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MORPHOLOGICAL CHARACTERIZATION AND GENETIC VARIABILITY ASSESSMENT WITH SSR MARKERS IN SEVERAL TOMATO GENOTYPES

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Abstract

A complete morphological description of the tomato genotypes is necessary either for the new cultivars under approval, or for the recommendation of proper tomato cultivars in certain cultural conditions. In this study, the morphological and molecular diversity of 13 tomato genotypes were analyzed to identify distinctness among them. The genetic diversity was evaluated with 8 SSR markers. The efficiency of these markers to reveal the genetic differences with tomato genotypes was proven by: a mean number of scorable bands per marker of 6.62, of which 81.5% were polymorphic bands and the polymorphic information content of 0.764. The cluster analysis grouped the 13 tomato genotypes into two distinct clusters, depending on their type of growth, and inside each group in correlation with parental origin. The evaluation of the relevant characteristics with specific descriptors demonstrated the differences between the genotypes analyzed in terms of their type of growth and the different aspects of the leaves and fruits. Combining the morphological description with molecular methods proved to be efficient for the assessment of distinctiveness among analyzed tomatoes and necessary for documented recommendations for tomato growers.

Key words*: dendrogram, polymorphism, Solanum lycopersicum, standard descriptors, variability.*

INTRODUCTION

Global population growth and climate change are the main reasons for researchers to accelerate their investigations and promote new plant varieties to meet increasing demand for high-quality foods, with good nutritional value.

Tomato (*Solanum lycopersicum* L.) is one of the most popular and economically advantageous crops in the world because of its widely cultivated area all over the world, under a wide range of agro-climatic conditions, its nutritional value (Todorovska et al., 2014; Renna et al. 2018), and its extensive use for culinary purposes. Therefore, the tomato is intensively studied not only as a model plant for studies of classical and molecular genetics (Titeli et al. 2021; Xia et al. 2021) but also as a crop plant for which the quantity and quality of tomato production in different climatic conditions are of global interest (Gerszberg & Hnatuszko-Konka, 2017).

The morphological characterization and the assessment of genetic diversity with different molecular markers are considered important tools in exploring tomato germplasm aiming for efficient use of the accessions in breeding programs (Sun et al., 2012; Wang et al., 2016; Ronga et al., 2018; Al-Shammari & Hamdi, 2021).

In Romania, the outstanding achievements of the last 10 years in the development of new tomato varieties and their promotion in the consumer market, or in the processing industry, require an estimate of the genetic diversity of the cultivated accessions (Felföldi et al., 2021). Therefore, aiming to have efficient utilization, conservation and management of tomato genetic resources, the present study was conducted for the followings: (i) to evaluate and register the diversity of morphological traits for 13 tomato genotypes necessary for documentation of each accession; (ii) to assess the genetic diversity using SSR markers; (iii) to apply the cluster analysis using markers (SSR) to prove the discrimination capacity of tested SSR markers for genotypes identification and also to show the degree of variability among the analyzed genotypes.

MATERIALS AND METHODS

Plant material. The tomato genotypes presented in Table 1 were studied at NR&DIB Ştefăneşti-Argeş, Romania. The seedlings were obtained by germination of selected seeds in plastic trays on peat substrate. All the quality seedlings, with normal development and a rich root system, healthy and hardened, were planted in unheated greenhouse conditions (the varieties with undetermined growth), or in field conditions (the varieties with determined growth).

