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Sorada - a tomato from grandma's garden a new hybrid created at BUASVM Timisoara

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Abstract **SORADA F1** is a tomato hybrid between two local landraces (Cruceni 102 ♀ × ♂ Rudna 143) from Timis County, created in order to combine their valuable traits, namely large, tasty and flavorful fruits, with a good tolerance to unfavorable conditions. The plant is very vigorous, with indeterminate growth, wealthy foliage, medium sized fruits and good firmness, uniform ripening, flavored, tasty and relevant nutritional qualities (vit. C, lycopen, Ca, Mg, Fe). The plants being tolerant to major diseases attacks provide the possibility to obtain reasonable yields without applying chemical treatments. The hybrid is suitable for cultivation both in the greenhouse as well as in the field, on supportive systems, using traditional crop techniques based on organic fertilization. As such, the hybrid is intended especially for traditional vegetable growers, organic and ecological farms.
Advantages. Being a hybrid adapted to the traditional organic and ecological farming, it contributes to environmental protection, by removing from the crop technology the chemical products such as fertilizers and pesticides. Also, the cultivation of this hybrid contributes to the increasing of the population health, being a traditional and organic product.
Applications. Thereby the consumer as the end user would benefit of healthy and high quality tomatoes "*from grandma's garden*", for both fresh consumption and processing.
Soarada exhibits the heterosis phenomenon for several important caracters: productivity, firmness of the pulp, sugar content, pericarp thickness, fruit height, caffeic acid and riboflavin content.

Key words

landraces, hybridization, heterosis, traditional farming, nutritional qualities

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops in the world. They are very widespread at the global level, accounting for 16% of the total area cultivated with vegetables. Tomatoes also recorded one of the most important increases in global production, 49% between 2000 and 2013. Tomato worldwide production is now 130 million tonnes, of which about 88 million tonnes are for fresh consumption and 42 millions of tons for processing. The top 5 tomato world producers are: China, EU, India, US and Turkey, covering about 70% of global production. China produced almost one-third of total tomato production, or about 43 million tons. In the European Union, Spain is the largest tomato producer for the fresh market, growing about a third of Europe's production. (https://www.eurofresh-distribution.com/news/around-world-tomat).

The growing demand of tomatoes year-to-year is driven by food consumption, the use of tomato-based products in medicine, cosmetics and pharmacy. In order to respond on market requirements, farmers expand the cultivated areas, and breeders are trying to create more and more performing cultivars (quantitative and qualitative).

Tomato (*Solanum lycopersicum*) originating from the Andean regions is among the species that has undergone intensive phenotypic and genetic selection[4]. The initiation of scientific breeding processes, the emergence of genomics, has triggered a real impulse in obtaining the data, knowledges and accurate tools that can be applied in creating of new genotypes and multiplying in tomatoes[2].

The main source of variability in tomatoes is represented by wild species, especially those self-incompatible such as *S. chilense* and *S. peruvianum*.

Compared to the rich gene pool of wild species, cultivated tomatoes have a low variability. Tomato cultivars and hybrid genomes are estimated to account for about 5% of the genetic variations in their wild relatives [13]. Therefore, at the phenotypic level, tomatoes had an opposite tendency manifested by the increases of diversity in the wild forms [14], especially in the case of morphological attributes of the fruits. Lack of genetic diversity in cultivated tomatoes can be highlighted by the use of DNA technologies. Very few polymorphisms within the cultivated tomato gene pool have been identified, even using sensitive molecular markers [15; 9; 22].

However, an important source of genes that is increasingly used in breeding programs of this specie is the local populations, the old varieties, adapted to the conditions of a specific pedoclimatic area [20]. Over the last decade, major advances have been achieved in exploring of genetic diversity in tomatoes, the efficient use of wildlife and local old populations to improve the main characters such as: adaptation to biotic and abiotic stress factors, yield components and fruit quality [3].

The existence of hundreds of wonderful tomato genotypes, adapted to grow and develop in almost all climatic conditions on Earth, in a wide variety of colors, sizes, shapes and uses, requires time and detailed research to select cultivars which are able to meets our needs.

