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Expressivity and Variability of the Cherry Tomatoes Main Characters from Core Collection of Plant Genetic Resources Bank Buzău

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RESEARCH ARTICLE

Abstract
The present study aims to phenotypically characterize cherry tomato varieties from core-collection to obtain a new morphotype with distinct phenotypic expressiveness and qualitative characters. Breeding procedures were carried out both in protected and open field for all genotypes under study and the breeding methods used were classical breeding, segregation, and selection. Following the breeding process, from the segregations obtained, a variety was selected in the F2 generation, genotype L 312 A, which proved to be phenotypically distinct. This genotype is part of the assortment of medium cherry tricolour tomatoes with high firmness, ovoid fruit weighing about 30 g on average, two seed lobes and a length/diameter ratio of 1-1.2. A new tricolour cherry tomato variety was genetically stabilized. It has distinct phenotypic expressiveness and superior quality traits. Prospects aim to approve and patent the variety.
Keywords: breeding, selection, segregation, morphotype, germplasm

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INTRODUCTION
Tomato ranks first among processed vegetables in the world (Prema, G., et al., 2011). Botanically classified as a berry, tomatoes originated in the South American Andes, ranging from northern Chile in the south, through Bolivia, Peru to Ecuador and Colombia in the north (Grubben and Denton, 2004; Bai and Lindhout, 2007). Initially, Peru had been proposed as the center of domestication of tomato, thus coinciding with its center of origin and genetic diversity. However, genetic evidence points to Mexico as the center of domestication, as modern cultivars appear to be closely related to a cherry tomato-like cultivar grown widely in Mexico and throughout Central America at the time of discovery by the Spanish (Ebert A.W. and Chou Y.Y., 2015). Cherry tomato (*Solanum lycopersicum var. cerasiforme*) is a botanical variety of the cultivated tomato. It is thought to be the ancestor of all cultivated tomatoes (Renuka D. M., et al., 2014). Cherry tomato is grown for its edible fruits; they are perfect for making processed products like sauce, soup, ketchup, puree, curries, paste, powder, rasam and sandwich. They also have good nutritional and antioxidant properties. The size of cherry tomatoes range from thumb tip to the size of a golf ball. And can range from being spherical to slightly oblong in shape. The possible exploitation of hybrid vigour in cherry tomato has been taken up at few research centres however very little systematic attention has been paid by plant breeders to study performance for yield and its components in cherry tomato. The genotypes performing well can be used further in heterosis breeding programme (Renuka D. M., et al., 2014). Tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*) is a highly autogamous species.

Cultivated tomato shows a low genetic diversity, but higher phenotypic diversity compared to S. pimpinellifolium (Miller and Tanksley, 1990) due to intensive human selection (Xu. J., et al., 2002). Although it is well known that cultivated germplasm resources provide an important genetic basis for both breeding and genetic research (Thornsberry et al., 2001). Conventional tomato (*Solanum lycopersicum* L.) descriptors are of great utility for gross morphological characterization but may not be practical for the precise fruit description required for distinguishing closely related cultivar groups (Figàs, M. R. et al., 2015). Most species within the *S. lycopersicum* complex can reciprocally hybridize with cultivated tomato, with the exception of *S. habrochaites* (Robertson and Labate, 2007). The loss of genetic diversity due to the replacement of local tomato varieties by improved cultivars has been in many cases mitigated by the collection and safe storage of germplasm in genebanks (Figas R. M. et al., 2015). It is a self-pollinating species with high genetic variability (Aguirre N. C., et al., 2017). Agricultural biodiversity is fundamental to production and food security, as well as for environment conservation (Corrado G., et al., 2014). Although it is well known that cultivated germplasm resources provide an important genetic basis for both breeding and genetic research (Flint G., et al, 2005). The evaluation of the diversity of a given collection can be based on phenotypic traits (Yan et al., 2007).