Table 1. Plant materials used in this study, their origin and main features

Tomato genotype	Important agronomic characters
Indeterminate growth	
Costate 21 Selection from the Pablo variety	-fruit weight (about 300 g per fruit) -special aspect of the fruit (sweet pepper appearance) -rich foliage density
Ştefăneşti 22 Rila variety selection	-high productivity per plant (over 3 kg). -tolerance to tomato-specific pests
Ştefăneşti 24 Rila variety selection	-could be grown in the greenhouse and in the field -good tolerance to disease and pest
HA1 Hybrid	-high tolerance to tomato-specific diseases -ability to adapt to different environmental conditions (type of soil and temperature) -sweet fruits with special taste
HA2 Hybrid	-produces many fruits in the bunches -special taste, very sweet
HA3 Hybrid	-the elongated shape of the fruit (capsicum appearance) -could be grown in protected areas and in the field
Determinate growth	
Argeş 20 Selection from the Argeş 11 variety	-fruit weight (about 200 g) -commercial appearance -good tolerance to tomato specific pests and different environmental conditions
Argeş 11 NotoriusxHeinz2274	-few seeds in the fruit -good productivity per plant
HB4 Hybrid	-long storage time, over 30 days - tolerance to the main diseases -recommended for growing in the field
HB5 Hybrid	-firmness of the fruits -resistance to cracking and sunburn -needs reduced numbers of practical works
HB6 Hybrid	-good production potential -tolerance to specific diseases and pests -recommended for broth production
HB7 Hybrid	-stability and uniformity of the main features -high content of carotenoid and vitamin C in the fruit
HB8 Hybrid	-good resistance to transport and storage -suitable for organic crop and greenhouse condition

Morphological description. Throughout the growth and development of the plants, observations were made and the morphological characteristics of the plants of each variety were registered according to the Descriptors for Tomato (*Lycopersicon* spp.) published by the International Plant Genetic Resources Institute (1996). This characterization includes measurements of whole plant height, size of floral components, fruit morphology and weight. All observations and measurements were carried out as indicated by the IPGRI descriptors, at certain plant organs, and at a certain phase of development.

DNA isolation. Young leaves were collected from adult tomato plants for total DNA extraction. This was performed according to the method recommended by Ahmed et al. (2009) with few modifications (Bădulescu et al., 2020). The total DNA extracted samples were verified for their quantity and quality by using a spectrophotometer BioPhotometer plus (Eppendorf).

DNA amplification. From each DNA sample were used 3 µl for amplification with 8 different SSR primers: SSR T-7, SSR47, SSR T-62, SSR63, SSR110, SSR111, SLM6-7, SLM6-12. The reaction mix contained: 5 µl of 5x FirePol Master Mix Ready to load (Solis BioDyne, Estonia), 2 µl of each forward and reverse primer, 3 µl DNA template, and water to a total volume of 25 µl. The amplification process was done with Techne TC-512 Thermal Cycler as follows: initial denaturation of 4 min at 94 °C; 35 cycles, each with 1 min denaturation at 94 °C, 1 min for primers annealing at 55 °C, and 2 min for elongation at 72°C; the final extension of 7 min at 72°C, and amplified products maintenance at 4°C.

Electrophoresis and DNA products visualization. After amplification were taken 5 µl for each sample to migrate on agarose gel (3.0% agarose with TAE buffer, and stained with ethidium bromide), at 75 volts for 1 hour. The migrated bands were visualized and photographed with Gene Flash Syngene Bio Imaging system under UV light. To estimate the size of amplified DNA bands was used the ladder Quick-Load Purple 50 bp DNA Ladder.

Data collection and analysis. For each SSR marker were recorded all scorable bands (the amplified DNA products), and were calculated the percentages of polymorphic bands: (number of polymorph bands/total number of bands) x100. The Polymorphism Information Content (PIC value) was calculated with the formula recommended by Botstein et al. (1980):

PIC = 1-$\sum p^2$, where p represents each allele frequency for an SSR in all analyzed genotypes.
The amplified products were scored as present (1), or absent (0), and the similarity Dice coefficients were calculated for each genotype in comparison with all the others and for each marker (Dice, 1945). The similarity analyses and the dendrogram were done and produced using NTSYS-pc version 2.2. (Rohlf, 2000).

