2^{defghi}	4.1 ± 0.9^{l}	3.1 ± 0.0^{cdefg}	275.1 ± 5.6^{abcdefg}
T38	19.2 ± 1.8^{abcde}	2.8 ± 0.8^{abc}	51.5 ± 2.5^{jkl}	58.6 ± 3.7^{jk}	2.3 ± 0.0^{abc}	3.4 ± 0.3^{a}	40.0 ± 5.9^{ghi}	3.0 ± 0.4^{fghij}	4.4 ± 0.6^{lmn}	133.8 ± 10.6^{abc}
T39	28.8 ± 5.2^{bcdefg}	7.8 ± 2.1^{cdefgh}	36.5 ± 2.6^{abcdef}	43.1 ± 4.2^{abcdefg}	4.0 ± 0.3^{efghij}	86.6 ± 12.3^{ef}	1.5 ± 0.2^{a}	2.6 ± 0.2^{abcdefghi}	2.9 ± 0.3^{cdef}	133.1 ± 26.0^{abc}
T40	47.1 ± 6.4^{h}	5.3 ± 0.2^{abcdef}	26.7 ± 1.9^{a}	34.9 ± 3.1^{a}	5.1 ± 0.4^{jklmno}	120.0 ± 50.9^{g}	1.9 ± 0.1^{a}	2.0 ± 0.0^{abc}	3.5 ± 0.0^{defghijk}	220.9 ± 79.4^{abcdef}

Means with the same alphabet in the same column are not significantly different by DMRT ($P \leq 0.01$)

PH plant height; Number of branches; *NDF* number of days to first flowering; *NDFFS* number of days to first fruit set; *NFC* number of fruits per cluster; *HFP* harvested fruits per plant; *AFW* average fruit weight; *NLF* number of locus per fruit; *PT* pericarp thickness; *FYP* fruit yield per plant

Table 5 Genotypic and phenotypic variances, genotypic and phenotypic coefficients of variation, heritability in broad sense, genetic advance and genetic advance as percent of mean of forty accession of tomato on ten quantitative traits

Traits	VG	VP	GCV (%)	PCV (%)	H^2B (%)	GA	GAM (%)
PH	37.18	66.74	24.94	33.11	55.71	5.62	22.99
NB	37.69	44.51	76.74	83.4	84.68	11.64	145.48
NDF	29.92	55.46	12.83	17.54	53.95	8.28	19.41
NDFFS	35.34	57.94	12.49	16.00	60.99	9.56	20.09
NFC	1.68	2.04	89.39	98.50	82.35	2.42	166.9
HFP	663.14	933.25	100.79	119.57	71.06	16.01	62.65
AFW	257.34	377.03	36.58	77.83	68.25	27.3	109.32
NLF	0.20	0.33	17.14	22.01	60.61	0.72	27.48
PT	0.76	0.95	26.66	29.81	80.00	1.61	49.12
FYP	20,903.3	30,904.6	44.77	54.44	67.64	244.95	75.85

PH plant height; number of branches; *NDF* number of days to first flowering; *NDFFS* number of days to first fruit set; *NFC* number of fruits per cluster; *HFP* harvested fruits per plant; *AFW* average fruit weight; *NLF* number of locus per fruit; *PT* pericarp thickness; *FYP* fruit yield per plant; *VG* genotypic variance; *VP* phenotypic variance; *GCV* genotypic coefficient of variation; *PCV* phenotypic coefficient of variation; H^2B heritability; *GA* genetic advance; *GAM* genetic advance as percent of means

yield per plant (r = − 0.41** and − 0.34**) at both the genotypic and phenotypic levels. Number of fruits per cluster was highly significant and positively correlated with fruit yield per plant (r = 0.44** and 0.42**) at both genotypic and phenotypic levels. Number of locus per fruit only showed significant correlation with fruit yield per plant (r = 0.36* and 0.32*) at both genotypic and phenotypic levels (Table 6).

The 40 accessions were divided into four major clusters. Cluster I consisted of five sub-clusters; A with one accession (T1), B with three accessions (T15, T13 and T6), C with five accessions (T23, T26, T34, T25 and T21), D with one accession (T19), and E with five accessions (T37, T29, T10, T16 and T4). Cluster II consisted of six sub-clusters; A with two accessions (T40 and T8), B, C and D with one accession each, T39, T32 and T14, respectively. Sub-cluster E had seven accessions (T38, T35, T31, T27, T11, T22 and T9) and F had three accessions (T28, T30 and T3). Cluster III consisted of four sub-clusters; A with three accessions (T20, T12 and T5), B with two accessions (T24 and T18), C with one accession (T36) and D with three accessions (T7, T17 and T2). Cluster IV consisted of only one accession (T33). Accessions in cluster I were moderate fruit yielders. Accessions in cluster II were low fruit yielders, accessions in cluster III were high fruit yielders while cluster IV had the accession with the highest yield. Sub-clusters IIB–IIF were majorly characterised by low number of main branches, similar number of days to first flowering and similar number of days to first fruit set (45.67 days in T3 to 58.70 days in T27). Sub-clusters IIA and IIB were characterised majorly by lowest average fruit weight (Fig. 1).