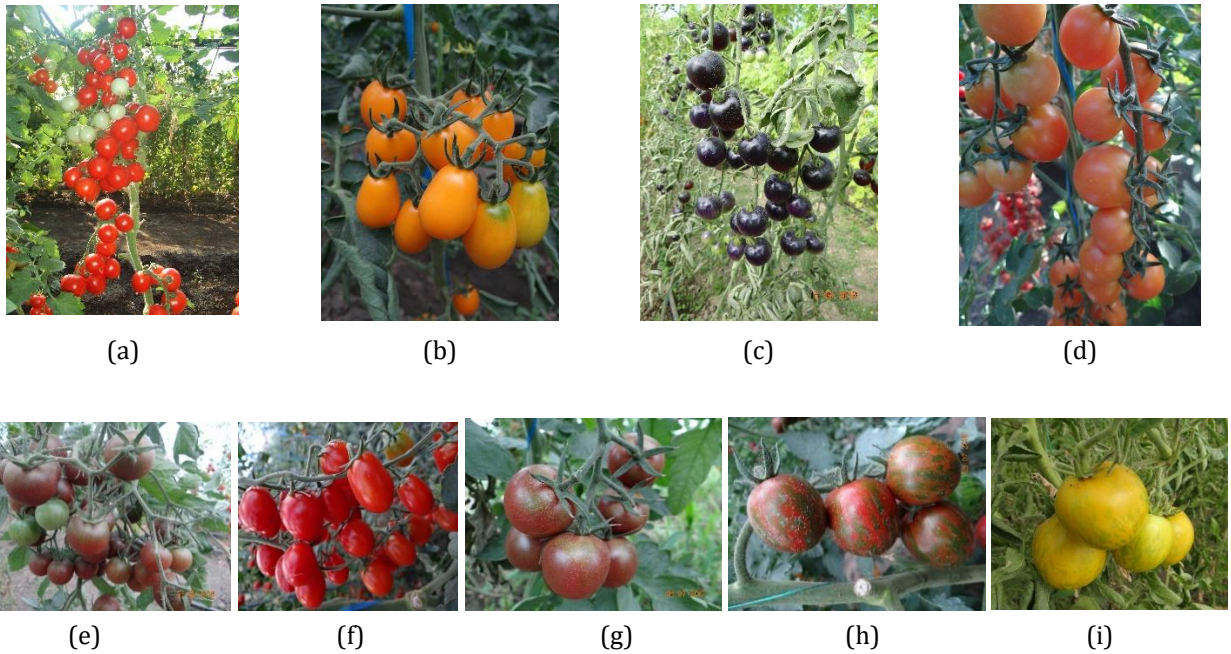
MATERIALS AND METHODS

The genetic material used in the present work consisted of the cherry tomato assortment from the BRGV Buzau germplasm collection. Nine lines with distinct phenotypic characteristics were selected and subjected to biometric measurements (Figure 1).

The methods used in the present study were classical hybridization, segregation and repeated individual selection. Hybridization consisted of the following steps: castration was performed when the petals began to open, forming an angle of 25-30° to the vertical axis of the flower and continued until the angle reached a maximum of 45°, the colour of the stamens was green. The procedure was carried out by grasping 2 petals with the fingers, as close to the base as possible, and by a vertical movement parallel to the flower axis the corolla was extracted, together with the stamens concentric to the lower part. Pollination was carried out by inserting the style into the pollen tube in such a way that the pollen grains covered the entire surface of the stigma. Pollinated flowers were marked with a specific mark. Pollen was collected directly from the pollen-generating crop for hybridisation. The stamens were harvested and dried for 15-20 hours at room temperature. The pollen obtained was placed in glass tubes of 3-5 cm length and 2-3 mm inner diameter. The pollen was used within 48 hours after extraction.

Selection was used as a method of genetic stabilization from F1 to F7, when one of the progeny resulting from hybridization was genetically stabilized.

The cherry tomato variety was grown using classical tomato breeding technology in two growing environments, both in the field and in protected areas. The L 312 progeny was genetically stabilized and characterized biometrically, phenologically, with harvests monitored in the field from July to September.



(a) (b) (c) (d)

(e) (f) (g) (h) (i)

Figure 1. Core collection cherry tomato assortment L 26(a), L 509(b), L 724(c), L 306(d), L 2013 A(e), L 80(f), L 311(g), L 709(h) si L 12(i).