For the creation of new cultivars, we must take into account several aspects in the process of selecting the genitors:

- the new cultivar must fit our purpose and needs.

What qualities should have the new cultivar ?

- to be vigorous and disease-resistant? Or is flavor more important than anything else?

All these depend on how it is used, for fresh consumption or for cooking, for processing and obtaining juice?

What are the growing conditions and challenges?

Romanian tomato growers will want varieties that grow well in low humidity and high insolation, genotypes that are known to be generally vigorous, tolerant to diseases and productive.

Tomatoes cultivar with indeterminate versus determinate growth?

Indeterminate tomato cultivars tend to produce longer vines and sprawling plants. Most of the famous local varieties are indeterminate. It can be more difficult to obtain good yields, especially under challenging soil and environmental conditions, with indeterminate varieties, but they can also produce indefinitely and over long periods of time,

Determinate tomato plants are usually more compact than indeterminate varieties; they grow until they flower and then set a single heavy crop. Determinate varieties are often favored by industrial processing of commercial farmers and anyone wanting a large harvest of tomatoes all at once.

Local varieties versus hybrids?

There isn't exact agreement on what makes a tomato variety an heirloom; generally heirlooms are old varieties, usually in cultivation for at least 40 to 50 years, are open pollinated, which means that when pollinated by themselves or by other plants of the same variety, they will come true from seed.

Heirloom tomato varieties tend to be less productive and produce a smaller quantity of fruits overall for a given planting area than hybrids. Their skins are usually thinner so that they crack and bruise more easily. On the other hand, local populations and old tomatoes varieties are better adapted to the biotic and abiotic stress-specific conditions of the area, are more tasty, flavorful and savory (https://www.gardenzeus.com/gardenzeus-guide-to-choosing-tomatoes).

Tomatoes hybrid tend to be vigorous and more productive, than local old varieties. It's important to select tomato genotypes that are resistant to diseases or pests that are prevalent in your local area.

Smaller tomatoes, are often but not always sweeter than larger-fruited varieties. Also light coloured tomatoes fruits (white, yellow, orange, pale red) have a high content of soluble sugars, being sweet, while the intense color (red, violet, blue, black) are more sour because of its high acid content.

The aim of this paper is to present a new tomato hybrid - Sorada – created at BUASVM from Timisoara and to describe the criteria that led to the choice of the genitors, the hybridization process techniques and some quantitative and qualitative characteristics of the fruits compared to the genitors, the mode of manifestation of the heterosis.

Material and Methods

The working method for obtaining the new cultivar was artificial intraspecific hybridization.

The research was carried out at Banat's University of Agricultural Science and Veterinary Medicine ”King Michael I st of Romania” from Timisoara (45.7489° N, 21.2087° E) in the greenhouses of the Department of Plant Physiology at the Faculty of Horticulture and Forestry in 2016-2017.

Hybridization technique

The emasculated of the motherline flowers is mandatory and is done before the opening of the sepals and petals (the sepals do not exceed an opening at 45 degrees to the axis of the flower).

The pollination itself consisted in depositing the paternal pollen on the mother flower stigma by means of a fine brush or by light touching the stigma of a smooth surface on which fresh pollen was shaken.

Immediately after pollination the motherline flowers were isolated in paper bags.

The pollen was harvested with a few minutes before of pollination because it has a short duration of viability (after harvesting it was used in maximum 20 minutes). At the beginning of the fruit formation, the isolation bag was removed and the fruit was labeled.

The parental lines used to obtain the hybrid Sorada (H5) were two local populations of Cruceni 102 (♀) × (♂) Rudna 143, identified in the traditional peasant farms in Timis county as part of the 2012 collection expeditions carried out through the research project " Screening for Salt Tolerance of Certain Local Vegetable Landraces for the Conservation of Genetic

Potential and Biodiversity "PN-II-PT-PCCA-2011-3, Contract. No.97 / 2012 financed by Romanian Executive Agency for Education, Research, Development and Innovation Funding. After collection, these cultivars were morpho-physiologically and molecularly characterized. They have been cultivated with traditional technologies for testing tolerance to drought, salinity, disease and pests, and then, based on results were used as genitors for obtaining new hybrids. In the first phase, 87 hybrid combinations were obtained, from which four hybrids were selected to be tested within the State Institute for Cultivars Testing and Approval (ISTIS) network for approval, Sorada being one of these hybrids .