Ten Principal Components were extracted for quantitative traits out of which the first three with eigen-values above 1.00 accounted for 80.76% of the total variation. The first PC was positively loaded with plant height (0.712), number of branches (0.786) and number of fruits per cluster (0.845). The second axis was positively loaded with average fruit weight (0.511), number of locus per fruit (0.461) and fruit yield per plant (0.788) (Table 7). The accessions on the right hand side of the bi-plot are stable and are high yielding. Also, the accessions closer to the bi-plot origin are more preferred. The traits on the right hand side of the bi-plot are the yield component traits: fruit yield per plant, plant height, number of fruits per cluster, number of branches and harvested fruits per plant (Fig. 2a). The polygon view of the bi-plot showing the vertex accessions is presented in Fig. 2b.

Among accessions, 5 markers out of 13 (representing 38% of the markers) did not show any polymorphism, and they included EST-SSR1, EST-SSR2, EST-SSR26, EST-SSR42 and EST-SSR83. Generally, markers did not display high level of polymorphism. The highest number of alleles was 2 and this was obtained in all the polymorphic markers. Among the polymorphic markers, the least gene diversity (0.05) was obtained in EST-SSR77 and EST-SSR23, while the highest (0.50) was obtained in EST-SSR25. The highest Polymorphic Information Content (PIC), 0.37, among polymorphic markers was obtained in EST-SSR19 and EST-SSR25, while the lowest (0.05) was obtained in EST-SSR23 and EST-SSR77 (Table 8).

The 40 accessions were divided into six major clusters. Cluster I consisted of four accessions (T28, T26, T40 and T39). Cluster II consisted of two sub-clusters A, with nine accessions (T38, T37, T36, T35, T33, T32, T31, T30 and T6) and B with three accessions (T2, T7 and T4). Cluster III had three sub-clusters, A with four accessions (T16, T15, T14 and T13), B with one accession (T17) and C with

Table 6 Genotypic (up) and phenotypic (down) correlations on ten quantitative traits of forty accessions of tomato

TRT	PH	NB	NDF	NDFFS	NFC	HFP	AFW	NLF	TP	FYP
PH		0.355*	− 0.885**	− 0.859**	0.626**	0.626**	− 0.489**	0.042	− 0.129	0.384**
		0.349*	− 0.785**	− 0.785**	0.563**	0.487**	− 0.399**	0.054	− 0.069	0.286
NB			− 0.659**	− 0.705**	0.737**	0.565**	− 0.631**	− 0.069	− 0.668**	0.348**
			− 0.589**	− 0.647**	0.703**	0.509**	− 0.567**	− 0.056	− 0.614**	0.305**
NDF				0.994**	− 0.767**	− 0.803**	0.729**	− 0.056	0.514**	− 0.332*
				0.956**	− 0.658**	− 0.661**	0.609**	− 0.001	0.389**	− 0.274
DFFS					− 0.837**	− 0.752**	0.685**	0.003	0.552**	− 0.412**
					− 0.742**	− 0.629**	0.584**	− 0.043	0.437**	− 0.344*
NFC						0.604**	− 0.577**	− 0.141	− 0.641**	0.442**
						0.538**	− 0.516**	− 0.121	− 0.589**	0.417**
HFP							− 0.701**	− 0.289	− 0.620**	− 0.038
							− 0.627**	− 0.248	− 0.564**	0.005
AFW								0.410**	0.731**	0.237
								0.365*	0.663**	0.272
NLF									0.181	0.361*
									0.191	0.324*
PT										0.098
										0.096
FYP										

PH plant height (cm); number of branches; *NDF* number of days to first flowering; *NDFFS* number of days to first fruit set; *NFC* number of fruits per cluster; *HFP* harvested fruits per plant; *AFW* average fruit weight (g); *NLF* number of locus per fruit; *PT* pericarp thickness (mm); *FYP* fruit yield per plant (g)

two accessions (T29 and T27). Cluster IV had three sub-clusters; A with three accessions (T34, T21 and T18), B with two accessions (T20 and T19) and C with six accessions (T22, T12, T11, T10, T9 and T8). Cluster V had four accessions (T1, T3, T5 and T23), while cluster six consisted of two accessions (T24 and T25). All accessions in cluster I were low and moderate fruit yielders. Accessions in cluster II and III were low and moderate fruit yielders, except for two accessions in cluster IIB (T7 and T2) which were high fruit yielders. All accessions in cluster IVA and B were moderate and high fruit yielders, while all in IVC were low fruit yielders except T12. Two of the accessions (T1 and T23) in cluster V were moderate fruit yielders (Fig. 3). The correspondence between the morphological traits and EST-SSR markers as revealed by Mantel Test was low (r = 0.14) and correspondence analysis showed two distinct groups for both traits (Fig. 4).

