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Full Length Research Paper

Diversity between and within farmers' varieties of tomato from Eritrea

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Tomato yields in Eritrea are low (15 Mg/ha) compared with 19 Mg/ha in Africa and 27 Mg/ha worldwide. This is partly caused by poor quality of varieties used. This study analysed the diversity among and heterogeneity within farmers' varieties of tomato from Eritrea and compared these varieties with other African and Italian varieties. Fifteen simple sequence repeat (SSR) markers were used for the genetic analysis. Genetic similarities among the varieties were calculated and an Unweighted Pair Group Method with Arithmetic Mean analysis was performed. Furthermore, individual plants of varieties were genotyped to evaluate uniformity within varieties. A high degree of diversity was observed among the Eritrean varieties. Thirteen out of the 15 SSRs were polymorphic, with 2 to 5 alleles per marker. The dendrogram showed two major types of varieties: San-Marzano and Marglob. Eritrean varieties were closely related to old Italian varieties in both types. Analysis of the within-variety variation showed that the Eritrean tomato genotypes were less uniform than the other varieties, probably because of deliberate mixing. A survey among farmers showed that some of them purposely mixed seeds to prolong the harvesting period, for yield stability and stress tolerance. Farmers value 'new material' as a source of influx.

Key words: Farmers' varieties, genetic diversity, genetic purity, rapid rural appraisal, *Solanum lycopersicum*, seed mixing, seed systems, simple sequence repeat.

INTRODUCTION

Eritrea is a country in the northeast of Africa bordering the Red Sea (coordinates: 12 to 18°N; 37 to 43°E). Its neighbouring countries are Sudan (in the west), Ethiopia (in the south) and Djibouti (in the southeast). Cultivated tomato, *Solanum lycopersicum,* is commercially one of the most important vegetables in Eritrea. Together with onion, potato and pepper, tomato is included in the top four priority vegetable crops in the country. The production is more equally distributed over all administrative zones than that of other vegetable crops grown in Eritrea.

Tomato production has a long tradition in Eritrea and dates back to the Italian colonial period. The average yield of tomato in Eritrea is about 15 Mg/ha (Ministry of Agriculture, 2000), whereas average yields are 19.1 Mg/ha in Africa, 23 Mg/ha in Asia and 27.2 Mg/ha in the entire world (Jones, 1999). Post-harvest losses of tomato, mainly caused by physiological deterioration and bruising, can amount to more than 30% of the production and in general the quality of tomatoes has never been given due attention (Ministry of Agriculture, 2000).

In addition to other production constraints, lack of an adequate seed supply system has contributed to the low productivity and quality of tomatoes in Eritrea. Most farmers maintain their own varieties and multiply their own seeds. In general, farmers classify tomato varieties into two major groups: Marglobe (round fruits) and San-

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Abbreviations: SSR, Simple sequence repeat; **SNP,** single nucleotide polymorphism; **QTLs,** qualitative trait loci; **EST,** expressed sequence tag; **PCR,** polymerase chain reaction; **PRA,** participatory rural appraisal; **CGN,** Centre for Genetic Resources of The Netherlands.

Marzano (angular fruits). The knowledge of the tomato genome has increased significantly in the last two decades (Vosman et al., 1992; Rus-Kortekaas et al., 1994; Arens et al., 1995; Smulders et al., 1997; Bredemeijer et al., 2002; Arens et al., 2010; see also the relevant websites of the International Solanaceae Genome Initiative), and molecular tools that facilitate genetic studies have been developed (Sifres et al., 2007). There are several approaches to DNA profiling (Cooke, 1999). At the moment, simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers are widely used in plant breeding and genomic research and are the bases for mapping of genes and qualitative trait loci (QTLs), marker assisted breeding, phylogenetic studies and comparative genomics (Hayden et al., 2008). SNPs are the most abundant types of DNA sequence polymorphisms and their higher availability and stability compared to SSR provide enhanced possibilities for genetic and breeding applications (Lijavetzky et al., 2007) with increased marker data quality and quantity (Jones et al., 2007). They are also suitable for automatic high-throughput analysis (Suliman et al., 2002). In recent years, because of the availability of large expressed sequence tag (EST) datasets for a number of plant species and the development of several bioinformatics tools, it has been possible to identify and develop SSRs markers from ESTs commonly known as EST-SSRs (Thiel et al., 2003; Tang et al., 2008). The development of such markers in con-trast to the earlier genomic SSRs, is easier, faster and cheaper (Varshney et al., 2005).