Cruceni 102 (29)
Old, local population collected from the village Cruceni (45.4747° N, 20.8769° E) municipality of Foeni, Timis county.

Previously tested in comparative crops and selected as female genitor for taste and productivity.
Characteristics: cultivar with indeterminate growth, in protected spaces has a defective pollination, the inflorescence is made up of many flowers, on average 13 of which bind a small percentage of 25-30%, fruits are irregular size, with a variable weight between 80 and 180 g, firmness of the fruit is reduced, it is sensitive to transport, it does not have a good resistance to cracking, can not be kept very long time after harvesting, in cross-section the fruit has a 5-6.5 mm pericarp thick and a variable number of seminal vesicles 4-8; the fruit has an attractive commercial appearance, with a very pleasant, balanced taste and a 5-5.5% sugar content (BRIX).

a. b.

Fig.1. Cruceni 102 ♀ (a. inflorescence with green fruits, b. riped fruit)

Rudna 143 (52)
Old, local population collected from the village Rudna (45.4980° N, 21.0058° E), municipality of Giulvaz, Timis county.

Tested in comparative crops and selected as male genitor for tolerance to disease and pests, fruit firmness and productivity.

Characteristics: Cultivar with indeterminate growth, in protected areas has a very good pollination, the inflorescence forms on average 6-8 round fruits with an average fruit weight of 65-90 g; strong fruits, resistant to transport and cracking, with very good storage capacity after harvest (over 14 days at room temperature), in cross-section the fruit has a pericarp with a thickness of 5.5-6.5 mm and 3-4 seminal lobes; the fruit has an attractive commercial appearance, with relatively faded taste and increased acidity and 4.7% sugar content (BRIX)

a b

Fig.2. Rudna 143 ♂ (a. inflorescence with green fruits, b. riped fruit)

SORADA F1

Intraspecific hybrid of tomato obtained by crossing parental forms Cruceni 102 (♀) × (♂) Rudna 143.

Characteristics: Hybrid with indeterminate growth, with a very good pollination very well in greeuses, high tolerant to diseases and pests, reacts very well to unilateral organic fertilization, the inflorescence forms between 8-10 round fruits with an average of fruit weight 75-90 g; strong fruit with resistance to crawling and cracking, very good storage capacity after harvest (over 7 days at room temperature); in cross-section the fruit has a 6-7 mm thick pericarp and 2-3 seminal lobes; with a reduced number of seeds (60-80), visibly covered with fine golden pearls;

The fruit has an attractive commercial appearance, with a pleasant, balanced taste, it can be harvested and this peduncle is short and delicate, thus increasing the shelf life after harvesting; fresh, ripe fruits have 5.5-6% sugar (BRIX)

a. b.

Fig.3. Sorada F1 (a. inflorescence with green fruits, b. riped fruit)

Have been made comparative analysis of the main morphological characters of the fruit; fruit weight (FW-g), fruit diameter (FD-mm), fruit height (FH-mm), pulp firmness (PF-N) (Force Gauge Penetrometer PTR-200- PCE Instruments), seminal lodges number (LN-no), pericarp thickness (PT-mm).

Fruit production (g/plant) was also determined from the first four floors of fructification and the values of the main biochemical components that determine the fruit quality, taste and flavor; carotene (CA-mg/g); ascorbic acid (AA-mg/g); riboflavin (RI-mg/g); pyrocatechol (PI- mg/g); rutine (RU-mg/g); caffeic acid (CA-mg/g) ; lycopene (LY- mg/g) and sugar content (Brix) .