Discussion

Diversity studies in tomato based on morphological traits has limited feedbacks due to influence of the environment. While qualitative morphological traits could be more reliable than quantitative, but problems of adequately scoring qualitative traits correctly may also affect the outcome of a diversity study. Therefore, marker assisted selection offers an efficient technique of choosing genotypes by limiting the influence of environment. High significant variations were observed among the forty accessions of tomato for all traits studied. Traits with highest levels of variability included number of harvested fruits per plant, average fruit weight, number of fruits per cluster, fruit yield per plant and number of main branches per plant. These higher levels of variations may be used as pointers to select materials in genetic enhancement programme. These are in agreement with the findings of Mazzucatoa et al. (2010), Pilar et al. (2015), Henareh et al. (2015). Accession T33 was outstanding in terms of fruit yield, and average fruit weight; accessions T1 and T40 had exceptional numbers of harvested fruits but with poor yield owing to the small sizes of their fruits. Accessions T21, T6, T25, T29, T30, T32, T34 and T38 with exceptional pericarp thickness had low to moderate yield among the evaluated accessions. Morphological traits have also been found useful in discriminating genotypes of tomato by Caramante et al. (2009), who utilized fifteen morphological traits on four tomato accessions, Hu et al. (2012) who used twenty six morphological traits on sixty seven varieties of tomato and Zhou et al. (2015) who utilized nine morphological traits of

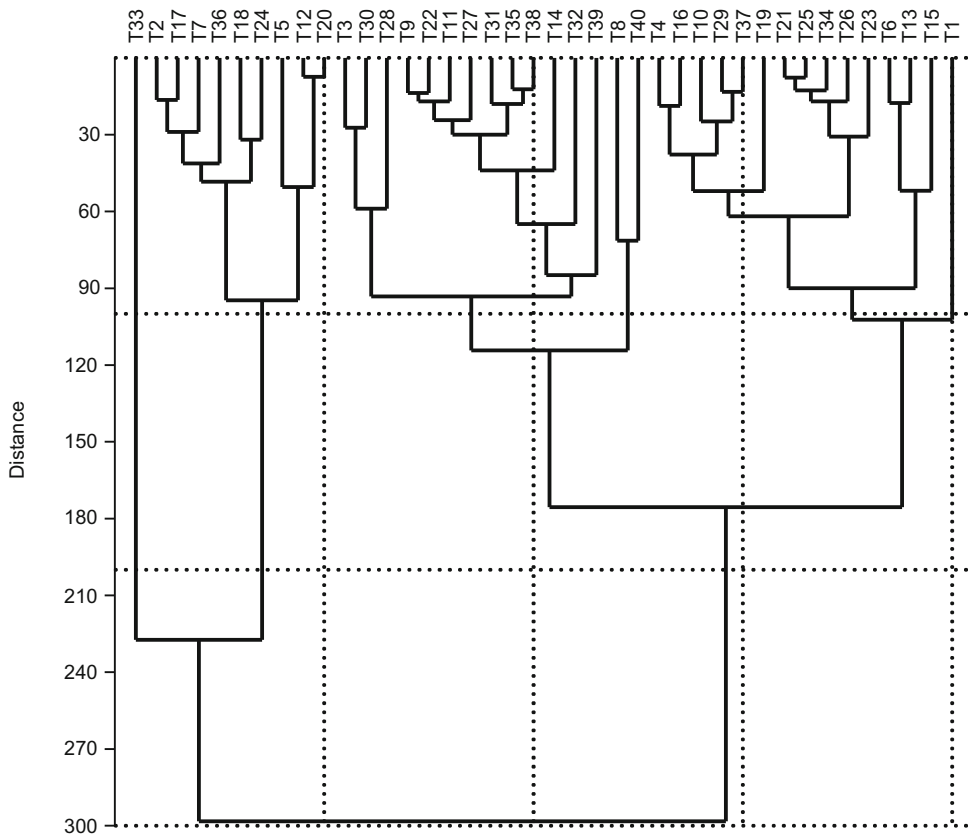

Fig. 1 Dendrogram of the 40 accessions of tomato based on quantitative traits. T1–T40 are codes for accessions of tomato evaluated

fifty accessions of tomato. High GCV and PCV were obtained in most traits except in number of days to first flowering and number of days to first fruit set, and only for GCV in number of locus. This is similar to the findings of Vijayan (2005) and Haydar et al. (2007). In contradiction to Khanom et al. (2008), most of the morphological traits displayed high differences between GCV and PCV except in number of days to first fruit set, number of locus per fruit and pericarp thickness, indicating that environmental influence was high on phenotypic expression of the accessions. Contrary to the findings of Haydar et al. (2007) and Mohamed et al. (2012), the highest GCV and PCV were obtained in number of harvested fruits per plant. Heritability was high for most traits and GAM was also high for all traits suggesting that selection based on these traits will be very effective in breeding programmes. This is in line with the findings of many workers on tomato diversity (Haydar et al. 2007; Khanom et al. 2008; Mohamed et al. 2012). High heritability accompanied by high GAM in traits such as number of main branches, number of days to first fruit set, number of fruits per cluster, number of harvested fruits, average fruit weight, number of locus per fruit, pericarp thickness and fruit yield indicate that additive gene component is at play for these traits. Direct selection for these traits will positively contribute to breeding objectives. These results are in line with the findings of Vijayan (2005) for number of main branches, number of locules per fruit, fruit weight and fruit yield per plant. Moderate heritability with high GAM in traits such as plant height and number of days to first flowering also indicate that a level of improvement could be achieved for these traits via selection.