SSRs are short (mostly 2 to 4 bp) tandem repeats of DNA sequences and are preferred molecular markers because of their properties of genetic co-dominance, high reproducibility and multi-allelic variation (He et al., 2003). SSRs are polymerase chain reaction (PCR) based markers that have been developed in many plant species including tomato. However, especially in crops like tomato where genetic diversity is limited (Miller and Tanksley, 1990), the molecular marker of choice must be highly informative. For tomato, several primer sets for SSRs analysis are available (Vosman et al., 2001). The utility of the technique in tomato has been shown by Smulders et al. (1997), Bredemeijer et al. (1998; 2002) and Areshchenkova and Ganal (1999). Jones et al. (1997) indicated the faithful reproducibility of SSRs being tested by a network of European laboratories. For an analysis of the diversity of farmers' tomato varieties from Eritrea we therefore used SSRs.

As Eritrean farmers maintain their own varieties without classifying or naming them, little is known about what types of farmers' varieties are grown, how uniform the varieties are and how large the diversity between varieties is. This study therefore aimed at evaluating genetic diversity within and between tomato varieties collected from different parts of Eritrea, to compare this to materials from other African and Italian sources and to relate the genetic analysis with information on the traditional seed management system obtained through a rural appraisal. The information from this study is useful to identify ways to improve the genetic material commonly grown in Eritrea.

MATERIALS AND METHODS

Plant material

For the genetic analysis, 25 farmers' varieties of tomato which were assumed to be maintained by self-pollination for several years were collected from farmers, who select and maintain tomato seeds, from the Northern Red Sea, Anseba and Debub regions of Eritrea (Figure 1). Additionally, two South African, two Zairian and twelve old Italian varieties, obtained from the Centre for Genetic Resources of The Netherlands (CGN) were included in the analysis for comparison. Tomato varieties Isola, Aranka, Nunhems 6328 and VNT cherry were used as genotyping references (Bredemeijer et al., 2002). For the heterogeneity test within a variety, twelve individual plants of six Eritrean and two Italian varieties were tested by selecting the most discriminative micro satellites based on the diversity test.

DNA isolation

Total DNA was extracted from one week old seedlings; six seedlings were bulked to extract DNA from each variety for the diversity test. Twelve seedlings of eight polymorphic varieties were treated individually for the uniformity test. The DNA extraction was performed according to Fulton et al. (1995). In brief, after buffered grinding, material was incubated in a 65°C water bath. Chloroform/isoamyl (24:1) was added, followed by centrifugation at 10,000 rpm for 5 min. The aqueous phase was collected and isopropanol was added to precipitate the DNA, immediately followed by a second centrifugation at 10,000 rpm for 5 min after which isopropanol was poured off, the pellet was washed with 70% ethanol and dried. DNA could then be re-suspended at 65°C followed by a third centrifugation at 10,000 rpm for 10 min after which the material was stored until analysis.

PCR conditions

Fifteen tomato micro satellite loci: TMS9, LE20592, LEE6, LEMDDNa, LED4, LED10, LE21085, LELE25, LEaat002, LED112A, LESATTAGA, TMS33, TMS22, JACKP1, and LEcag003, were selected based on information from Bredemeijer et al. (2002) and He et al. (2003). PCR amplifications were carried out on an MJ Research PTC-200 DNA Engine Thermal Cycler (Scientific Support, Hayward, CA, USA) using the conditions described by Esselink et al. (2003), including an annealing temperature of 50°C for all primers. Forward primers were fluorescently labelled with 6FAM, Hex or Ned. Reverse primers were PIG-tailed (Brownstein et al., 1996) to increase the scorability of the profiles (Bredemeijer et al., 1998). Fluorescent amplification products were detected using an ABI Prism 3700 DNA Analyzer (Perkin Elmer Biosystems, Massachusetts, USA) and all samples were genotyped in accordance with reference alleles for each locus as described in Vosman et al. (2001), using Genotyper Software (version 3.5 NT, Perkin Elmer Biosystems, Massachusetts, USA).

