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Analysis of Diversity among and Heterogeneity within Tomato Cultivars from Eritrea

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Introduction

Tomato production has a long tradition in Eritrea and dates back to the Italian colonial period. Yet, the average yield of tomato in Eritrea has remained low, 15 Mg ha^{-1} (Ministry of Agriculture, 2000) compared to 19.1 Mg ha^{-1} on average in Africa, 23 Mg ha^{-1} in Asia and 27.2 Mg ha^{-1} on average in the entire world (Jones, 1999). Moreover, post-harvest losses of tomato can amount to more than 30% of the production and in general the quality of tomatoes has never been given a due attention. In addition to other production constraints, lack of an adequate seed supply system has contributed to the low productivity and quality (mainly storability) of tomatoes in Eritrea. Most farmers maintain their own varieties and multiply their own seeds. However, the performance of these varieties is generally poor partly due to the existing inadequate seed system. In general, farmers classify tomato varieties into two major groups: Marglobe (round fruits) and San-Marzano (angular fruits). Cultivated tomato is a species in which genetic markers, like isozymes and RFLPs (Restriction Fragment Length Polymorphism), yield limited information due to lack of variability, which is a consequence of self-pollination in combination with the narrow genetic base of the modern cultivars (Alvarez et al., 2001). Sequence tagged microsatellites, also known as the Simple Sequence Repeats (SSRs) approach, has proven to be particularly useful for variety identification and testing of several crops (Bredemeijer et al., 2002). Vosman et al., 1998 indicated the technique as efficient in the identification of tomato cultivars. SSR techniques are expensive if the sequence information to design the primers has not been developed yet. However, for tomato the primer sets for SSR analysis have already been developed. Besides, recently SSR has given highly informative genotyping sets in other crops such as barley (Macaulayet et al., 2001). Jones et al. (1998) indicated the faithful reproducibility of SSRs being tested by a network of European laboratories. Thus, this method was selected to study the genetic diversity of tomato varieties from Eritrea. This study aims at evaluating genetic diversity and heterogeneity of tomato varieties collected from different parts of Eritrea; comparing diversity and heterogeneity of Eritrean genetic material with materials from other African and Italian sources; relating the genetic analysis with information on the traditional seed management system obtained through a rapid appraisal; and finally coupling genetic information with the traditional seed system and proposing possible improvements of the seed system in Eritrea for tomato.

Materials and methods

Plant material: For the genetic analysis, 25 local cultivars of tomato, which were assumed to be maintained by self-pollination for several years by the farmers, were collected from the Northern Red Sea, Anseba, and Debub regions of Eritrea, in which traditional farmers who select and maintain tomato seeds were encountered. Additionally, two South African, two Zairian and twelve old Italian cultivars, obtained from the Centre for Genetic Resources of The Netherlands (CGN, Wageningen, The Netherlands) were included in the analysis for comparison. Tomato cultivars Isola, Aranka, Nunhems 6328 and VNT cherry were used as genotyping references (Bredemeijer et al., 2002). For

the heterogeneity test within cultivars 12 individual plants of 6 Eritrean and 2 Italian varieties were tested by selecting the most discriminative microsatellites 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 8 polymorphic varieties were treated individually for the heterogeneity test. The DNA extraction was performed according to Fulton et al. (1995).

PCR conditions: Fifteen tomato microsatellite loci were selected from Bredemeijer et al. (2002) and He et al. (2003). PCR amplifications were carried out on an MJ PTC200 using the conditions of Esselink et al. (2003) for rose microsatellite markers, including a uniform Tm of 50 ^{o}C. 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).

Participatory Rapid Appraisal (PRA): Prior to the genetic diversity analysis, a diagnostic survey was carried out on current status, constraints and potentials of tomato production in all tomato producing areas of Eritrea. The traditional seed management system was addressed as a part of the survey. Another Participatory Rapid Appraisal (PRA) was carried out after 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 supplied seeds. The appraisal aimed at obtaining insight into drivers for possible sources of contamination of the varieties encountered during the genetic analysis in the informal seed system of tomato in Eritrea.

Analysis of genetic data: Average numbers of alleles per microsatellite locus was calculated by taking the major alleles (based on size) for the varieties tested. The presence/absence (1/0) of each major fragment/peak (allele) was scored and stored in a database and the similarity index was calculated using the formula $D = 1 - \Sigma Pi^2$ where Pi is the frequency of the i^{th} allele in the 45 varieties examined. A dendrogram showing the genetic relatedness among the varieties used was constructed using the unweighted pair group method with arithmetic mean (UPGMA) module of NTSYS. For the uniformity test within a variety individual plants were genotyped and allele frequency of the major picks scored and the percentage of occurrence of the specific microsatellite allele calculated.