Positive correlations were observed to be higher at genotypic level for all traits, while negative correlations were higher at the phenotypic level for all traits. Phenotypic correlation deals with the extent of association between two traits among entities of a population; and its components are genotypic and environmental correlations. Genotypic correlations on the other hand deals with the magnitude to which a gene or group of genes influence two attributes. Nevertheless, genotypic correlation is of greater importance in any breeding schemes (Ajayi et al. 2017). Plant height had positive correlations with number of branches at both genotypic and phenotypic levels; it had high positive correlations with number of fruits per cluster and number of harvested fruits per plant at genotypic and phenotypic levels, and with fruit yield only at the genotypic level. Number of branches was also highly positively correlated with number of fruits per cluster, number of harvested fruits per plant (at genotypic and phenotypic levels); and positively correlated with fruit yield at both levels. Number of fruits per cluster was observed to be highly

Table 7 Eigen vectors of three principal components on ten quantitative traits of forty accessions of tomato

Traits	Principal component 1	Principal component 2	Principal component 3
PH	**0.7116**	**0.3731**	**− 0.5083**
NB	**0.7862**	− 0.0099	**0.4318**
NDF	**− 0.8967**	− 0.2035	0.2685
NDFFS	**− 0.9201**	− 0.2498	0.1567
NFC	**0.8448**	0.1073	0.2718
HFP	**0.7828**	− 0.2400	− 0.1393
AFW	**− 0.7565**	**0.5111**	0.1172
NLF	− 0.1329	**0.6891**	0.1498
PT	**− 0.6515**	**0.4605**	**− 0.4659**
FYP	0.2609	**0.7875**	**0.3512**
Eigen values	5.1800	1.8800	1.0100
Prop of var. (%)	51.8400	18.8100	10.1100
Cum. Var. (%)	51.8400	70.6500	80.7600

Bold represent traits with high loadings or high contributions

PH plant height (cm); number of branches; *NDF* number of days to first flowering; *NDFFS* number of days to first fruit set; *NFC* number of fruits per cluster; *HFP* harvested fruits per plant; *AFW* average fruit weight (g); *NLF* number of locus per fruit; *PT* pericarp thickness (mm); *FYP* fruit yield per plant (g)

positively correlated at both genotypic and phenotypic levels with number of harvested fruits and fruit yield per plant. Number of locules was positively correlated with fruit yield per plant. These results are in agreement with the findings of Vijayan (2005) for number of locules per plant and number of branches per plant. No significant correlations were found between number of fruits with fruit yield, and average fruit weight with fruit yield contrary to the findings of Qumer et al. (2014) and Henareh et al. (2015). Therefore, selection based on plant height, number of branches, number of fruits per cluster and number of locules per plant will significantly improve fruit yield in tomato. Furthermore, negative genotypic correlation between number of days to flowering and fruit yield, and high negative genotypic correlation between number of day to first fruit set and fruit yield indicate that accessions which flowered early had higher fruit yield, therefore selection for number of days to flowering and number of days to first fruit set will contribute significantly to fruit yield. However, high significant positive correlations at genotypic and phenotypic levels between number of days to flowering and pericarp thickness, and number of days to first fruit set and pericarp thickness suggest that accessions which flowered late had thicker pericarps.

Detailed study of important traits for selection in tomato has been hindered for lack of genetic markers that can pinpoint differences effectively among tomato breeding lines. Deployment of SSR markers has enable to further garner more information on important traits which could help in selection of tomato (Milevska et al. 2011). Compared to morphological analyses, SSR procedure is faster and more appropriate for thorough-put study and highly traceable (Caramante et al. 2009). Genetic diversity study that avoids vague genotype discrimination relies heavily on the use of molecular markers to enhance both breeding objectives and efficient germplasm conservation of tomato and other crop species (Caramante et al. 2009). Analyses that combine morphological traits with molecular markers have been found to provide better information in genetic diversity assessments (Zhou et al. 2015). Nevertheless, morphological diversity is not always revealed at the molecular level (Hu et al. 2012). The worth of SSR markers and their relationships to morphology in tomato accessions have been documented by Caramante et al. (2009).

In the present study, eleven out of thirteen EST-SSR primers used on the forty accessions were able to generate PCR products. Five primers out of thirteen (representing 38 percent) primers used displayed no polymorphism. This led to the low genetic distances observed among many of the accessions involved in the study, higher number of markers displaying higher polymorphism would be more desirable perhaps for future work. Generally, polymorphic markers did not show high degree of polymorphism as opposed to the findings of Caramante et al. (2009) and Kwon et al. (2009). Degree of polymorphism has been previously classified into three groups; high if mean PIC is greater than 0.5; medium if mean PIC is greater than 0.25 but less than 0.5 and low if mean PIC is less than 0.25 (Xie et al. 2010). Hence, in this study, EST-SSR makers identified low locus polymorphism (mean PIC = 0.13) in the forty accessions of tomato contrary to the findings of Mazzucatoa et al. (2010), Zhou et al. (2015) on tomato and Herraiz et al. (2015) on pepino. Higher number of markers