RESULTS AND DISCUSSIONS

Morphological characterization. All collected data with the main and distinct descriptors are presented in Table 2. In the first stage of evaluation, the seedling phase, we found that out of the total of 5 descriptors (7.1.1.), only for 2 descriptors (measurements for the length and width of the primary leaves) there were differences, the other characteristics regarding the green color of the hypocotyl, the intermediate coloration intensity and the presence of pubescence were the same in all genotypes.
In the case of genotypes with indeterminate growth, in greenhouse conditions, the plants were directed to a maximum height between 167 cm (Ştefăneşti 24) and 250 cm (Costate 21), and in the case of genotypes with determinate growth, the plant heights varied from 49 cm (Argeş 11 and Argeş 20) to 77 cm (HB7).
The morphological descriptors for stem and leaf (7.1.2.) highlighted several differences between genotypes: (i) the density of pubescence on the stem was slightly different between the genotypes with indeterminate growth (rare in HA2 and intermediate in the other 5 genotypes), and genotypes with determinate growth that expressed variations from rare to very dense (HB7); (ii) specific for the genotypes with determinate growth is the short length of the internodes, denoted by 3; (iii) dense foliage was noticed for the genotypes Costate 21, HA2 and HB7; (iv) the shape of the mature leaf is very different: potato-type (HB8), standard (Argeş 20, Ştefăneşti 22, HA1, HA2 and HB6), peruvian (Costate 21, HB4 and HB7), pimpinellifolium (Argeş 11, Ştefăneşti 24, HA3 and HB5).
A larger number of descriptors were used to characterize the inflorescences (7.2.1.), but obvious differences between genotypes were noticed only for the position of the style compared to that of the stamens. The Costate 21 variety stood out with its slightly exserted position at the moment of anthesis.
For a consumer, very important is the aspect of the fruit, its color, and shape.
The descriptors for the shape (7.2.2.5.) and size (7.2.2.6.) of the fruits revealed particularities of each tomato genotype: flattened (1) for Costate 21; slightly flattened (2) for Ştefăneşti 22, HA1, HB4, and HB7; round (3) for Argeş 20 and HB8; round elongated (4) for Ştefăneşti 24, Argeş 11 and HB6; cylindrical/long oblong (6) for HA2, HA3, and HB5. In correlation with this characteristic is the latitudinal cross-section shape of the fruit (7.2.2.29), which varied from irregular in Costate 21 variety, angular in Ştefăneşti 24 and HA3, to round in the other studied genotypes. Specific for each one is the color of the fruit at maturity (7.2.2.11), which varied from yellow for HA2, slightly orange for Ştefăneşti 24 and HB7, to red for the rest of described tomatoes. In correlation with this descriptor, the color of the pericarp (7.2.2.26) was noted, which varied from yellow (2) for HA2 and HB7 to orange (3) for Ştefăneşti 24, pink (4) for Ştefăneşti 22, HB4, and HB5, red (5) for all the other genotypes showing the same red external color of the fruit.
The differences among the harvested fruits of the same genotype in terms of fruit size (length /width), the average weight/fruit, and external color, were the basic criteria for assessing the homogeneity of fruit size, as follows: low (3) for Argeş 20, Ştefăneşti 24 and Costate 21; intermediate (5) for Argeş 11, HA1, HB4, HB5, and HB7; high (7) for Ştefăneşti 22, HA2, HA3, HB6, and HB8.
The descriptors for the average quantifiable evaluations regarding the weight and size of the fruit (7.2.2.8, 7.2.2.9, and 7.2.2.10), as well as the ones referring to the number of places, aspect of pistil scar, and fruit blossom end shape, are the specific ones of each genotype.
The presented results show the differences in terms of morphological descriptors among the analyzed genotypes, and the repetition of the records 3 years in a row proved the stability and genetic uniformity of the seed material.

Table 2. Evaluation of the morphological traits in tomato analyzed genotypes with standard descriptors

Genotype	Descriptors for tomato									
	7.1.2.1	7.1.2.2	7.1.2.3. (cm)	7.1.2.4.	7.1.2.5.	7.1.2.6.	7.1.2.9.	7.2.1.7.	7.2.2.5.	7.2.2.6.
Stefanesti 22	4	7	212	5	3	5	3	2	2	3
Stefanesti 24	4	5	167	5	5	5	5	2	4	3
Costate 21	4	7	250	5	5	7	4	3	1	3
HA1	4	7	198	5	5	5	3	2	2	3
HA2	4	5	175	3	3	7	3	2	6	2
HA3	4	7	210	5	5	5	5	1	6	4
Arges 11	2	3	50	3	3	3	5	2	4	3
Arges 20	2	3	50	3	3	3	3	1	3	5
HB4	2	3	69	3	3	5	4	1	2	5
HB5	2	3	65	5	3	5	5	1	6	3
HB6	2	3	65	5	3	5	3	1	4	3
HB7	2	3	77	7	3	7	4	1	2	5
HB8	2	3	68	5	3	5	2	2	3	3

