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Assessment of the Diversity of Tomato (*Solanum Lycopersicum* L.) Accessions in the Nigeria National Gene Bank Using Simple Sequence Repeat (SSR) Markers

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ABSTRACT

Tomato is one of the most cultivated vegetable crops worldwide, with a global production of over one hundred and thirty million tons. Its multi-purpose uses range from being consumed in various forms, including salads, pastes, sauces, and soups, to its culinary and antioxidant properties. Assessment of variability among genetic materials is essential for maintaining and utilizing genetic resources. A fundamental requirement for the effective use of these genetic materials in breeding and crop improvement is a thorough analysis of the genetic variability and structure of the accessions. This study aims to assess the genetic diversity of tomato accessions in the Nigeria gene bank using Simple Sequence Repeat (SSR) markers. DNA was extracted from fifty tomato accessions using the CTAB protocol. The DNA was quantified using a Nanodrop Spectrophotometer and quality-checked on 1%w/v agarose. High-quality DNA was then used for amplification with five polymorphic SSR markers. The total number of alleles was 23, with a mean of 3.20. The polymorphic information content (PIC) values ranged from 0.35 (ODT4) to 0.50 (ODT1), with a mean value of 0.43, while the gene diversity ranged from 0.45 to 0.57, with a mean value of 0.51. The cluster analysis based on the Unweighted Pair-Group Method using Arithmetic Means (UPGMA) grouped the accessions into two major clusters, each with two subclusters. The first major cluster comprised two subclusters of three and thirty tomato accessions, respectively, while the second major cluster had one and sixteen tomato accessions in its subclusters. The results showed that SSR markers were successfully used to discriminate among the tomato accessions based on the PIC and genetic diversity values, hence promoting their use for future crop improvement and contributing to food security.

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Introduction

Solanum lycopersicum L. (tomato) belongs to the family Solanaceae. This family has approximately 100 genera and 2500 species, including several plants of economic value, such as potato, eggplant, pepper, and tobacco. These species in the tomato clade are all diploid with the same chromosome number (2n = 24) (Al *et al*., 2018). Tomato is one of the most cultivated vegetable crops around the world, both in the temperate and tropical regions, due to its multi-purpose uses, with global production reported to be around 130 million tons (Kulus, 2018; Wang *et al*., 2016). This vegetable can be consumed in various forms, including cooked, processed, and

fresh; therefore, it serves as an ingredient/condiment in culinary, which is rich in components that are of health benefits (Hussain *et al*., 2018).

Assessment of genetic material variability is essential to maintaining and utilizing germplasm resources (Mungai *et al*., 2018). A fundamental requirement for the effective use of these genetic materials for breeding and crop improvement is the thorough analysis of the genetic variability and structure of germplasm accessions (Zhou *et al*., 2015). Diversity is important to crop improvement, which involves quality enhancement, tolerance to abiotic and biotic stresses, and adaptability of crop plants to varied environments, especially in changing climatic conditions (Bhandari, 2017). Various methods, including morphological, biochemical, and molecular markers, were used to assess the genetic diversity of plants (Khan *et al*., 2020). Molecular marker is a powerful tool preferred to study genetic diversity as it allows a comprehensive knowledge of the relationships between species within the same genus without the influence of the environment or plant development stage (Henareh *et al*., 2016; Zhou *et al*., 2015). The different molecular markers that have been used in genetic diversity study include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat or microsatellite (SSR), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequence (CAPS), sequence-related amplified polymorphism (SRAP), and single nucleotide polymorphism (SNP) markers (Henareh *et al*., 2016; Mata-Nicolás *et al*., 2020; Todorovska *et al*., 2014). Simple Sequence Repeats (SSR) are genetic markers with tandem repeated motifs of two to six bp; they are used in many cultivated plants and are found in all prokaryotic and eukaryotic genomes (Zane *et al.,* 2002). SSR markers are considered to be the preferred molecular markers in crop breeding because of their properties of genetic co-dominance, high reproducibility, and multiallelic variation, among others (Al-Shammari and Hamdi, 2021; He *et al*., 2003). The use and efficiency of SSRs for the study of genetic diversity and variability in the genus *Solanum* and tomato cultivars identification have been confirmed in previous studies (Smulders *et al*., 1997; Bredemeijer *et al*., 2002; He *et al*., 2003; Frary *et al*., 2005; Tam *et al*., 2005). This is due to the discrimination capacity of SSR markers for genotype identification. The present study uses Simple Sequence Repeat markers to assess the genetic diversity of tomato accessions in the Nigeria gene bank.