Diagnostic survey and participatory rural appraisal (PRA)

Two surveys were carried out. Prior to the genetic analysis, a general

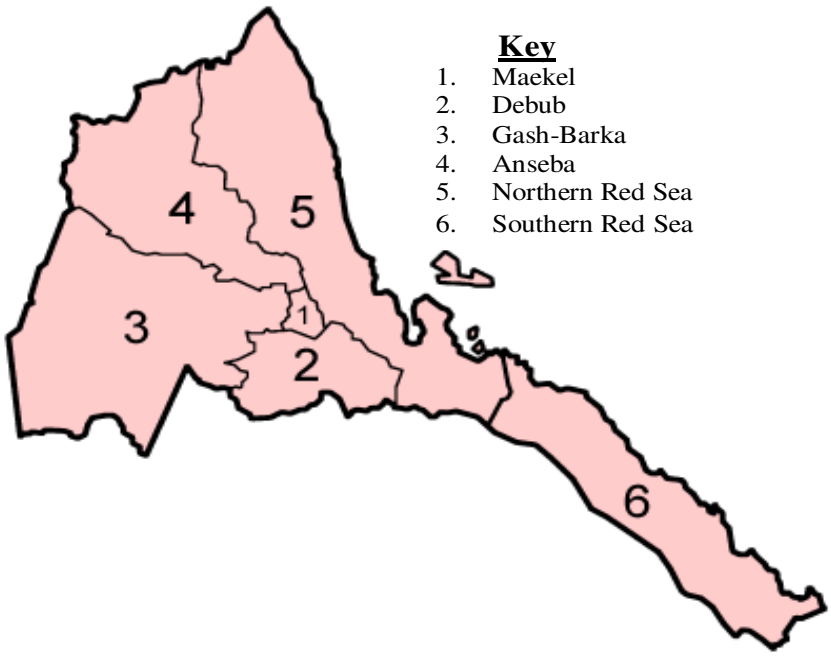

Figure 1. Map of Eritrea with different agro-ecological regions. Farmers' tomato varieties analysed were collected from regions 2, 4 and 5.

diagnostic survey was carried out on potentials and constraints of tomato production in Eritrea. The traditional seed management system was addressed as a part of the survey. Another PRA was carried out following the genetic analysis to investigate and trace back the reasons for the observed diversity among and heterogeneity within varieties. The PRA included 24 farmers, who had supplied seeds. The appraisal aimed at obtaining insight into drivers for possible sources of contamination of the varieties in the informal seed system of tomato in Eritrea encountered during the genetic analysis.

Analysis of genetic data

The presence/absence (1/0) of each peak was scored and stored in a database. A peak was considered as allele when the relative peak area was larger than 15% of the total peak area (Bredemeijer et al., 2002). A distance matrix was calculated using the Jaccard similarity index (Tan et al., 2005). A dendrogram showing the genetic relatedness among the varieties was constructed using the unweighted pair group method with arithmetic mean (UPGMA) module of NTSYS-pc version 2.1 software package (Biostatistics Inc., USA, Rohlf, 2000). Average numbers of alleles per variety were calculated. For the uniformity test, individual plants were genotyped and the allele frequencies calculated. Percentage of non-uniformity was calculated based on the relative presence of specific alleles for each variety according to Cooke et al. (2003).