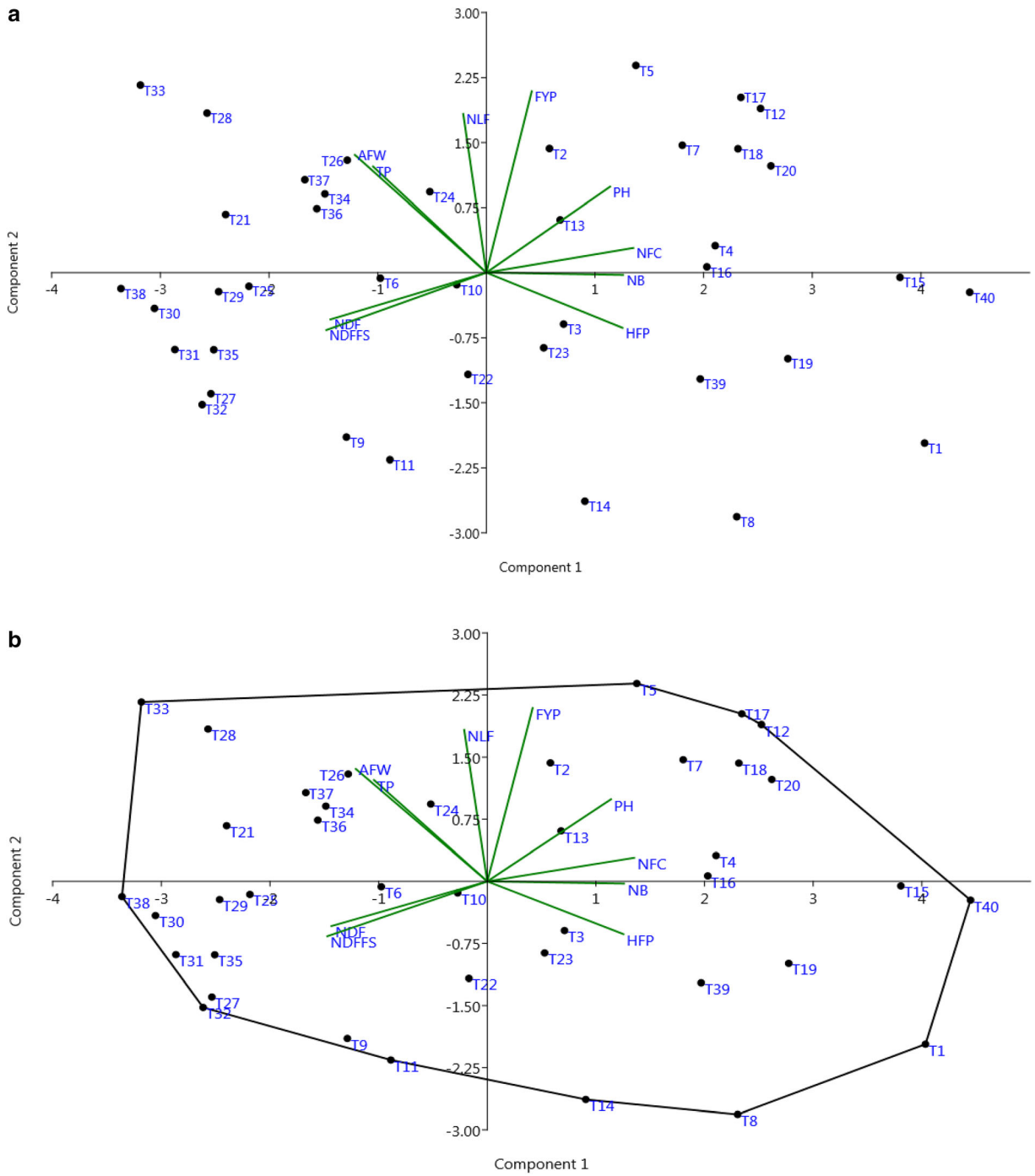

Fig. 2 **a** Bi plot based on the Principal Components axis 1 and 2 showing the interrelationships among different traits of 40 accessions of tomato. T1–T40 are codes for accessions of tomato evaluated. *PH* plant height (cm); number of branches; *NDF* number of days to first flowering; *NDFFS* number of days to first fruit set; *NFC* number of fruits per cluster; *HFP* harvested fruits per plant; *AFW* average fruit weight (g); *NLF* number of locus per fruit; *PT* pericarp thickness (mm); *FYP* fruit yield per plant (g). **b** Polygon view of the genotype x trait bi-plot of 40 accessions of tomato. T1–T40 are codes for accessions of tomato evaluated. *PH* plant height (cm); number of branches; *NDF* number of days to first flowering; *NDFFS* number of days to first fruit set; *NFC* number of fruits per cluster; *HFP* harvested fruits per plant; *AFW* average fruit weight (g); *NLF* number of locus per fruit; *PT* pericarp thickness (mm); *FYP* fruit yield per plant (g)

would be preferred. Many of these accessions might also have belonged to similar genetic background as the fact that they were collected from different locations does not rule out the fact that they might be related.