Sample preparation-lycopene and polyphenols from tomatoes (addapted from Motilva)

Samples of tomato (1 g) were grinded in a mortar and pestle. 10 ml solvent mixture n-hexane/acetone/ethanol (50/25/25, v/v/v) were added. The mixture was homogenised with a vortex for 1 min and centrifuged at 5000 rpm for 20 min at 5 °C. The supernatant was collected and the extraction repeated until a colourless supernatant was obtained.

The extract containing lycopene was kept in the dark!

Three extractions were necessary to complete extraction of the lycopene and the phenolic compounds from the tomato. The supernatants obtained from the extractions were put in an amber separation funnel and 5 ml of pure water were added. The funnel was shaken and two layers allowed to form; the upper layer (orange), the organic phase (hexane), containing the carotenoid, lycopene, and a lower layer (colourless) was the aqueous phase (acetone and ethanol) which contained the phenolic compounds. The two phases were collected in separate bottles, using an amber bottle for the the organic phase. The organic phase was again put in the separation funnel and 5 ml of pure-water was added to remove any residue of water-soluble compounds. After shaking, the two layers were collected in their respective bottles. After cleaning up the organic phase, the aqueous phase was put in a separation funnel and 5 ml of n-hexane was added in order to recover any residual lycopene. The separation funnel was shaken and again two layers allowed to form and collected in their respective bottles. This process was repeated one more time, and phases were collected and placed in their respective bottles. Ultimately, two bottles were obtained, the first containing the organic phase (lycopene), which was orange in colour, and the second containing the aqueous phase (phenolic compounds), which was colourless. Before analysis samples were filtered using 25 mm Syringe filters (0,45µm PTFE membrane).

HPLC analysis

Analysis was performed using an ultra high-performance liquid chromatograph (Nexera X2, Shimadzu, Tokyo, Japan) equipped with a diode array detector (M30A, Shimadzu, Tokyo, Japan) and a Nucleosil 100-3-C18 reversed-phase column (4.0 mm i.d. x 125 mm column length, 3 µm particle size, Macherey-Nagel GmbH, Duren, Germany).

For polyphenols from the aqueous phase

The column temperature was maintained at 30 ºC and the flow rate of 0.430 mL min^{-1}. The elution solvents used for the chromatographic analysis consisted of ultra-pure water with 0.1% TFA (A) and acetonitrile (B). The chromatographic elution program used was as follows: 95% A and 5% B, then the linear gradient grew to 42% B and maintained for 5 min, followed by a linear gradient of 35% B in 30 min. Afterwards, the eluent was changed to 95% A and 5% B linear gradient for 5 minutes. Total run time 35 minutes. The injected volume of samples and standards was 10 µL and it was done automatically using an auto-sampler. The spectra were acquired in the wavelength range: 200 - 600 nm.

For lycopene from the organic phase

The column temperature was maintained at 30 ºC and the flow rate of 0.500 mL min^{-1}. The elution solvents used for the chromatographic analysis consisted of methanol (A) and acetone (B). The chromatographic elution program used was as follows: 50% A and 50% B, then the linear gradient grew to 75% B and maintained for 3 min, followed by a linear gradient of 50% B in 3 min. Total run time 12 minutes. The injected volume of samples and standards was 10 µL and it was done automatically using an auto-sampler. The spectra were acquired in the wavelength range: 200 - 600 nm.

For carotenoids from the aqueous phase

The column temperature was maintained at 20 ºC and the flow rate of 1 mL min^{-1}. The elution solvents used for the chromatographic analysis consisted of ultra-pure water with 0.1% TFA (A) and acetone (B). The chromatographic elution program used was as follows: 25% A and 75% B, then the linear gradient grew to 100% B and maintained for 3 min, followed by a linear gradient of 75% B, the gradient was kept in 75% B, 5 minutes. Total run time 25 minutes. The injected volume of samples and standards was 10 µL and it was done automatically using an auto-sampler. The spectra were acquired in the wavelength range: 350 - 700 nm.

Spectrophotometric analysis were made using a double beam spectrophotometer Specord 200 (Analytik Jena AG, Germany), with a 10 mm quartz cuvette. Absorbance was measured at specific wavelength: for the organic phase: 450, 470, 503 nm, and for aqueous phase: 350, 470 nm.