RESULTS AND DISCUSSION

Diagnostic survey on traditional seed management system

The survey indicated that many farmers produce their own tomato seeds because they feel that F_1 hybrid seeds are expensive, unreliable in terms of adaptability and unreliable in terms of resistance to local abiotic and biotic stresses. Thus, many farmers prefer to keep their own material individually by selecting stable and disease resistant varieties based on field performance. This is also common in other crops and other parts of Africa (Zannou et al., 2004; Kudadjie et al., 2007; Richards et al., 2009; Offei et al., 2010). However, in the tomato production of Eritrea, the selected seeds are not shared or sold. Traditional maintenance of varieties and multiplication of selected seeds by individual farmers over the years on the one hand and the fact that they are not shared or sold on the other hand could result in diversity of present day farmers' tomato varieties in Eritrea. This diversity could be larger than in many other parts of the world where new high-yielding hybrids are planted and farmers exchange varieties freely. Most Eritrean farmers using the traditional seed system are not benefiting from the emerging high-yielding varieties, yet farmers seem comfortable with their local varieties. In all cases, it is important that farmers attain an improved and sustainable yield. The varieties used by farmers had different names and characteristics and there was no information whether they were true-to-type, contaminated and/or purposely mixed.

The diagnostic survey showed that the seeds were selected mostly from the 1st or 2nd harvest of tomato, locally called *'Bokuri'* which are believed to be vigorous and of good quality. In most cases, large and good-looking ripe fruits were selected and seed extraction was

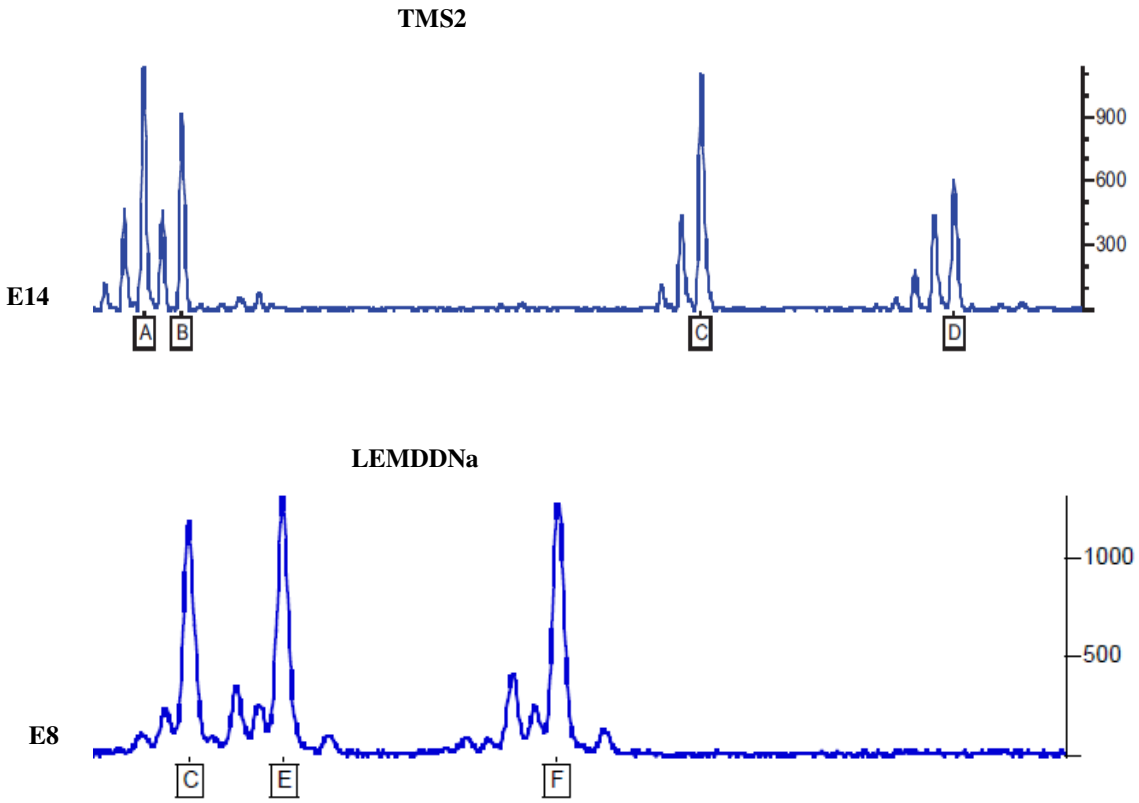

Figure 2. Allele pattern generated for the Eritrean farmers' varieties E14 and E8 for TMS22 and LEMDDNa loci, respectively.

done by slicing the fruit, washing the seeds on a sieve, mixing the seeds with ash and finally seeds were sun-dried. Mixing with ash is believed to protect the seeds against pests and pathogens, whereas the drying of the seeds improves their storability. The seeds were then kept in a dry place (Asgedom et al., 2011). Seed selection and storage is solely a woman's task, pointing at strong gender aspects related to the functioning of the seed system (Asgedom et al., 2011).