The components of clusters from morphological traits were similar to some from the ones established by EST-SSR markers in agreement to the findings of Caramante et al. (2009) and Herraiz et al. (2015) but disagrees with the

Table 8 Polymorpic information of thirteen EST-SSR markers utilised in characterisation of forty accessions of tomato

SSR marker	Major allele frequency	Allele number	Availability	Gene diversity	Polymorphic Information content (PIC)
EST-SSR1	1.00	1	1	0.00	0.00
EST-SSR2	1.00	1	1	0.00	0.00
EST-SSR7	0.70	2	1	0.42	0.33
EST-SSR19	0.58	2	1	0.49	0.37
EST-SSR23	0.98	2	1	0.05	0.05
EST-SSR25	0.53	2	1	0.50	0.37
EST-SSR26	1.00	1	1	0.00	0.00
EST-SSR35	0.83	2	1	0.29	0.25
EST-SSR42	1.00	1	1	0.00	0.00
EST-SSR67	0.90	2	1	0.18	0.16
EST-SSR71	0.95	2	1	0.10	0.09
EST-SSR77	0.98	2	1	0.05	0.05
EST-SSR83	1.00	1	1	0.00	0.00
Mean	0.88	1.62	1	0.16	0.13

Samle size = 40; number of observation = 40

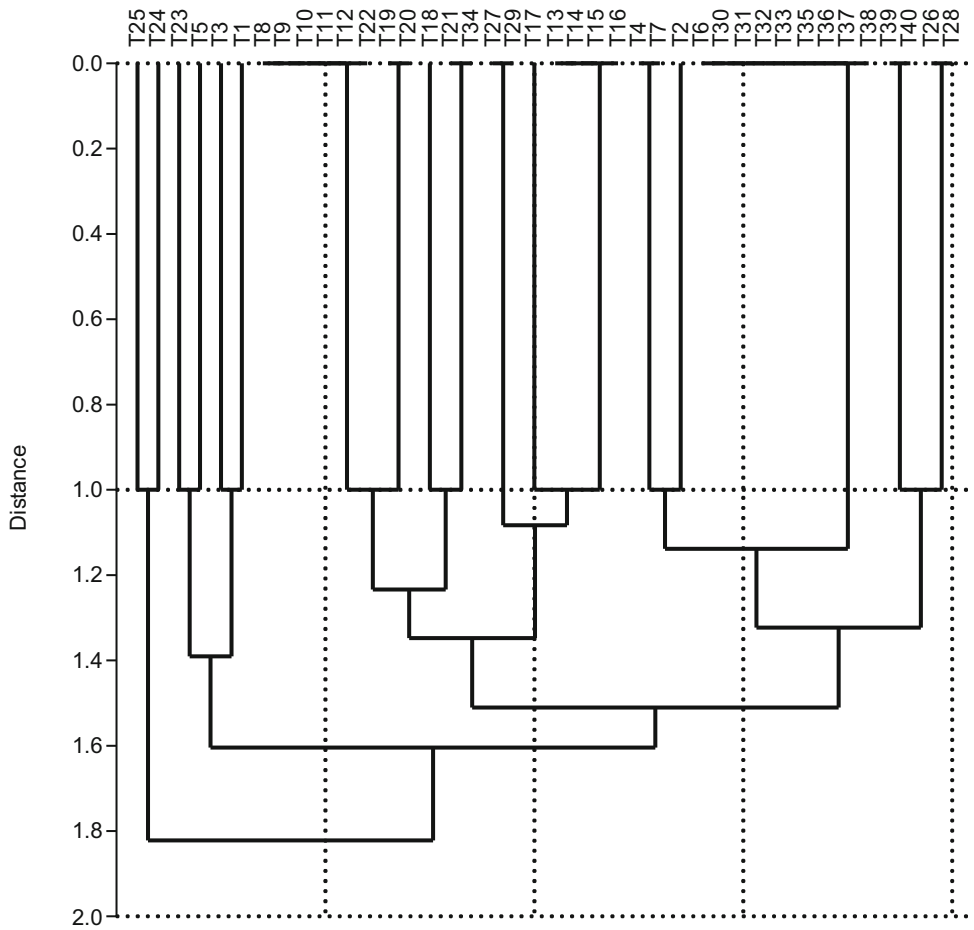

Fig. 3 Dendrogram of the 40 accessions of tomato based on 13 EST-SSR molecular markers. T1–T40 are codes for accessions of tomato evaluated

findings of Mazzucatoa et al. (2010), Hu et al. (2012) and Zhou et al. (2015). Some of the accessions that fell into similar clusters in both dendrograms include the following: Accessions T40, T39 and T28 all of which were low fruit yielders shared similar clusters in both. Accessions T38, T35, T31, T30 and T32 (low yielders), some moderate