Results and Discussion

Participatory Rapid Appraisal (PRA): Traditionally, many farmers produce their own tomato seeds by selecting stable and disease resistant varieties, because F_1 hybrid seeds are expensive, unreliable in terms of adaptability and disease resistance; and are kept secret individually without sharing or selling to other farmers which could indicate diversity of present day tomato varieties in Eritrea. Farmers focusing on the traditional seed system are not benefiting from the emerging high-yielding varieties and it is important that farmers attain improved and sustainable yield.

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, having 13 polymorphic SSR loci with 2 - 5 alleles, which is unusual for true–to–type cultivars, while the cultivars obtained from the CGN were more or less homogeneous with monomorphic microsatellite loci. The average number of alleles for Eritrean varieties was 1.3, for the varieties from the CGN it was 1.04, and for the control cultivars it was 1.4. The 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 varieties. Thus, further experiments are required to support our result, as our experiment was conducted by bulking six individuals for each variety tested.

Relationship between varieties: The dendrogram (Fig. 1) showed two major clustering of cultivars, group 1 consisting of E1 up to and including IT9, and group 2 consisting of E2 up to and including E7. Five Italian varieties: IT6, IT8, IT9, IT10 and IT11 are found in this cluster suggesting a genetic relationship between Eritrean and the old Italian varieties. Nevertheless, none of the Eritrean varieties was identical to the Italian varieties. This could probably be due to seed contamination during

maintenance of varieties and selection of seeds over the years or else it could also mean that the original varieties that were shipped to Eritrea are not in the set studied.

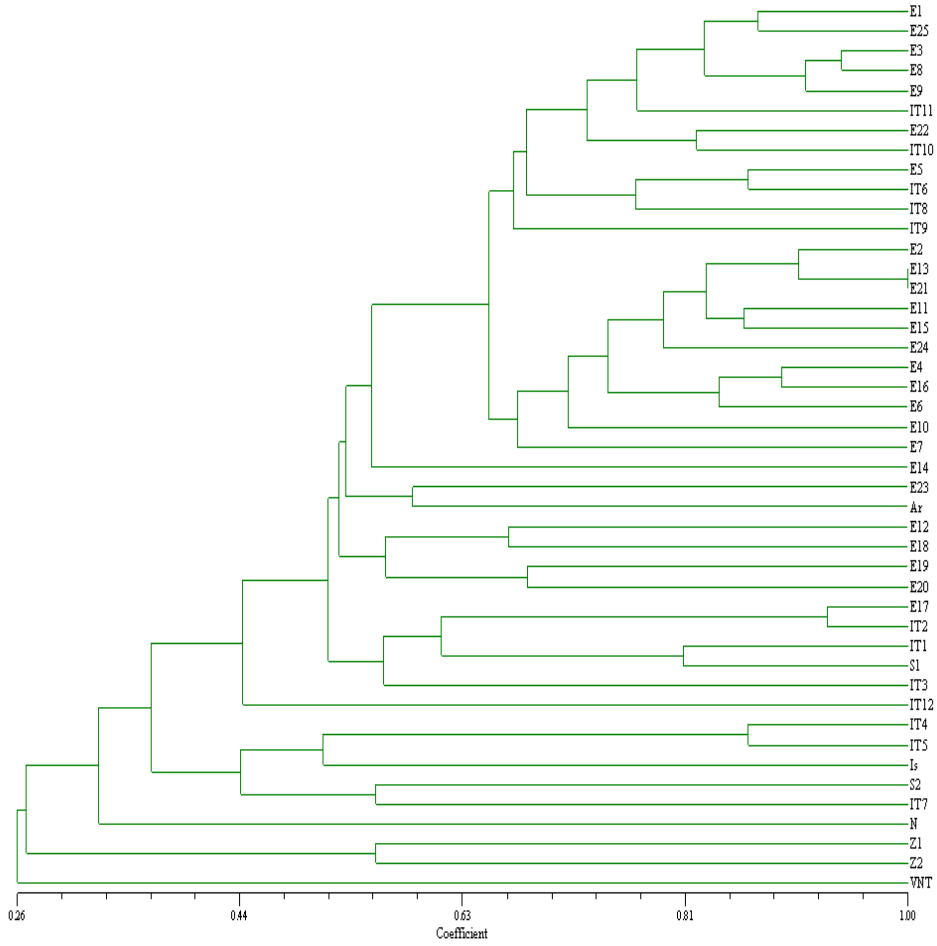

***Fig.1**, Dendrogram representing the genetic relationship of E (Eritrean), IT (Italian), S (South African) and Z* (Zarian) cultivars in x-axis based on 15 SSR markers.

Group 2 included merely Eritrean varieties, all of them San-Marzano types and were obtained from different agro-ecological zones of Eritrea. Down in the dendrogram, group 3, the grouping of the varieties became obscure. However, all Marglob varieties studied fall in this part of the dendrogram; all African, and the control varieties also fell in this group. Furthermore, the analysis clearly distinguished the San Marzano and the Marglob types of tomato and the dendrogram clearly showed the genetic relationship between old Italian cultivars and Eritrean varieties in both types.