Genetic diversity analysis

One of the most interesting but challenging results from the genetic diversity analysis was that most of the varieties obtained from Eritrea were found to be heterogeneous. Often more than one allele was found per variety for any of the 13 polymorphic SSR loci with 2 to 5 alleles (Figure 2), which is unusual for true–to–type varieties. The SSR loci showed a higher degree of heterogeneity in the Eritrean varieties than with the old Italian and other African varieties (Table 1). Only two of the 15 loci (LED 10 and LEcag003) analysed were homozygous in all varieties tested except for the control Nunhem6328.

Average numbers of alleles per micro satellite locus for the varieties tested are presented in Table 1. The average number of alleles for Eritrean varieties was 1.3, for the varieties from the CGN it was 1.0. Eritrean varieties E7 and E23 scored the highest average number of alleles: 1.7; most of the non-Eritrean varieties showed the lowest, close to 1.0. A t-test showed a significant difference between Eritrean and Italian varieties in the number of alleles ($P < 0.001$). These results clearly confirmed that there was a high heterogeneity among the Eritrean varieties compared with the samples from the CGN suggesting genetic contamination among the Eritrean genotypes.

Relationship between varieties

The dendrogram (Figure 3) showed three major clusterings of varieties, group 1 consisting of E1 up to and including IT9, group 2 consisting of E2 up to and including E7 and group 3 contains the remaining varieties. Group 1 includes Eritrean San-Marzano type varieties collected from the Debub region (where there is long tradition of tomato cultivation), except E22 which is from the Northern Red Sea region. Five Italian varieties: IT6, IT8, IT9, IT10 and IT11 were found in this cluster suggesting a genetic relationship between Eritrean and the old Italian varieties. Nevertheless, none of the Eri-

Table 1. Description of tomato varieties and the average number of alleles/micro satellite loci based on 15 SSR markers. E1- E25 Eritrean, IT1-IT12 Italian, Z1–Z2: Zairian and S1-S2: South African tomato varieties.