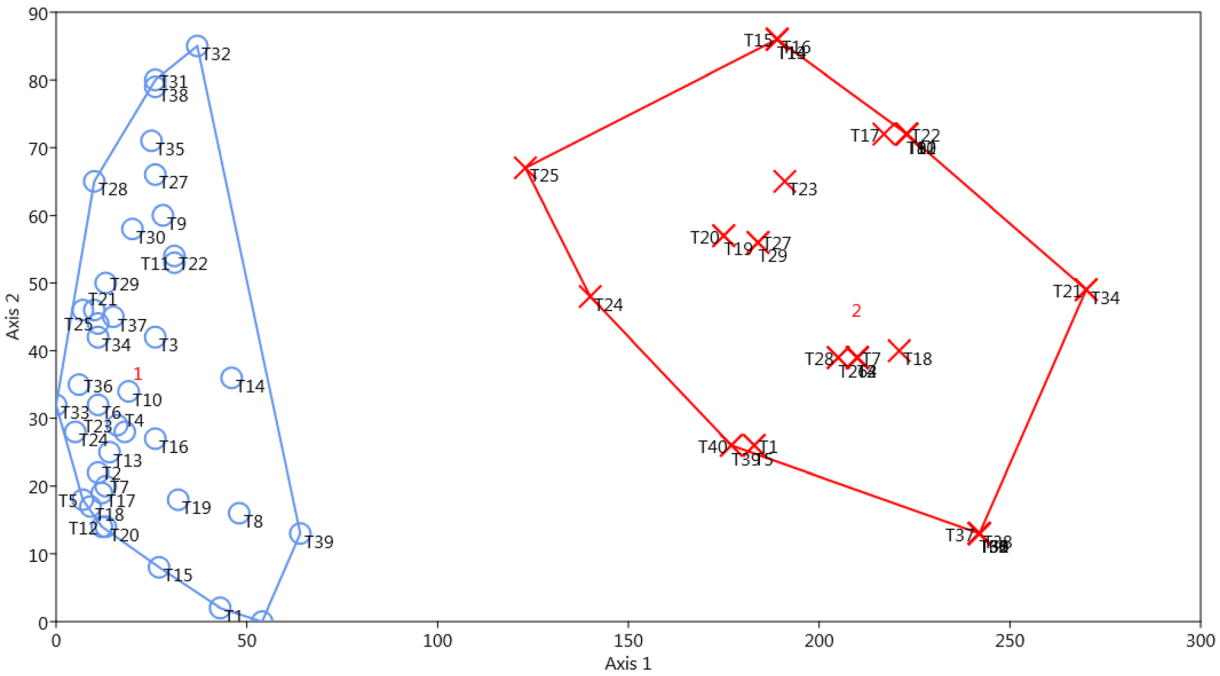

Fig. 4 Relationship between morphological (circle) and EST-SSR markers (X) for 40 accessions of tomato using correspondence analysis. Relationship based on morphological traits (group 1; left hand) and relationship based on EST-SSR markers (group 2; right hand side). T1–T40 are codes for accessions of tomato evaluated

yielders (T4, T37 and T6) and high yielders (T7 and T2) were also found in similar clusters of both dendrograms. Accessions T16, T15, T13 and T29 (moderate yielders) were domiciled in same clusters of both dendrograms. Accessions T34, T21 and T19 moderate yielders; accessions T12, T18 and T20 (high yielders); and accessions T11, T22, T8 and T9 (low yielders) all share similar sub clusters in both dendrograms. Accessions T1 and T23 also shared similar clusters in both dendrograms. Similarity of clusters shared may suggest that some of the primers could be linked to quantitative traits that characterised these clusters. High similarities have been reported between morphological traits and EST-SSR markers (Kwon et al. 2009; Herraiz et al. 2015). Hu et al. (2012) did not find any significant association between molecular makers and quantitative traits. Also, results showed that SSR markers were able to discriminate among those which had similar quantitative traits on one hand and also on the other hand they did not despite the fact that genetic diversity of tomato is limited (Caramante et al. 2009).

The Principal Component Analysis (PCA) is very efficient at examining the relative input of specific trait in tomato breeding programme (Vijayan 2005). PCA identifies a minor set of variables that explain a large percentage of the entire difference in the original data (Ajayi et al. 2017). In the present study, the first PC accounted for 51.84 percent of the total variation observed by the ten traits involved in this study. The second and third PCs accounted for 18.81 percent and 10.11 percent, respectively. Positive contributors in PC1 included plant height, number of main branches, number of fruits per cluster, number of harvested fruits per plant and fruit yield per plant, while others were negative contributors. The positive contributors in PC2 included all traits except number of main branches, number of days to first flowering, number of days to first fruit set and number of harvested fruits per plant. All traits were also positive contributors in PC3 except plant height, harvested fruits per plant and pericarp thickness. Except for number of locules per fruit and fruit yield per plant, all traits examined had high contribution to PC1 with the highest positive contribution coming from number of fruits per cluster and highest negative contribution from number of days to first fruit set. Therefore, these traits are very important in the yield improvement of tomato. Plant height and plant spread have been confirmed to be important in tomato improvement programmes (Vijayan 2005). The high loadings for number of fruits per cluster and number of harvested fruits per plant will contribute positively to increase yield, also high negative loadings coming from number of days to first flowering and number of days to first fruit set will also make positive contribution to fruit yield, as this indicate that accessions which flowered early produced fruits early and fruited for a longer time compared to late fruiters. The highest contributors to PC2 included fruit yield per plant, number of locules per fruit, average fruit weight and pericarp thickness. Highest

contributors in PC3 included plant height, number of main branches per plant and pericarp thickness. Therefore, PCs 1 and 2 can be called the vegetative, phenology and reproductive axes. PC3 however can be termed the vegetative axis. These findings agree with the results of Qumer et al. (2014), Zhou et al. (2015).