Materials and Methods

Sample collection and DNA extraction

This research was conducted at the Molecular Biology Laboratory at the National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan. The fifty accessions of tomatoes were collected from the Seed Gene Bank of NACGRAB (Table 1). The accessions were catalogued and given a unique identifier. The tomato species were planted in pots. After three weeks, genomic DNA was extracted from them using the cetyl trimethyl ammonium bromide (CTAB) protocol. DNA was extracted directly from the seeds for some that did not germinate. The CTAB protocol used is as described by Oduoye *et al.,* 2020. The genomic DNA was quantified using a Nanodrop Spectrophotometer, and quality was checked using 1% agarose gel electrophoresis.

DNA amplification

Five SSR primer pairs were synthesized by Inqaba Biotec for the amplification. Each lyophilized primer was reconstituted to 100 µM with distilled water. About 50 ng of DNA was amplified in an Eppendorf thermocycler nexus gradient using 10 pM, primer (Table 2), 10 µl of one *Taq* Quick-load 2x master mix with standard buffer (New England BioLabs® Inc.) mixed with six µl of nuclease-free water (Sifau *et al*., 2017). The cycling conditions include initial denaturation at 92 °C for three minutes followed by 35 cycles of (denaturation at 92 °C for 30 sec., annealing at 60 - 63 °C for 20 sec., and extension at 72 °C for 30 sec.), ending with a final extension at 72 °C for 5 minutes. Five µl of amplification products were run through 1% w/v agarose gel using a 1xTBE (Tris-Borate-EDTA) buffer-filled electrophoresis tank with 101 volts and a running time of 40 minutes. The size of the

polymorphic fragments was determined using three µl of appropriate DNA ladder at both ends and viewed by staining the gel with Safeview™ Classic stain (Applied Biological Materials Inc, Canada) in GA9000/9010 version 12 transilluminator.

Table 1. List of tomato accessions

S/N	AN	Code	S/N	AN	Code	S/N	AN	Code
1	NGB00698	T1	18	NGB00718	T18	35	NGB00710	T37
2	NGB00727	T2	19	NGB00717	T19	36	NGB00728	T38
3	NGB00734	T3	20	NGB00732	T20	37	NGB00750	T39
4	NGB00714	T4	21	NGB00742	T21	38	NGB00692	T40
5	NGB00740	T5	22	NGB00707	T22	39	NGB00730	T41
6	NGB00733	T6	23	NGB00724	T23	40	NGB00726	T42
7	NGB00722	T7	24	NGB00735	T24	41	NGB00738	T43
8	NGB00720	T8	25	NGB00694	T25	42	NGB00739	T44
9	NGB00737	T9	26	NGB00713	T26	43	NGB00741	T45
10	NGB00708	T10	27	NGB00748	T27	44	NGB00745	T46
11	NGB00696	T11	28	NGB00711	T28	45	NGB00746	T47
12	NGB00697	T12	29	NGB00744	T29	46	NGB00749	T48
13	NGB00736	T13	30	NGB00743	T32	47	NGB00751	T49
14	NGB00699	T14	31	NGB00729	T33	48	NGB00752	T50
15	NGB00725	T15	32	NGB00716	T34	49	NGB00756	T51
16	NGB00747	T16	33	NGB00715	T35	50	NGB00762	T52
17	NGB00721	T17	34	NGB00719	T36	-	-	-

AN= Accession numbers

Table 2. List of SSR primers and the annealing temperature

Primer names	Forward sequence (5'→3')	Reverse sequence (5'→3')	Temp (°C)
ODT1	GAGCAGTGGAGTCCAGCATT	GAGTCCAGCTACGAGCAGTG	62
ODT2	CAAGGTCCTGCTGTCCATCT	TCGTATTTGGCCCTGTCAGTC	62
ODT3	GCTCTCTGTTGACAGACCCA	AGTGGTTTCGTATTTGGCCCT	62
ODT4	GGTCCAGTGGCAGAGGATATG	CCAGCATAAGGGCTATCGGT	62
ODT5	TGCTCTCTGTTGACAGACCC	ATTTGGCCCTGTCAGTCTTGT	62

Data analysis

The data was scored based on one or the presence and zero for the absence of an amplified DNA band for each locus across all the samples, thus generating the 0 and 1 matrix for the five primers. The basic genetic measurement (number of alleles, PIC and gene diversity) using the five SSR markers was calculated using Power Marker (Lui and Muse, 2005) and the relation matrix between the tomato accessions was shown graphically in the form of a dendrogram using a UPGMA for all the tomato accessions constructed with MEGA6.