Code	Name of farmer	Source (Place)	Status	Type	Region	Average no. of alleles
Varieties from Eritrea						
E1	Sereke Araya	Temagila	F	S	Debub	1.1
E2	Haile Hadish	Adilogo	F	S	Debub	1.5
E3	Tekla Asfaha	Kakibda	F	S	Debub	1.2
E4	Sibhat Teklu	Kakibda	F	S	Debub	1.3
E5	Amanuel Berhe	Temagila	F	S	Debub	1.1
E6	Habtom Gebregergis	Kakibda	F	S	Debub	1.2
E7	Habtom Libsikal	Temagila	F	S	Debub	1.7
E8	Gebrehiwot Simun	Kakibda	F	S	Debub	1.3
E9	Birhane Gilay	Temagila	F	S	Debub	1.2
E10	Yosief Amehasion	Adifenin	F	S	Debub	1.5
E11	Mehari T/tsion	Adifenin	F	S	Debub	1.6
E12	Askalu G/meskel	Gindae	F	M	Northern Red Sea	1.2
E13	Abdu Hiyabu	Gindae	F	S	Northern Red Sea	1.5
E14	Abrham Sielu	Adiquala	F	M	Debub	1.5
E15	Tekla Tesema	Adiquala	F	S	Debub	1.4
E16	Tsegay Gebreab	Adiquala	F	S	Debub	1.1
E17	Sheab Elaberid	Elaberid	F	M	Anseba	1.3
E18	Dawit Wolday	Debresina	F	M	Anseba	1.3
E19	Dawit Wolday	Debresina	F	M	Anseba	1.3
E20	Estifanos Berhe	Debresina	F	M	Anseba	1.5
E21	Keshi Tesfalem Abrehe	Adi tekelezan	F	S	Anseba	1.5
E22	Tekle Fisihatsion	Adi tekelezan	F	S	Anseba	1.1
E23	Merhawi Keshi abreha	Adi tekelezan	F	M	Anseba	1.7
E24	Abdela Jaber	Hamelamalo	F	S	Anseba	1.3
E25	Fishatsion Abraha	Adimengoti	F	S	Debub	1.1
Average						**1.3**
Varieties obtained from CGN						
IT1	Indefitable	Italy	-	R	16737	1.1
IT2	Cuor-di-Bue	Italy	-	SF	19113	1.0
IT3	Pirro	Italy	-	SF	17127	1.0
IT4	Giaron	Italy	B	HR	15880	1.0
IT5	Sonora	Italy	B	SF	15882	1.0
IT6	San-Marzano	Italy	B	S	14452	1.0
IT7	Egizia	Italy	-	SF	19149	1.0
IT8	Sirio	Italy	B	S	15881	1.2
IT9	Mendoza-07	Italy	B	SF	14440	1.1
IT10	Mendoza-44	Italy	B	SF	14441	1.1
IT11	Burba	Italy	B	SF	15980	1.1
IT12	Gringo	Italy	B	SF	15504	1.0
S1	Sunneva	South Africa	-	SF	17134	1.0
S2	Karinke	South Africa	B	R	14548	1.0
Z1	MI-1	Zaire	F	SF	15311	1.0
Z2	MII -3	Zaire	F	R	15319	1.1
Average						**1.0**

B: Breeders' variety and F: farmers' variety; Type: S: San-Marzano; M: marglobe; SR: Slightly flattened; R: round; HR: highly round.