Statistical analysis was performed using SPSS, ANOVA followed by Duncan's post-hoc test with 95% confidence interval and p-values < 0.05. Evaluation of the germplasm core collection was carried out by performing biometric measurements for the main characters based on the international UPOV and IPGRI descriptors. The amount of total soluble solids was measured by refractometer.

RESULTS AND DISCUSSIONS

Cherry tomato core collection biometric evaluation

The 9 lines taken in the study showed distinct phenotypic traits (Table 1). The accessions have indeterminate growth. L 26 is an accession characterized by pronounced firmness, concentrated ripening and an intense red gloss characteristic of the fruit. L 12 is distinguished by the colour of the fruit, green streaked with yellow and green flesh. L 509 are some of the sweetest cherry tomatoes, with bright yellow fruit, multiple truss showing concentrated ripening and a distinctive pyriform fruit shape.

L 2013 A is a black cherry tomato, with a distinctive, strawberry-like aroma and low firmness. L 724 are cherry tomatoes with strong anthocyanin colouring of the fruit, the immature fruit being black and at maturity turning cherry black with a characteristic red spot. L 80 are the sweetest cherry tomatoes in the germplasm collection, with high yields, reaching 1500 g per plant in the field. L 306 are orange cherry tomatoes with concentrated ripening and linear truss. L 311 are similar to the 2013 A accession but have larger fruit size, being part of medium cherry tomato group. L 709 is a medium tricolour cherry tomato, with high firmness.

Table 1. Main biometric characteristics of genotypes -averaged open field values

Accession	Plant height (cm)	Leaf lenght (cm)	Petiole lenght (cm)	Florets no./flowering stem (unit)	Fruit weight (g)	Fruit no./plant (unit)	Fruit height (cm)	Fruit width (cm)	TSS (°Brix)
L26	181.6±2.4 f	29.8±1.9 e	-	6±0.7 d	29.4±2.1 c	93.2±1.9 e	3.54±0.4 c	3.54±0.5 c	1.84±0.2 g
L12	55±1.6 g	15.34±0.5 g	-	4.4±0.5 e	80±1.6 b	25.4±1.1 i	5.56±0.2 a	5.98±0.1 a	4.9±0.2 f
L509	195.8±2.6 e	26.6±1.1 f	2.22±0.2 cd	8±0.7 b	9.82±0.1 g	30.2±1.3 h	2.8±0.2 d	2.9±0.2 de	8.18±0.2 c
L2013A	235±1.6 a	34±1.6 c	8.66±0.2 a	10±1.6 a	15±0.2 f	117±1.6 a	2.84±0.1 d	2.86±0.1 e	6.14±0.1 e
L724	210±1.6 d	35.8±0.8 b	2.54±0.1 c	6.4±0.9 cd	23.8±2.6 d	85.4±2.7 g	2.8±0.8 d	3.24±0.1 cd	5.8±0.2 e
L80	210±1.6 d	44±1.6 a	1.26±0.2 e	7.8±0.8 b	18.74±0.1 e	318±1.6 a	3.68±0.1 bc	2.14±0.1 f	11±0.2 b
L306	215.2±1.3 c	32.6±0.2 cd	2±0.2 d	7.4±0.5 bc	18.56±0.2 e	312.6±1.1 b	2.62±0.2 d	2.7±0.2 e	7±0.7 d
L311	211±1.6 d	25.2±1.3 f	3±0.7 b	8.4±0.9 b	40±1.6 b	110±2.2 d	3.5±0.2 c	4.22±0.1 b	5.52±0.1 e
L709	218±1.6 b	32.2±0.8 d	-	10.8±0.8 a	10.78±0.9 g	87.8±1.3 f	4.12±0.1 b	1.6±0.5 g	15.6±1.1 a

Note: Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$, 95% confidence level)

Cherry tomato hybridization

From the tomato assortment described above, L 26 C and L 12 accessions were selected to achieve the following hybridization: L26C ♀ x L12♂. This resulted in a number of 4 different progenies, similar to the parental forms, which were given code numbers as L 16, L 510, L 312 and L 524 of which L 312 was selected (Figure 2).