Results and Discussion

Assessing genetic diversity and relationships among gene bank crop accessions is important as this will facilitate effective collection and evaluation and promote utilization. SSR markers are efficient for assessing diversity in the *Solanaceae* (Khan *et al*., 2020). For the 50 tomato accessions, high-quality genomic DNA was obtained for the amplification reactions. The polymorphism between the tomato accessions was determined using five polymorphic SSR markers based on the allele frequency (Fig.1) at each locus, and these were used to analyze the genetic diversity of 50 tomato accessions. PIC provides an estimation of the discriminating power of the molecular marker based on the number of detected alleles and their frequency. The SSR polymorphism in the tomato accessions was measured in terms of the PIC, gene diversity and the number of alleles using Power marker software.

The Nei's genetic distance for the markers was calculated, and the relation matrix between the accessions was shown using a dendrogram. The major allele frequency (MAF) was between 0.54 to 0.66, with a mean of 0.62, while the PIC ranged between 0.35 to 0.49 and an average of 0.43. The highest gene diversity was obtained from ODT3 (0.57), and the least was from ODT4 (0.45), with an average of 0.51 (Table 3). All

five SSR markers were moderately informative. The mean number of alleles per locus was 3.2, which is similar to what was reported by Athinodorou *et al*. (2021) for one hundred and ninety Cypriot tomato genotypes using ten SSR markers (Athinodorou *et al*., 2021).

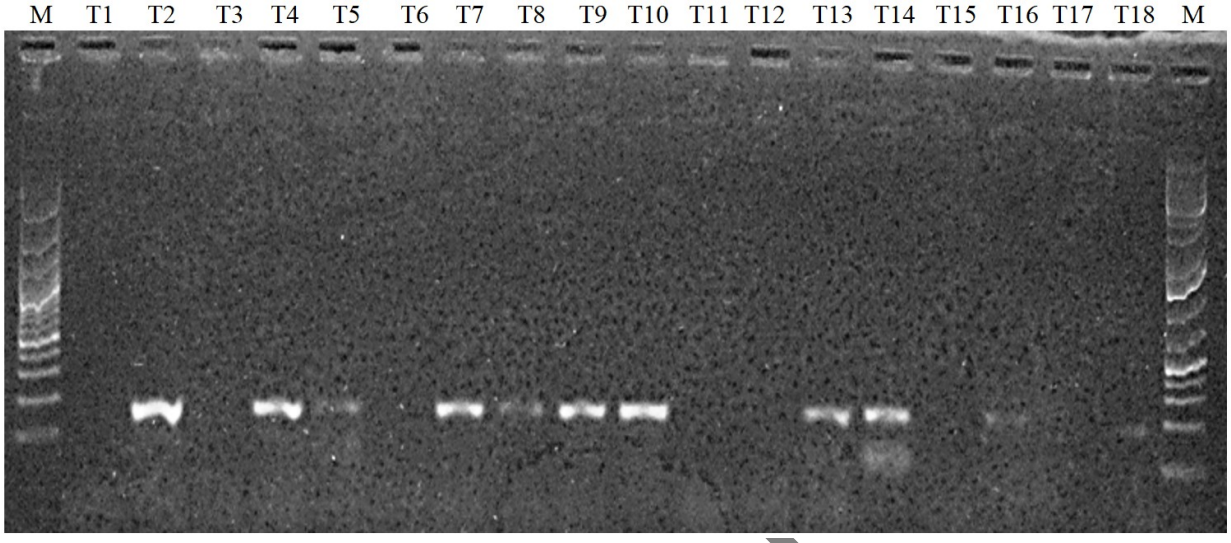

Fig. 1. Profile of amplified DNA by *ODT1* primer from different samples: T1-T18 are samples; M= 1kb DNA marker.