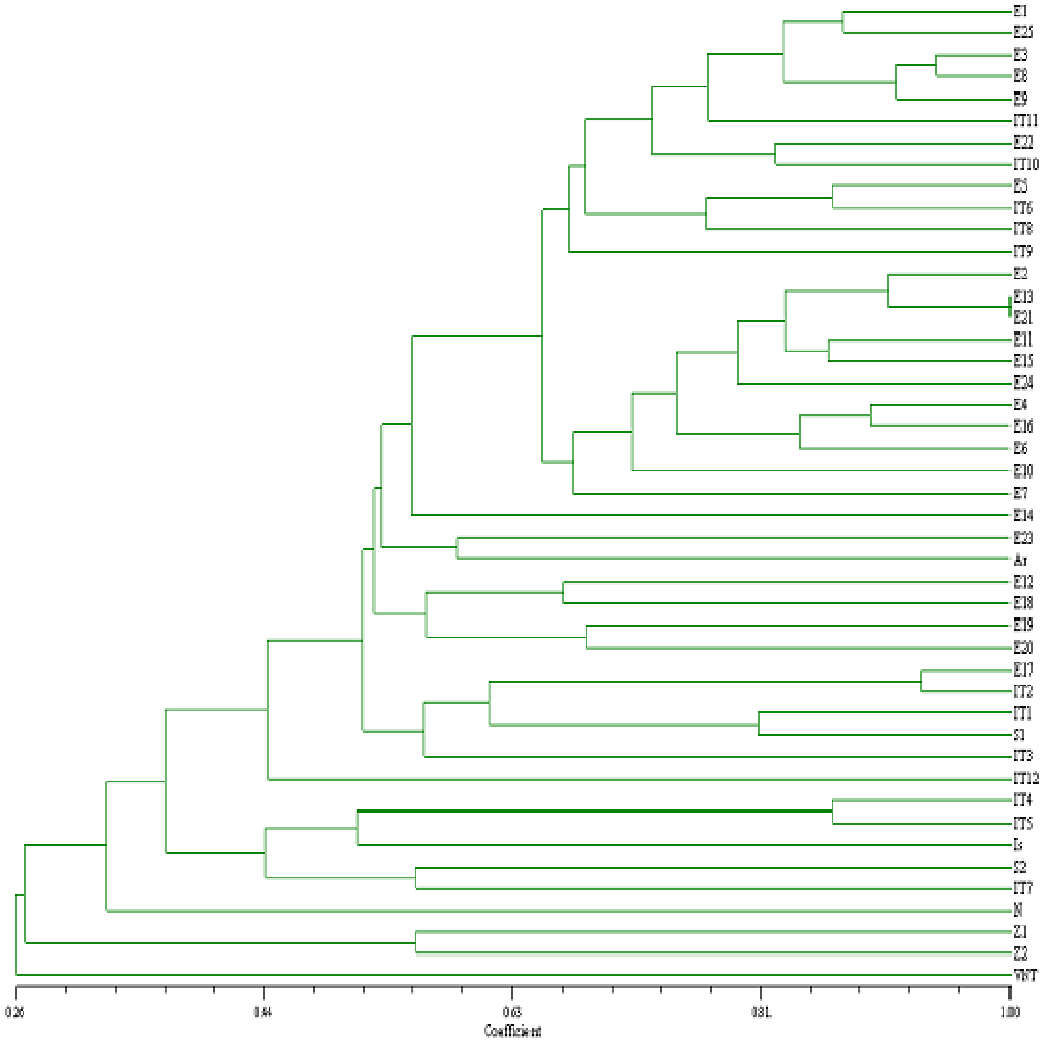

Figure 3. Dendrogram representing the genetic relationship among the 45 varieties tested: E (Eritrean); IT (Italian); S (South African); Z (Zairian); N (Nunhem6328); Is (Isola); Ar (Aranka) and VNT (VNT Cherry) varieties in y-axis based on 15 SSR markers.

trean varieties was identical to the Italian varieties. This could be due to influx of new material into the original varieties (which were originally sourced from Italy) during maintenance of varieties and selection of seeds over the years, or it could also mean that the original varieties that were shipped to Eritrea were not present in the set studied. Group 2 included merely Eritrean varieties, all of them San-Marzano types. These were obtained from different agro-ecological zones of Eritrea unlike those in group 1. In this group, varieties E13 and E21 were genetically identical (Figure 3) al-though they were obtained from different regions. These two varieties might be among those that have been originally distributed by the Ministry of Agriculture to all regions of Eritrea and then maintained by farmers found at different localities.

Down in the dendrogram, group 3, the grouping of the varieties became obscure. However, all Marglob varieties studied fell in this part of the dendrogram; all African and the control varieties also fell into this group. The Eritrean variety E17 (Marglob) and the Italian variety IT2 clustered together in the dendrogram, which again suggests that varieties from Eritrea often have Italian predecessors. None of the African varieties clustered with the Eritrean varieties.

Furthermore, the analysis distinguished the San-Marzano and the Marglob types of tomato and the dendrogram clearly showed the genetic relationship between old Italian and Eritrean varieties in both types. However, it was difficult to establish correlations between regions and farmers' varieties.

Uniformity of the Eritrean varieties

The varieties from Eritrea demonstrated a higher and

Table 2. Uniformity of 6 selected polymorphic Eritrean (E) and two Italian (IT) varieties for 6 informative SSR loci. A, B, C, D represent different alleles identified. (x): number of individual plants showing a particular allele.

Variety	SSR marker						% Non-uniformity
	TMS 9	LE20592	LEMDDNa	JACKP1	LEaat002	TMS 33	
E1	B(1) C(11) BC(1)	B(6) C(6)	A(6) D(4)	B(4) C(3) D(1)	C(10)	A(9) B(1)	27
E25	C(10)	B(11) C(1)	A(3) D(5)	C(10)	C(11)	A(9) B(2)	10
E3	B(1) C(8)	B(9) C(4)	A(7) D(4)	B(6) C(3)	C(12)	A(11) B(1)	20
E8	C(10)	B(4) C(8)	A(7) D(1)	B(3) C(6)	C(12)	A(9) B(2)	16
E9	C(9)	B(4) C(7)	A(7)	B(3)	C(11)	A(9) B(1)	10
E5	C(11)	B(4) C(10)	A(11) D(2)	B(4) C(7)	C(12)	A(12)	14
IT9	C(12)	B(11)	D(10)	C(12)	D(11)	B(11)	0
IT10	C(12)	B(12)	A(12)	C(12)	C(12)	B(10)	0

