International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2012 IJFAS Journal-2012-1-4/91-96
ISSN 0000-0000 ©2012 IJFAS

In Vivo And In Vitro Production of Some Genotypes of Cherry Tomato Solanum Lycopersicum Var. Cerasiforme (DUNAL`)

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ABSTRACT: Cherry tomato is a variety that is poorly present at Macedonian fields, mainly due to the traditional habits of the consumers and the commercial tomato producers to grow tomato varieties with large fruit. Cherry tomato - *Lycopersicon esculentum* Mill. var. *cerasiforme* (Dunal) is a tomato variety with small fruit, while having different shapes and colors, and it is used mainly for fresh consumption. The features of this variety are portrayed its sweetness and aroma, which further enriche the taste of food. During this research, a comparative analysis of the morphological traits in this type of tomato in outdoor production conditions, as well as in protected environment was performed. The possibilities for production and maintenance in plant tissue culture *in vitro* were researched as well, with the goal of improving the morphological and biological features of cherry tomato.

***Keywords*:** Lycopersicon esculentum Mill. var. cerasiforme (Dunal), in vitro organogenesis, plant characteristics, fruit characteristics

INTRODUCTION

Tomato is one of the most important vegetable crop planted worldwide (Rick, C.M., 1980). The production, processing industry and trade of this crop represent businesses worth billions of dollars and provides employment for a huge number of people (De Vriend, H., 2011). Tomato production in the Republic of Macedonia is present on more than 5700 hectares, being the leading vegetable crop in the region.

Cherry tomato however, is extremely poorly present at our fields, mainly due to the traditional habits of the consumers and the commercial tomato producers to grow tomato varieties with large fruit and the lack of available information on cherry tomato production. Rodriguez, G., (2007) names three types of tomatoes exist: cultivars for fresh consumption, cultivars for processing and cherry tomatoes. Cherry tomato - *Lycopersicon esculentum* Mill. var. *cerasiforme* (Dunal), is a tomato variety with small fruit, with different shapes and colors and it is used mainly for fresh consumption. Stertz, S.C., et al., 2005, describe cherry tomato fruits as juicy and meaty berry, similar to cherry, with red color at maturation and bigger than 1.5 cm in diameter. Characteristic for this variety is its sweetness and aroma, which further enriches the taste of food. During this research, analysis of the morphological traits of the fruits of this type of tomato in in vivo production conditions was conducted, in an attempt to select for the genotypes which fulfill the local markets demands and eligible for commercial production. Quality of tomato fruit is a category that is defined on the basis of what their purpose is (He, Y., et al., 2005). Fruit quality of cherry tomatoes, when being used as fresh vegetables is determined accordingly to the content of chemical components such as: dry matter content (in degrees Brix), total content of sugars, organic acids and other organic compounds (Thybo, A.K., 2006). Since the content of total sugars and acidity are largely responsible for the taste of tomato the focus of research of the quality of tomatoes should be positioned on these components. Increasing the content of total sugars and acidity contributes significantly to the sweet and sour taste of tomatoes (Stevens, M.A., et al., 1979). Beside these, in their composition tomatoes contain a number of other organic compounds in low

concentrations that have a major impact on their flavor (Baldwin, E.A., et al., 1991).

In addition, the possibilities for production and maintenance of cherry tomato in plant tissue culture were researched, with the goal of further improvement of the morphological and biological features of cherry tomato. Tissue culture is a very important technique these days for rapid obtaining pathogen free plants. Two promising genotypes of cherry tomato have been placed in culture *in vitro* in order to evaluate their ability to regenerate and the effect that different cherry tomato genotypes and different plant growth regulators have.

MATERIALS AND METHODS

Seeds from four cherry tomato genotypes (Ch 1/4; Ch 1/5; Ch 7/2; Ch 9/2) were obtained from the genebank at the Goce Delcev University (Figure 1). Sowing was in open plastic trays with dimensions 50.7 x 33 x 4.4cm and 70 cells, on April 15th, 2010. After the first true leaves developed, the seedlings were transferred to square pots size 8 x 8 cm filled with mixture of peat and perlite in 3 : 1 ratio by volume. Seedlings were transplanted on June 6th 2010 at plant spacing of 35cm in rows and 80cm between rows. Standard cultural practices for tomato were applied during the experiment. Length and width (diameter) of the fruit was measured in at least 10 fruits of each genotype.