The PIC value correlates with that reported by Pozharskiy *et al*. (2023) for three out of 13 SSR markers used to investigate the genetic structure of 68 tomato varieties from the Fruit and Vegetable Research Institute of Kazakhstan (Pozharskiy *et al*., 2023), which indicated moderate information content while Pidigam *et al.* (2021) had a higher average PIC value. The least genetic similarity value was 0.20 and associated with NGB00732, NGB00727, NGB00726, NGB00722, NGB00744, NGB00743, NGB00736, NGB00735, NGB00720, NGB00714, NGB00708, NGB00694, NGB00692, and NGB00751, indicating close relationship between these accessions. This value is similar to what Khan *et al.* (2020) reported for twenty-six tomato genotypes using thirty SSR markers. However, the highest genetic similarity was associated with NGB00742 and NGB00692, NGB00735 and NGB00745, and a couple of others, which indicate the suitability of hybridization to achieve the highest possible variability among populations.

The hierarchical cluster analysis was done using UPGMA (Unweighted Pair-Group Method using Arithmetic Means) method and the graphical representation was generated using Power Marker software (version 3.25) for the 50 tomato accessions using the five primers. The dendrogram showed the phylogenic relationship among the tomato accessions and two major clusters were showed in Fig. 2.

Table 3. Statistical values and parameters of polymorphism information content

Marker	MAF	Gene Diversity	PIC
ODT1	0.62	0.55	0.49
ODT2	0.66	0.50	0.44
ODT3	0.54	0.57	0.49
ODT4	0.66	0.45	0.35
ODT5	0.60	0.48	0.36
Mean	0.62	0.51	0.43

MAF= Major Allele Frequency; PIC= Polymorphic information content.

The main cluster was further divided into two subclusters with three and thirty accessions each, while the second major cluster had two subclusters with one and sixteen accessions each. The highly diverse cluster may have good breeding potential for tomato crop improvement and broaden the genetic base of commercial tomatoes, which have low genetic diversity. Okumus and Dağidir (2021) also had two main clusters in their study on the genetic relationship among 24 tomatoes using 40 SSR markers (Okumuş and Dağidir, 2021), while Al-

Shammari and Hamdi (2012) had six main clusters in their study on twenty-four tomato accessions using fifteen polymorphic SSR markers (Al-Shammari and Hamdi, 2021).

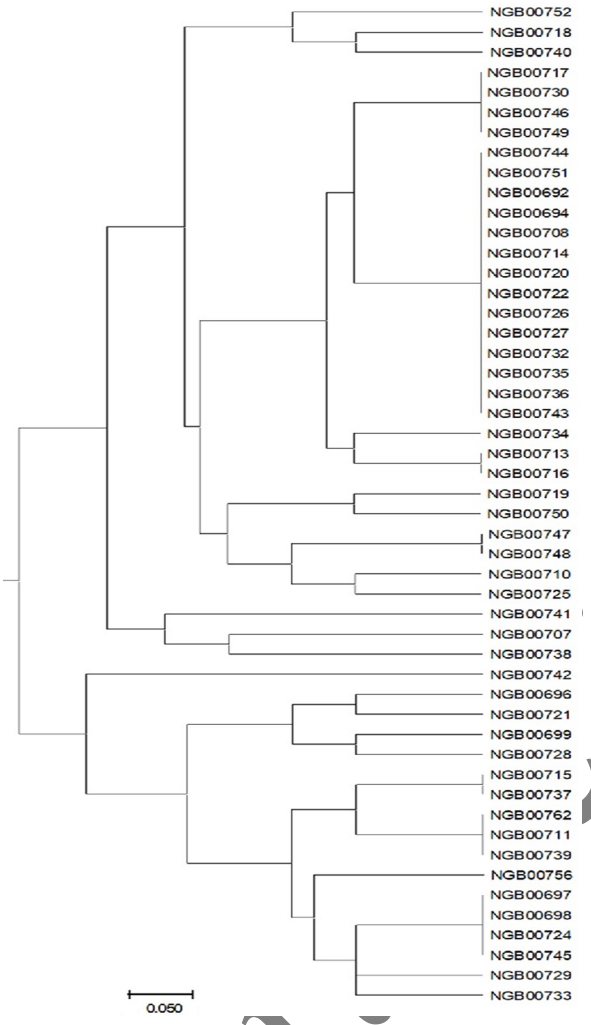

Fig.1 Dendrogram showing the genetic relationship of 50 tomato accessions using UPGMA

Conclusion

Food and nutrition security is important in the face of the changing climatic conditions. Therefore, assessing the genetic diversity of genetic resources is necessary to broaden the genetic base of available cultivars and ensure effective conservation. The use of molecular markers to detect variations within tomato germplasm is vital to improving the low genetic diversity associated with cultivated tomatoes. The efficacy of SSR markers for assessing the genetic diversity in the family Solanaceae has been reported in several studies. The current research has shown that five SSR markers revealed two main clusters among the fifty tomato accessions, which could be explored to identify duplicated accessions, create a core collection in the gene bank, and have good breeding potential for tomato improvement. Also, genetic similarity showed the suitability of some of the accessions for hybridization. Further investigation using more SSRs could reveal the pattern of diversity and population structure of the gene bank tomato accessions.