The bi-plots of PCs 1 and 2 captured 70.65% of the total variation with the genotypes and genotype by trait interactions. For bi-plot analyses, angle formed between two traits is depicted to be the correlation between those traits. If the angle between two traits is less than 90° (i.e. acute), the traits are positively correlated. Conversely, two traits apart at more than angle 90° (i.e. obtuse), are negatively correlated. However, if the angle between two vectors is exactly at 90°, such traits do not have any correlations (Atnaf et al. 2017). In the present study, fruit yield per plant had positive correlation with plant height in agreement with Qumer et al. (2014) and a weak positive correlation with number of fruits per cluster and number of main branches per plant. Plant height was highly positively correlated with number of clusters per plant and number of main branches, but weakly correlated with number of harvested fruits per plant. Pericarp thickness and average fruit weight were highly positively correlated. Number of days to first flowering and days to first fruit set were highly positively correlated. Improvement in any of these traits will positively contribute to the improvement of others. For instance, selection based on plant height, number of fruits per cluster and number of main branches will significantly contribute to fruit yield. However, negative correlations between yield and phenology characters (number of days to first flowering and number of days to first fruit set) suggest that selection based on earliness to flower and setting of fruits will contribute significantly to improved fruit yield of tomato. Negative correlations between yield and number of harvested fruits per plant suggest that selection based on number of harvested fruits will not add any value to yield of tomato, perhaps sizes of tomato matter when considering yield. In this study, many of the accessions with high numbers of harvested fruits had very small fruits. Results of the genotypic and phenotypic correlations also showed similar relationships.

The extent of the trait trajectory projected from the source displays the discriminating capacity of a trait among genotypes. Traits with longer trajectories possess high discriminating potential whereas, traits with short trajectories are weak at differentiating genotypes (Atnaf et al. 2017). In the present study, fruit yield, average fruit weight, number of days to first flowering and number of days to first fruit set had longer projections, and therefore higher discrimination capacities. However, plant height, number of branches per plant, number of harvested fruits, pericarp thickness and number of fruits per locus had shorter projected trajectories, hence indicating their inability to discriminate effectively among genotypes. The bi-plot pinpointed the best accessions for specific traits. In line with this, accessions T5, T17 and T12 were vertex accessions in the fruit yield, plant height, number of fruits per cluster and number of main branches sector, hence best for these traits. They can be selected for breeding objectives. Accessions T40, T1, T8 and T14 were vertex accessions in the number of harvested fruits per plant sector, hence possessed the higher number of fruits harvested. They may however not be good for breeding objectives for their small sizes of fruits. Accessions T11, T32 and T38 were vertex accessions in the number of days to first flowering and number of days to fruit set sector, and hence were late maturing accessions. They are poor in yield and can however be improved by crossing them with the accessions T5, T17 and T12 in the first sector. The vertex accession in the fruit weight and pericarp thickness sector is T33, hence was the best for fruit weight. Vertex accessions display the greater value for the trait or traits falling within the same sector in the bi-plot (Yan et al., 2007). Other authors who have utilized bi-plots analysis in tomato variability studies include Pilar et al. (2015) and Bhattarai et al. (2016).

Conclusion

Marker assisted selection is a veritable tool in the improvement of crop varieties because the discriminatory power of morphological markers diminishes with increased number of genotypes, as in the present study. The result from this study clearly revealed that accessions T5, T17 and T12 were better in fruit yield, plant height, number of main branches as well as number of fruits per cluster; and these traits were positively correlated with positive interactions. These are therefore selected for the next phase of the breeding programme for the improvement of yield in tomato. The information on the level of correspondence between morphological and EST-SSR data may be useful in future breeding work of tomato.

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Authors' contributions All authors contributed to the study conception and design. Collection of field data were performed by Oluwatoyin Sunday Osekita and Abiola Toyin Ajayi. DNA extraction and molecular characterisation were performed by Idowu Olaposi Omotuyi. Data analysis was handled by Abiola Toyin Ajayi. Alaba Emmanuel Gbadamosi supervised the work. The first draft of the manuscript was written by Abiola Toyin Ajayi and all authors made

their comments on it. Alaba Emmanuel Gbadamosi edited the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest Alaba Emmanuel Gbadamosi declares that he has no conflict of interest. Abiola Toyin Ajayi declares that he has no conflict of interest. Oluwatoyin Sunday Osekita declares that he has no conflict of interest. Olaposi Idowu Omotuyi declares that he has no conflict of interest.

Ethics approval This article does not contain studies with human participants or animals performed by any of the authors.

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