Calculation of lycopene levels. Lycopene levels in the organic phase were calculated according to:

Lycopene (mg/kg fresh wt.) = (A_{503}x537x10x0.55)/(1x172)

where:

537 g/mole is the molecular weight of lycopene,

10 mL is the volume of mixed solvent,

0.55 is the volume ratio of the upper layer to the mixed solvents,

1 g is the weight of tomato added,

172 mM^{-1} is the extinction coefficient for lycopene in hexane.

Data were statistically processed using analysis of variance and t test. The significance of differences was expressed based on letters, being considered as significant the differences between variants marked with different letters[6].

The values of mid parent heterosis was estimated as formulated by[8]. Heterosis % = (F1-MP) x 100/ MP;, where F1, MP denote the performance of hybrid, and average performance of parents, respectively.

Results and Discussions

Considering the morphological characteristics of the fruit (weight, diameter and seminal lodges number) it is observed that the Sorada hybrid showed significantly equal values to those of the Rudna 143 cv. and significantly lower than the Cruceni 102. The height of the fruit and pericarp thickness in the Sorada hybrid showed significantly higher values than in the parental forms. As far as pulp firmness concerned, the Sorada hybrid recorded a close relative value to the motherline forms, associated to a significantly increased value compared with father.

Table 1

Values of some fruits traits for Sorada hybrid and its parents under greenhouse conditions during winter

Genotype	Traits	FW (g)	FD (mm)	FH (mm)	PF (N)	LN	PT (mm)
Rudna 143	$\bar{x} \pm s_{\bar{x}}$	91.60±8.90b	53.10±3.95b	45.70±3.72b	2.10±0.15a	2.0 b	6.20±0.24b
♂	$s_{\%}$	9.72	7.45	8.14	6.94		3.81
Sorada F_1	$\bar{x} \pm s_{\bar{x}}$	90.8±5.58b	54.14±2.49b	49.03±3.15a	2.02±0.20a	2.0 b	6.58±0.45a
	$s_{\%}$	6.41	4.60	6.42	9.87		6.87
Cruceni 102	$\bar{x} \pm s_{\bar{x}}$	104.4±6.95a	57.46±2.22a	47.18±0.78b	1.36±0.07b	5.67±0.33a	5.78±0.39c
♀	$s_{\%}$	6.65	3.87	1.65	4.82	5.88	6.76
	LSD 5%	7.17	2.15	1.55	0.37	1.90	0.38
	Heterosis	-7.35	-2.02	5.53	17.44	-39.13	9.73

The means with the same letters do not differ at P = 0.05

FW-Fruit weight; FD-Fruit diameter; FH-Fruit height; PF-Pulp firmness; LN-Seminal lodges number; BR-Brix; PT-Pericarp thickness.

The Sorada hybrid showed a positive heterosis for pulp firmness, pericarp thickness and fruit height, with values ranging from 17.44% for pulp firmness and 5.53% for fruit height. Also, there was a negative heterosis associated with lower parental average values for seminal lodge number, fruit weight and diameter, but for the last two characters the deviations from the mean parts were reduced and insignificant.

Regarding the plant production and its components (tab.2), it is noticed that the Sorada hybrid recorded values significantly higher than the parental forms, associated with increases of 784-1666 g. The superior production potential of the hybrid also manifested itself at the level of the four stages of fructification, on the background of higher significant increases for the first two floors. At the level of the last two floors the production of the hybrid was significantly higher than that of the father (Rudna 143 cv.)

Table 2

Values of plant yield for Sorada hybrid and its parents under greenhouse conditions during winter

Genotype	Plant yield (g)	Cluster 1 (g)	Cluster 2 (g)	Cluster 3 (g)	Cluster 4 (g)
Rudna 143 ♂	1736 c	616 b	447 c	395 b	277 b
Sorada F_1	3402 a	1332 a	833 a	720 a	517 a
Cruceni 102 ♀	2618 b	747 b	652 b	689 a	530 a
LSD 5%	783	358	181	168	134
Heterosis	56.31	95.51	51.50	32.73	28.30

The means with the same letters do not differ at P = 0.05

The productive performance of the hybrid is also underlined by the high positive heterosis values, so that the superiority of the hybrid to the parents' average was between 28.30% for the fourth floor and 95.51% for the first stage of fructification, being proportional to the moment of binding fruit (tab. 2).