Conflict of Interests

There is no conflict of interest associated with this paper.

References

Al-Shammari, A. M. A., Hamdi, G. J., Al-Mahdawi, M. A. S., & Mohammed, N. K. (2021). Genetic diversity analysis and DNA fingerprinting of tomato breeding lines using SSR markers. *Journal of Agricultural Science*, 1-7. https://doi.org/10.15159/jas.21.13

Al Shaye, N., Migdadi, H., Charbaji, A., Alsayegh, S., Daoud, S., Wala, A. A., & Alghamdi, S. (2018). Genetic variation among Saudi tomato (*Solanum lycopersicum* L.) landraces studied using SDS-PAGE and SRAP markers. *Saudi Journal of Biological Sciences*, 25(6), 1007-1015. https://doi.org/10.1016/j.sjbs.2018.04.014

Athinodorou, F., Foukas, P., Tsaniklidis, G., Kotsiras, A., Chrysargyris, A., Delis, C., ... & Nikoloudakis, N. (2021). Morphological diversity, genetic characterization, and phytochemical assessment of the Cypriot tomato germplasm. *Plants*, 10(8), 1698. https://doi.org/10.3390/plants10081698

Bhandari, H. R., Bhanu, A. N., Srivastava, K., Singh, M. N., & Shreya, H. A. (2017). Assessment of genetic diversity in crop plants - An Overview. *Advances in Plants and Agriculture Research*, 7(3), 279-286. https://doi.org/10.15406/apar.2017.07.00255

Bredemeijer, G., Cooke, R., Ganal, M., Peeters, R., Isaac, P., Noordijk, Y., ... & Vosman, B . (2002). Construction and testing of a microsatellite database containing more than 500 tomato variet- ies. *Theoretical and Applied Genetics*, 105, 1019 -1026 https://doi.org/10.1007/s00122-002-1038-6

Frary, A., Xu, Y., Liu, J., Mitchell, S., Tedeschi, E., & Tanksley, S. (2005). Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. *Theoretical and Applied Genetics*, 111(2), 291-312 https://doi.org/10.1007/s00122-005-2023-7

He, C., Poysa, V., & Yu, K. (2003). Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. *Theoretical and Applied Genetics*, 106, 363-373. https://doi.org/10.1007/s00122-002-1076-0

Henareh, M., Dursun, A., Mandoulakani, B. A., & Haliloglu, K. (2016). Assessment of genetic diversity in tomato landraces using ISSR markers. *Genetika*, 48 (1), 25-35 https://doi.org/10.2298/gensr1601025h

Hussain, I., Aslam-Khan, S., Ali, A., Farid, A., Ali, N., Ali, S. A. R. D. A. R., ... & Raza, H. (2018). Genetic diversity among tomato accessions based on agro-morphological traits. *Sains Malaysiana*, 47(11), 2637- 2645. https://doi.org/10.17576/jsm-2018-4711-06

Khan, R. T., Abbas, S. R., Gerdezi, S. D. A., Javed, G., & Mehmood, A. (2020). Assessment of genetic diversity of tomato genotypes by using molecular markers. *Pure and Applied Biology*, 9(2), 1532-1540 https://doi.org/10.19045/bspab.2020.90160

Kulus, D. (2018). Genetic resources and selected conservation methods of tomato. *Journal of Applied Botany and Food Quality*, 91, 135-144. https://doi.org/10.5073/jabfq.2018.091.019

Lui, K. (2005) PowerMarker: Integrated analysis environment for genetic marker Data. *Bioinformatics*, 21, 2128-2129. http://dx.doi.org/10.1093/bioinformatics/bti282

Mata-Nicolás, E., Montero-Pau, J., Gimeno-Paez, E., Garcia-Carpintero, V., Ziarsolo, P., Menda, N., ... & Díez, M. J (2020). Exploiting the diversity of tomato: the development of a phenotypically and genetically detailed germplasm collection. *Horticulture Research*, 7, 66 https://doi.org/10.1038/s41438-020-0291-7