Isolation of initial explants

As an initial material to work from the meristem explants, apical buds and meristem with size up to 3 mm for the buds, and 0.5 mm for the meristem were used. From the non meristematic explants were used whole cotyledones or parts from cotyledons, hypocotyls, nods, internods and peper anthers for haploid regenerants. All initial explants isolated from seeds were previously subject to sterilization.

Sterilization of plant material

Cherry tomato seeds were sterilized with tap water for ten minutes, and then were immersed in distilled water for 2 hours. The seeds were surface sterilized for 15 seconds in 70% C_2H_5OH, following 10 minutes in 1% NaClO, followed by few rinses in sterile distilled water.

Plant growth medium ingredients

The surface sterilized cherry tomato seeds were germinated on MS medium (Murashige, T., and Skoog, F., 1962) containing 30 $g \cdot l^{-1}$ sucrose, 0.7% agar, 100 $mg \cdot l^{-1}$ inositol, 200 $mg \cdot l^{-1}$ casein hydrolysate, 0.1 $mg \cdot l^{-1}$ vitamin B_1, 1.0 $mg \cdot l^{-1}$ vitamin B_6 и 0.5 $mg \cdot l^{-1}$ nicotinic acid. The following phytohormones were used: IAA-indole-3-acetic acid, NAA-α-naphtaleneacetic acid, BAP-6-benzylaminopurine, KIN kinetin-6-furfuryl aminopurine, 2iP-N^6-2-isopentyl adenine) and 2.4 D-2,4-dichlorophenoxyacetic acid. The initial explants from Ch 1/4 and Ch 1/5 were cultivated on MS medium with the following combinations and concentrations of phytohormones:

MS1 = MS + 2.0 $mg \cdot l^{-1}$ BAP + 2.5 $mg \cdot l^{-1}$ 2.4 D
MS2 = MS + 2.5 $mg \cdot l^{-1}$ BAP + 1.5 $mg \cdot l^{-1}$ NAA
MS3 = MS + 2.0 $mg \cdot l^{-1}$ 2iP + 0.5 $mg \cdot l^{-1}$ IAA
MS4 = MS + 0.5 $mg \cdot l^{-1}$ KIN + 1 $mg \cdot l^{-1}$ IAA.

Initial explant isolation

Initial explants were excised from 2-3 weeks old plants. Apical buds, hypocotyls and cotyledons with 1-3 mm size, were excised and cultured on MS medium supplemented with varying concentrations of different plant growth regulators.

Growth conditions

All tissue culture were kept in climate chamber with controlled conditions:

- temperature of 25 ± 2ºC,
- 16h photoperiod,
- 50% relative humidity, and
- 50 μmol m^{-2} s^{-1} light intensity.

Total sugar determination

Total sugar content was determined by the phenol-sulphuric acid method (Dubois, M., et al. 1956) Absorbance was measured with a JENWAY 6300 spectrophotometer at a wavelength of 480 nm and total sugar content was calculated on fresh weight basis.

Total acidity

Titratable acidity was estimated by a standard titrimetric method. 20 grams of extracted juice was mixed with 250ml of distilled water. In the presence of phenolphtalein as an indicator, the mixture was titrated by adding 0.1 N NaOH until the break of light pink color. The volume of NaOH added to the solution was multiplied by the correction factor of 0.064 for the calculation of total acidity as percentage of citric acid.

Dry matter determination

Dry matter was determined with a hand-held refractometer KRUSS HR10, calibrated with distilled water. Measurements were made on at least 10 fruit at botanical maturity, taken from the second cluster. Each fruit was longitudinally cut at two and juice was extracted. Approximately equal number of drops of tomato juice was placed on the prism of the refractometer and the percentage was directly noted from the measuring scale of the instrument.

Statistical analysis

All results obtained during the research were statistically processed and analyzed using analysis of variance (ANOVA) and Duncan's post-hoc comparison of differences between means at 0.05 probability level with the statistical software IBM SPSS Statistics 19.