Genotype	Descriptors for tomato								
	7.2.2.8. (g)	7.2.2.9. (mm)	7.2.2.10. (mm)	7.2.2.11	7.2.2.26	7.2.2.29.	7.2.2.31.	7.2.2.32	7.2.2.33
Stefanesti 22	199	6.6	7.1	5	4	1	5	2	2
Stefanesti 24	194	6.6	6.7	3	3	2	4	4	2
Costate 21	233	6.5	7.7	5	5	3	7	4	2
HA1	125	5.3	6.3	5	5	1	5	2	2
HA2	52	7.7	3.4	2	2	1	3	3	3
HA3	190	9.9	6	5	5	2	5	2	3
Arges 11	140	6.9	6	5	5	1	3	2	2
Arges 20	183	6.6	6.4	5	5	1	5	2	2
HB4	196	6.7	7.3	5	4	1	5	2	2
HB5	91	7.1	4.7	5	4	1	3	1	2
HB6	103	6.1	5.5	5	5	1	3	2	2
HB7	208	6.3	7.7	3	2	1	6	4	2
HB8	94	5.2	5.4	5	5	1	3	2	2

Genetic diversity using SSR markers. The reproducible amplification products obtained after PCR amplification were analyzed to evaluate the efficiency of the selected primers in confirming the genetic diversity of the analyzed tomato genotypes.

From the beginning, we chose to use only SSR primers that produce multiple bands with tomato varieties (Rajput et al., 2006; Saravanan et al., 2014). Also, for allele scoring, we considered the minor and shadow bands for proving the differences among genotypes, similar to Rodriguez et al. (2001), and Diklesh et al. (2016). After migration on agarose gel were noticed a total number of 315 bands, migrated at a distance corresponding to a base-pair number between 100 and 425. All the 8 tested SSRs had with the 13 tomato genotypes a mean number of 6.6 bands/primer and produced polymorphic bands in a proportion of 81.5%. Our results (Table 3) showed the efficiency of these SSRs for revealing the differences among analyzed genotypes. Moreover, the mean value for the polymorphic information content (PIC) for all SSRs of 0.764, is another reason to consider all tested SSRs as efficient markers in detecting the genetic polymorphism. The highest PIC values calculated for SSR 110, SSR 47, SSR 63, and SLM 6-7 are directly correlated with their highest values for the number of alleles and also, for the lowest value of the sum of the square for the allele frequencies of all genotypes. Thus, according to Serrote et al (2020), these markers could be considered the most informative ones for the efficient discrimination of the tomato genotypes.

The matrix with similarity coefficients (data not shown) was obtained by using UPGMA method, and the constructed dendrogram is presented in Figure 1. This dendrogram is based on the genetic similarity calculated for the tested SSRs and clearly revealed the clustering of tomato genotypes depending on their type of growth. The first cluster includes only genotypes with indeterminate growth (Costate 21, Ştefăneşti 22, Ştefăneşti 24, HA1, HA2 and HA3) and a genetic similarity ranging from 0.50 to 0.87. Distinct could be considered HA3, which has very low similarity coefficients with all the other tomato genotypes (0 with Ştefăneşti 24, or 0.25 with Ştefăneşti 22 and HA1, or 0.37 with HA2).

The second cluster grouped the tomato genotypes with determinate growth (Argeş 20, Argeş 11, HB4, HB5, HB6, HB7, and HB8), for which the similarity was ranging from 0.12 to 0.62. Within this group of genotypes, the HB4 hybrid is distinguished, with the lowest recorded values of similarity coefficients, from 0 to only 0.25 with the two varieties of Argeş.