Within line 312 in F1 both indeterminate and semi-determinate but also intermediate forms appear. Thus, selection was continued, the line was grown until genetic stabilization and 100% indeterminate plants were obtained.

L26C ♀

X

L12♂

F1

(a) (b) (c) (d)

Figure 2. Cherry tomato hybridization and F1 generation: L 16(a), L 510 (b), L312 (c) and L 524(d)

Biometrical description of L 312

In F1, line 312 had 15% indeterminate forms (SP) and 23% semi-determinate forms (Sp), with 62% being intermediate forms that were eliminated. In F4 and F5, there was a marked decrease in semi-determinate forms, with the percentage of indeterminate forms increasing to 45% and 47% respectively. In F7, 312 accession was genetically stabilized, 100% SP plants being achieved (Figure 3).

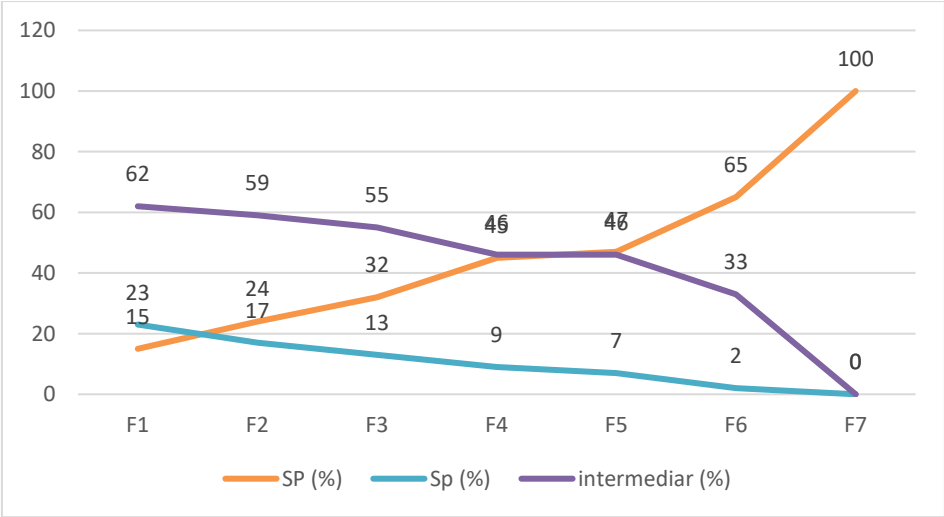


Figure 3. SP, Sp and intermediary plants evolution from F1 to F7

L 312 (Figure 4) was biometrically characterized according to Table 2 in two crop environments, both in the field and in the greenhouse. The accession showed indeterminate growth, with a height of 140 cm in the field and 220 cm in the greenhouse, with leaves of 27 cm average length, bipinnate leaves, fruits in linear trusses, with concentrated ripening, good firmness, aroma and taste typical of classical tomatoes. The immature fruits are light

green with dark green stripes and when ripe they become burgundy red with green and yellow streaks. The fruit has an average weight of 30 g and a height/diameter ratio of 1-1/2.

(a) (b) (c)

Figure 4. L 312: (a) plant detail, (b) immature and mature fruits, (c) fruit cross-section

The results of the Duncan test demonstrate the differences between the two growing environments and their influence on the measurable characters of the plants, identifying significant differences in plant height, leaf length and fruit weight features, which recorded higher values in the greenhouse than in the field. The other characters were not influenced by the growing medium, thus proving the typicality of the line.