more varying degree of non-uniformity than the other varieties. The highest percentage of non-uniformity within a variety for the analysed SSR loci was 27% for E1 and the lowest percentage was 10% for E9 among the Eritrean varieties with an average of 16% (Table 2), while the old Italian varieties showed no non-uniformity at the SSR loci tested. This reconfirms the results of the genetic diversity analyses, where average numbers of alleles surprisingly reached levels up to 1.7 (Table 1) and where as many as five distinct peaks were observed. These findings clearly show that the Eritrean varieties are often mixtures or are contaminated.

Cooke et al. (2003) who studied modern European varieties found that seven out of ten varieties were uniform and the other three were non-uniform. Non-uniform varieties were in a range of only 0.5 to 1.4% non-uniformity. It is relevant to find out why Eritrean farmers' varieties are genetically contaminated and to investigate whether farmers intentionally mix varieties and finally to assess the impact of contamination on genetic diversity and conservation of tomato varieties in Eritrea. However, intentional mixing of varieties is a common phenomenon in many crops in Africa (Hirpa et al., 2010).

Participatory Rural Appraisal (PRA) on possible sources of contamination

About 37% of the interviewed farmers confirmed that farmers kept on experimenting with different varieties until they would get varieties which are high-yielding, disease-resistant and well adapted. Other farmers selected their best varieties on the basis of one or two of the aforementioned characteristics. According to the survey conducted about 50% of the farmers got their original seeds from the market, 37% from other farmers and 13% from the Ministry of Agriculture. Farmers did not make deliberate crossings between varieties but they did carry out selection within their varieties and maintained seeds from superior plants. They also introduced on purpose (and some farmers unwillingly) admixtures to their varieties, which is in agreement with the results from the genetic analysis. They kept or tried to maintain seed for the coming year with the influx of new genetic material into their original variety. This new influx could enhance the performance of the original farmers' variety but it could also reduce it if there was no proper selection of the new materials for desirable characters.

The survey showed that farmers' knowledge on diversity, selection and specifically on maintenance of varieties was limited. Kudadjie et al. (2007) also showed that farmers are not always aware of or understand the different sources of variation within their crop. The survey also showed that about 63% of the interviewed farmers knew that their varieties were not uniform. The main sources of variety contamination are given further in descending order based on the evaluation of the interviewed farmers: 1) Farmers get seedlings from their neighbour when they face shortage of seedlings or loss of seedlings due to heavy rain, hail, diseases and insects or farmers did not raise enough seedlings followed by non-selective seed production (33% of the interviewees); 2) animal manure carrying tomato seeds (25% of the interviewees); 3) seeds bought from other farmers were already polluted (25% of the interviewees); 4) some farmers mixed seeds purposely to prolong harvest and for shading purpose (17% of the interviewees); 5) seeds dropped from past season grew together with present season (negligible).

Implication for conservation and seed system of tomato in Eritrea

Utilization of already established genetic resources could be altered to the advantage or disadvantage of the farmers. The survey showed that farmers value 'new material' as a source of influx. Some farmers purposely mix seeds to prolong the harvesting period, for yield stability and stress tolerance. This mixing contributes to the genetic diversity in what farmers cherish as their own 'secret seed'.

In Eritrea, there was no genetic information in the di-

versity and heterogeneity of farmers' tomato varieties although it was known that farmers do select and maintain their own seeds. However, *in situ* conservation of the selected varieties needs to follow proper selection, maintenance and multiplication procedures on the consecutive years. Farmers should only introduce new influx to their selected farmers' varieties if the newly introduced materials are found to positively add up in terms of desirable characters. In Eritrea, although a relatively wide diversity of tomato genetic resources are conserved *in situ*, the use of already established genetic resources should be improved by upgrading farmers' knowledge in genetic diversity.

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