Tomatoes and tomato products are rich in antioxidant compounds such as carotenoids (particularly lycopene), ascorbic acid and phenolic compounds.

In fresh tomatoes, sensorial quality analysis requires a complex and costly assessment, the sensory profile based on the taste of several trained experts. While some characteristics such as taste may be related to the ratio of soluble and organic acid content [10; 11], flavor and texture characteristics are difficult to quantify by instrumental analysis [17; 5]. In addition, most quality features present a polygenic heritage and are strongly influenced by environmental conditions.

The negative relationship between the soluble carbohydrate content and the size or yield of the fruit limits genetic progression[19].

The quality of fruit can be characterized by a number of physico-chemical properties, e.g., soluble solids content, titratable acidity, soluble solid/titratable acidity ratio, vitamin C content, lycopene and carotene content, caffeic acid etc[7].

Table 3

Values of some chemical compounds of the fruits for Sorada hybrid and its parents under greenhouse conditions during winter

Genotype	CA (mg/g)	AA (mg/g)	RI (mg/g)	PI (mg/g)	RU (mg/g)	AC (mg/g)	LY (mg/g)	Brix
Rudna 143 ♂	1.310 b	0.0534 b	0.0272 b	0.0556 a	0.00019 b	0.0037 a	0.0409 a	4.70 b
Sorada F_1	1.454 b	0.0561 b	0.0528 a	0.0346 b	0.00021 b	0.0035 a	0.0257 b	5.50 a
Cruceni102 ♀	1.671 a	0.0716 a	0.0352 b	0.0423 b	0.00039 a	0.0025 b	0.0381 a	5.17a
LSD 5%	0.171	0.0092	0.0123	0.01	0.0001	0.0006	0.0076	0.38
Heterosis	-2.45	-10.24	69.23	-29.32	-27.59	12.90	-34.94	11.49

The means with the same letters do not differ at P = 0.05

CA-Carotene; AA-Ascorbic acid; RI-Riboflavin; PI-Pyrocatechol; RU-Rutine; AC-Caffeic acid; LY-Lycopene.

Lycopene is the most abundant carotene in tomatoes and accounts for up to 90% of total carotenoids. Typical mature red fruits contain less β-carotene and other carotenoids. β-carotene appears in tomato fruits in different amounts from 0.23 to 2.83 mg 100 g-1 [1].

The Sorada hybrid showed a significantly higher riboflavin content compared with parental forms and a caffeic acid content close to the Rudna 143 and significantly higher than Cruceni 102. In terms of the carotene, ascorbic acid and routine content, hybrids recorded similar values with father and significantly inferior to the motherline.

Tomatoes are a valuable source of ascorbic acid (vitamin C); however, ascorbic acid in tomato fruit varies greatly, depending on genotype and cultivation conditions [18; 16].

The pyrocatechol content of hybrid and motherline was significantly inferior to the father's form. Concerning lycopene content, the hybrid exhibited significantly lower values for both parental forms.

Therefore, Sorada showed a positive heterosis for the caffeic acid and riboflavin content, with values between 12.90 and 69.23%. A negative heterosis associated with lower parental average values for the other biochemical components was found, with the limits of -2.45% for carotene content to -34.94% for lycopene content. The sugar content of the Sorada hybrid showed superior values to the genitors associated with a significant increase compared to Rudna 143.

Conclusions

Creating hybrids with high heterosis is a constant goal in the process of breeding tomatoes, but the best results are obtained when the parental forms are genetically removed, increasing the chance of complementarity and allelic heterozygosity in the cross.

Sorada is a hybrid that exhibits heterosis towards parents, both through productivity and in terms of the main morphological and quality characteristics of the fruit. The main qualities of the new hybrid are related to fruit firmness, high productivity, high content of soluble sugars and riboflavin, all associated with a very good tolerance to biotic stressors.

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