Table 2. Main biometric measurements of L 312 both in field and greenhouse

Trial	Plant height (cm)	Leaf lenght (cm)	Fruit weight (g)	Fruit no./plant (unit)	Fruit height (cm)	Fruit width (cm)
L 312 F	138,4±27,7 [b]	27,8±4,02 [b]	30.5±1,71 [b]	174±95,8 [a]	3,8±1,16 [a]	3,5± 0,71 [a]
L 312 G	220,4±14,7 [a]	36,2±4,98 [a]	32,3±2,36 [a]	217,6±93 [a]	3,9±0,56 [a]	3,7±0,8 [a]

Note: Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$, 95% confidence level), F-open field, G-greenhouse

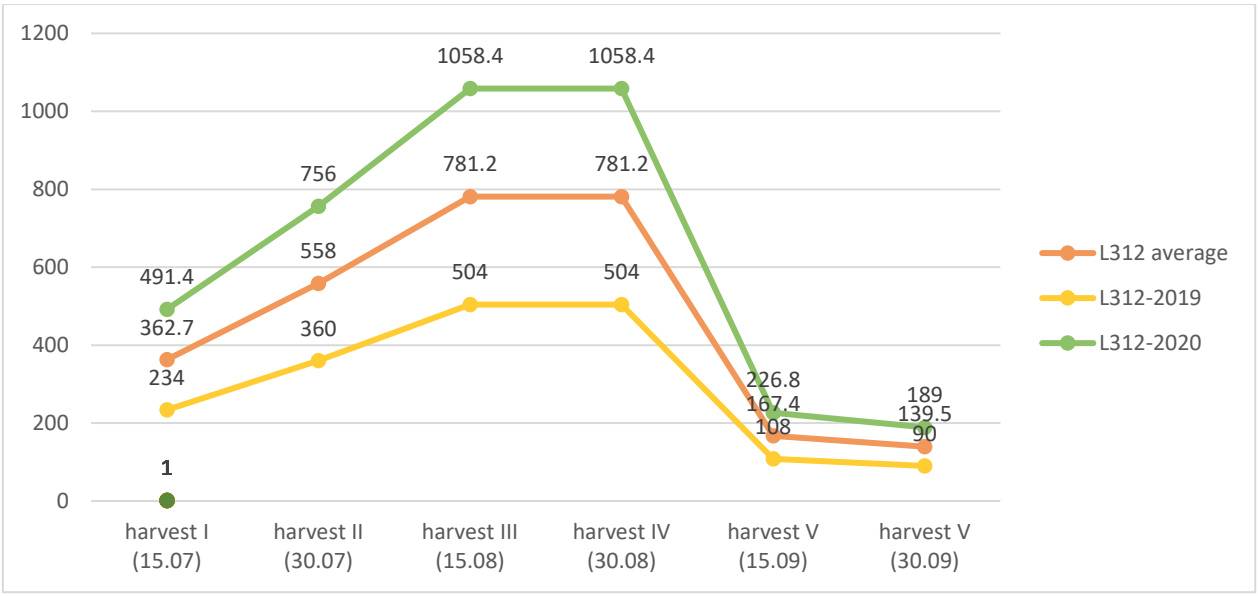


Figure 5. Open field harvest dynamics of L 312 (g/plant)

The staggered harvesting of field production has been monitored, which provides a dynamic that can be easily followed using Figure 5. Weighing was carried out during the growing season in two consecutive years, 2019 and 2020, and then averaged over the two years. Thus, L 312 had a staggered production from July to September, with a maximum production recorded in 2020 of 1058 g/plant.

CONCLUSIONS

The germplasm collection was evaluated phenotypically and biometrically. All 9 analyzed accessions showed characteristic distinctiveness composing a diverse range in terms of main morphological characteristics.

Two lines, L 26 and L 12 were selected to perform a hybridization which resulted in 4 progenies of which L 312 was of major interest in terms of obtaining genetic stabilization.

The line was subjected to selection from F1 to F7, with only the indeterminate growth forms being kept.

The cherry tomato variety has been enriched by obtaining genetically stable line 312 which will be proposed for approval and patenting at BRGV Buzau.

Author Contributions: Costel Vînatoru coordinated the entire study, being the holder of the *Solanum lycopersicum* spp. germplasm collection, Bianca Musat, Camelia Bratu and Geanina Negosanu contributed to the phenotypic observations, biometric measurements and Matilda Popescu and Florin Burlan performed the laboratory analyses (TSS).

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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