Uniformity of the Eritrean varieties: All Eritrean varieties are inbreds, thus one expects to find homozygosity at each locus and only one allele in each variety. All individual plants analyzed for the Eritrean varieties turned out to be heterozygote on average for all the microsatellite loci except for LEaat002 loci and had 0% to 31.2% percentage of heterogeneity with an average of 15.9% while that of the Italian varieties were all homozygote with 0% heterogeneity (Table 1). Also, uniformity within an Eritrean variety turned out to be low, see Table 1. For most of the markers more than one allele was found in the Eritrean varieties, whereas the two Italian varieties analysed turned out to be uniform at all loci. As the Eritrean varieties showed more than 1 allele at several loci, they are most likely impure or mixtures of varieties. Among the Eritrean varieties the highest percentage of non-uniformity within a cultivar for the analysed SSR loci reached 26.5% for E1 and the lowest percentage was 7.7% for E9 with an overall average of 15.9% (Table 1), depicting the genetic contamination of varieties in Eritrea. Yet, the percentage of non-uniformity for the old Italian cultivars was 0%.

Table 1. ***Uniformity of selected polymorphic Eritrean and Italian cultivars.***

Group 1	**SSR Loci**						
Variety	**TMS 9**	**LE20592**	**LEMDDNa**	**JACKP1**	**LEaat002**	**TMS 33**	**% Non-uniformity within a variety**
E1	B(2) C(11)	B(6) C(6)	A(6) D(4)	B(4) C(3) D(1)	C(10)	A(9) B(1)	**27.6**
E25	C(10)	B(11) C(1)	A(3) D(5)	C(10)	C(11)	A(9) B(2)	**10.7**
E3	B(1) C(8)	B(9) C(4)	A(7) D(4)	B(6) C(3)	C(12)	A(11) B(1)	**20.0**
E8	C(10)	B(4) C(8)	A(7) D(1)	B(3) C(6)	C(12)	A(9) B(2)	**16.2**
E9	C(9)	B(4) C(7)	A (7)	B(3)	C(11)	A(9) B(1)	**7.7**
E5	C(11)	B(4) C(10)	A(11) D(2)	B(4) C(7)	C(12)	A(12)	**13.4**
% H	**4.4**	**31.2**	**23.6**	**25.5**	**0**	**10.8** →	**Mean: 15.93** **Mean: 15.91**
	TMS 9	**LE20592**	**LEMDDNa**	**JACKP1**	**LEaat002**	**TMS 33**	**% Non-uniformity within A variety**
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**
% H	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0** →	**Mean: 0.0** **Mean: 0.0**

% H: Percentage of Heterogeneity

Based on the results from the genetic diversity analyses and the heterogeneity test using the SSR technique conducted, we can conclude that many of the Eritrean varieties tested were heterogeneous. But it was also important to verify the source of this genetic contamination of the varieties and investigate whether farmers were aware of this fact and finally assess its impact on genetic diversity and conservation of tomato varieties in Eritrea.

Survey on traditional seed management systems: About 62.5% of the interviewed farmers knew that their varieties were not uniform. The main sources of variety contamination are given below on descending order based on the evaluation of the interviewed farmers:

1. Farmers get seedlings from their neighbours 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 (33.3% 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 (16.7% of the interviewees).
5. Seeds dropped from past season grew together with present season (negligible).

We are convinced that farmers have been adding admixtures to their secret varieties over the years of maintenance and selection, either knowingly or unknowingly. As a consequence, the conservation of biodiversity and the establishment of a functional seed system of tomato in Eritrea are impeded.

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 due to lack of knowledge on genetic diversity. In Eritrea there was no genetic information on the diversity and heterogeneity of 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. '*Too much heterogeneity is confusion and too much homogeneity is sterility'*. Farmers should not introduce new influx to their old secret seeds, once they found the cultivar superior, unless the newly introduced materials are found to positively add up in terms of some desirable characters.

Conclusions and Outlook

Based on the genetic diversity analyses and the heterogeneity test conducted using the SSR technique we can conclude that many of the Eritrean varieties tested were heterogeneous. The diversity test clearly confirmed the genetic relationship between old Italian cultivars and Eritrean varieties in both types. Results for the genetic diversity analysis and heterogeneity of tomato cultivars as well as the PRA afterwards matched perfectly indicating the usefulness of SSR, as important techniques to investigate and analyze diversity and heterogeneity despite the fact that, crops like tomato have low levels of variation. Utilization of already established genetic resources could be altered by lack of knowledge on genetic diversity. Thus, selected genetic resources should be conserved in-situ by upgrading farmers' knowledge on genetic diversity and conservation and/or introducing a mechanism of supplying true-to-type seeds to farmers. Generally the results showed that selected genotypes are facing genetic contamination, which partly explains the low yield and quality of tomato in Eritrea.

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