Mungai, G. W., Owino, W., Ambuko, J., Giovannoni, J. J., Nyende, A. B., & Michuki, G. (2018). Genetic diversity within African tomato using next generation sequencing. *Plant Genetic Resources*, 169 (4), 296- 305. https://doi.org/10.1017/S1479262117000314

Oduoye, O. T., Oluwasanya, O. A., Arikawe, O. O., Sunday, A., Ayekun, O. A., Oyenpemi, O. S., ... & Okechukwu, E. C. (2020). Genetic variation via SSR polymorphic information content and ecological distributions of Nigerian sesame. *African Journal of Biotechnology*, 19(4), 165-170. http://dx.doi.org/10.5897/AJB2019.16980

Okumuş, A., & Dağidir, Ş. (2021). Assessment of genetic diversity on tomato (*Lycopersicon esculentum* Mill.) landraces using SSR molecular markers in Turkey. *Frontiers in Life Sciences and Related Technologies*, 2(2), 51- 59. https://doi.org/10.51753/flsrt.957055

Pakhtunkhwa, K. (2018). Genetic diversity among tomato accessions based on agro-morphological traits. *Sains Malaysiana* 47(11), 2637- 2645. http://dx.doi.org/10.17576/jsm-2018-4711-06

Pidigam, S., Thuraga, V., Munnam, S. B., Amarapalli, G., Kuraba, G., Pandravada, S. R., ... & Sudini, H. K. (2021). Genetic diversity, population structure and validation of SSR markers linked to Sw-5 and I-2 genes in tomato germplasm. *Physiology and Molecular Biology of Plants*, 27(8), 1695-1710. https://doi.org/10.1007/s12298-021-01037-8

Pozharskiy, A., Kostyukova, V., Khusnitdinova, M., Adilbayeva, K., Nizamdinova, G., Kapytina, A., ... & Gritsenko, D. (2023). Genetic diversity of the breeding collection of tomato varieties in Kazakhstan assessed using SSR, SCAR and CAPS markers. *PeerJ*, 11, e15683. https://doi.org/10.7717/peerj.15683

Sifau, M. O., Oduoye, O. T., Oluwasanya, O.A., & Aladele, S. E. (2017). Assessment of genetic variability in Sorghum accessions (*Sorghum bicolor* L. Moench) at the national centre for genetic resources and biotechnology, Ibadan, Nigeria. *Journal of Applied Science and Environmental Management,* 21 (6), 1143- 1147. http://dx.doi.org/10.4314/jasem.v21i6.25

Smulders, M. J. M., Bredemeijer, G., Rus-Kortekaas, W., Arens, P., & Vosman, B. (1997). Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon*

esculentum cultivars and accessions of other Lycopersicon species. *Theoretical and Applied Genetics*, 94, 264-272 https://doi.org/10.1007/s001220050409

Tam, S. M., Mhiri, C., Vogelaar, A., Kerkveld, M., Pearce, S. R., & Grandbastien, M. A. (2005). Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon-based SSAP, AFLP and SSR. *Theoretical and Applied Genetics*, 110(5), 819-31 https://doi.org/10.1007/s00122-004-1837-z

Todorovska, E., Ivanova, A., Ganeva, D., Pevicharova, G., Molle, E., Bojinov, B., ... & Danailov, Z. (2014). Assessment of genetic variation in Bulgarian tomato (*Solanum lycopersicum* L.) genotypes, using fluorescent SSR genotyping platform. *Biotechnology and Biotechnological Equipment*, 28(1), 68- 76. http://dx.doi.org/10.1080/13102818.2014.901683

Wang, T., Zou, Q. D., Qi, S. Y., Wang, X. F., Wu, Y. Y., Liu, N., ... & Li, H. T. (2016). Analysis of genetic diversity and population structure in a tomato (*Solanum lycopersicum* L.) germplasm collection based on single nucleotide polymorphism markers. *Genetics and Molecular Research*, 15(3), 1-12. https://doi.org/10.4238/gmr.15038209

Zane, L., Bargelloni, L., & Patarnello, T. (2002). Strategies for microsatellite isolation: A review. *Molecular Ecology*, 11(1), 1-16 https://doi.org/10.1046/j.0962-1083.2001.01418.x

Zhou, R., Wu, Z., Cao, X., & Jiang, F. L. (2015). Genetic diversity of cultivated and wild tomatoes revealed by morphological traits and SSR markers. *Genetics and Molecular Research*, 14(4), 13868- 13879. https://doi.org/10.4238/2015.october.29.7