Our evaluations with 8 polymorphic SSR markers, the data recorded in the matrix with similarity coefficients, and the obtained dendrogram, brought forward the following: (i) the two varieties of Ştefăneşti have a similarity coefficient of 0.5, both varieties coming from selections of the same variety, Rila; (ii) the similarity coefficient between the two Argeş varieties is only 0.25, due to the origin of the variety Argeş 20 from the variety Argeş 11, which is a complex hybrid.

Table 3. Results with relevant parameters proving the efficiency of tested SSR markers

Marker identifier	Band size intervals	Number of scorable alleles	Polymorphic alleles %	Polymorphic information content (PIC)
SSR 110	100-375	8	87.5	0.866
SSR 111	100-300	5	80.0	0.695
SSR T-7	100-275	4	75.0	0.635
SSR 47	100-350	8	87.5	0.806
SSR 62	100-425	6	83.3	0.734
SSR 63	100-350	9	85.7	0.847
SLM 6-7	100-400	9	77.8	0.837
SLM 6-12	100-350	4	75.0	0.697
Average±St.dev		6.62±2.1	81.5±5.2	0.764±0.08

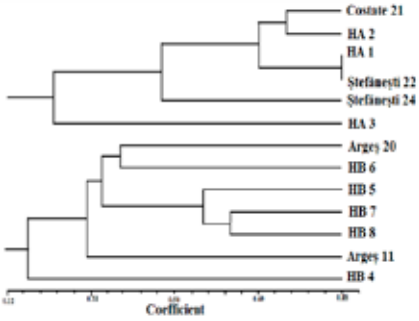

Figure 1. Dendrogram showing the genetic similarity among the 13 tomato genotypes derived by SSR markers

General considerations resulting from the research. The standard descriptors are the most used tool for the characterization and identification of tomato varieties (Vînătoru et al. 2016; Muşat et al. 2019; Salim et al. 2020). During the growing season different cultural conditions (soil nutrition, water supply, diurnal temperature variation, and different light spectral composition) are essential to optimize plant growth (Mihnea et al. 2019; Uleanu & Bădulescu, 2017; Vînătoru et al., 2015). Depending on these, the same genotype may have different morphological features. Thus, it is recommended to repeat at least 2 years consecutively the same procedure of analysis and evaluation of the plant organs from germination to fruit harvest, following a certain methodology. Similar to other economically important species, the assessment of genetic diversity among tomato varieties is performed based on morphological descriptors, and molecular characterization (Garcia et al., 2004). Starting from the generally accepted ideas that a high similarity value indicates a low degree of genetic variability (Arrufitasari et al., 2022), and that SSR markers producing multiple alleles per locus are considered suitable to characterize genetic diversity within or between populations (Kosman & Leonard, 2005), we consider the applied procedure to be appropriate for highlighting the genetic polymorphism among the analyzed tomato genotypes. The genetic similarity with the 13 tomato genotypes, based on 8 SSRs, ranging between 0.13 and 0.88, shows the great genetic variability among studied tomato genotypes and indicates a very useful genetic resource for breeding purposes.

Obviously, we agree with Benor et al. (2008), Kim et al. (2017), Castellana et al. (2020), Aziz et al. (2021) considering that a higher number of SSR markers would contribute to an even better characterization of the germplasm in tomatoes and to successful exploitation of the agronomically important traits.

CONCLUSIONS

The assessment of genetic diversity among 13 tomato genotypes with standardized morphological descriptors and molecular markers highlighted the high diversity of the main characteristics of the genotypes as a result of interaction between genetic information and the environment. Phenotypic diversity is valuable for a breeder, mainly for the selection of new genitors to improve the fruit quality traits and yield potential, but correlations with the genetic base of these traits increase its usefulness in breeding programmes.

The chosen SSRs for genetic evaluation and the results obtained after amplification confirmed that these markers are efficient and adequate for highlighting the distinctiveness among tomato genotypes. Interpretation of the results obtained with the eight SSR markers, as well as the similarity coefficients results, proved a very high degree of genetic diversity (between 13% and 88%) among the analyzed genotypes.

If the diversity of morphological characteristics is important for the curator (to identify accessions) and the breeder (to choose the appropriate material for certain breeding purposes), the assessment at the molecular level is that which brings certainty to the correct identification and characterization of tomato genotypes.

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