RESULTS

Morphological characteristics of fruit

The results from the analysis of the morphological traits of the fruits of cherry tomato are most important from production point of view. Differences between genotypes for the morphological traits fruit weight, fruit width, fruit length and fruit index were observed. Fruit weight varied in the range 14.81 - 28.92 grams with the tomatoes grown at open field and 14.80-31.56 grams for the cherry tomato genotypes grown under plastic. Regarding individual genotype performance, with both growing environments genotypes Ch 7/2 and Ch 1/5 had largest value for fruit weight, while genotype Ch 9/2 had the lowest weight registered in both growing environments.

The means for fruit width (4.03 cm) and fruit length (3.76 cm) of the fruits from the genotypes in protected environment varied within a narrow range for all genotypes and were higher in comparison to fruits grown under plastic tunnel (Table 1 and 2).

As all genotypes were with red color and round fruit shape, the fruit shape and color of the fruits in our research proved as the most stable properties, along with pericarp thickness and number of locules.

Chemical composition

In our tests the content of total sugar varied in the range 2.84 - 3.26%. Maximum content of total sugars was observed with genotype Ch 9/2 (3.26%) and Ch 1/5 (3.20%), and lowest for genotype Ch 7/2 (2.84%). According to these results, all genotypes in our study can be considered as genotypes with high content of total sugar. Differences among genotypes for titratable acidity of cherry tomato were significant and varied from 0.26% with genotype Ch 9/2 to 0.42% with Ch 7/2 (Table 3).

In addition, there were significant differences between the dry matter content in our experiment in the content of soluble solids. The highest value was obtained with genotype Ch 1/5 (6.78%) and Ch 9/2 (4.75%), followed by the control variety C1 with 4.80% and Ch 1/4 (4.33%). Genotype Ch 7/2 showed smallest value for dry matter content of 3.10% (Table 3).

In vitro culture of cherry tomato

Highest percentage of root formation was observed when apical buds were used as explants on the MS + 2.0 $mg \cdot l^{-1}$ 2iP + 0.5 $mg \cdot l^{-1}$ IAA medium while MS + 2.0 $mg \cdot l^{-1}$ BAP + 2.5 $mg \cdot l^{-1}$ 2,4 D showed to best for shoot formation. Medium MS + 2.5 $mg \cdot l^{-1}$ BAP + 1.5 $mg \cdot l^{-1}$ NAA turned out to be the best media for root formation (Table 4).

DISCUSSION

In early fruit development phase, many factors - physiological, hormonal, genetic and plant nutrition itself, may affect fruit growth and development (Stertz, S.C., et al., 2005). Domestication and breeding of cultivated tomato resulted in the creation of varieties with different shapes and sizes of fruit (Paran, I. and Van der Knaap E, 2007). The fruit shape and color of the fruits in our research have proven as the most stable properties. All genotypes were with red color and round shape. These results are consistent with those Koleva Gudeva, L. and Trajkova, F., 2010, obtained. The color of the fruit is one of the most important and most complex fruit characteristics of cherry tomato. The complexity of this property tomato arises from the presence of different carotenoid pigment system whose appearance is determined by pigment type and concentration, and is subject to genetic and environmental regulation (Arias, R., et al., 2000; Lopez Camelo, A.F. and Gomez, P.A., 2004).
The size and shape of the tomato fruit, as the number of locules per fruit and fruit index are very important in the selection of tomatoes for fresh consumption (Maluf, W.R., et all., 1989). Bertin, N., 2005, notes that high temperature may influence fruit growth through indirect effects on plant development, maintenance respiration and assimilate availability. The fruits from the plants in the plastic house in our experiment showed higher average of fruit weight, which could be consequence to the higher temperatures maintained durind season in the protected environment. High relative humidity can reduce plant transpiration, and this was another factor in the protected environment, as it can reduce plant transpiration and cause differences in fruit weight.

Fruit index was calculated as the ratio between the length and width. Contrary to fruit weight, fruit length and width showed to have higher values in the fruits from the plants grown in open field over fruits grown in protected environment. This could be attributed to the altering growing factors as Warnock, S.J., 1990, suggests that the variation in the length and width (diameter) of the fruit, is genetically conditioned by environmental factors such as temperature and humidity.
Tomato fruits, except cherry tomato which are bilocular usually have more locules. All genotypes in our research were bilocular.

One of the biggest challenges for plant breeders is creating varieties of tomatoes that will meet the expectations of consumers regarding their organoleptic quality. (Carli, P., et al. 2011). More research (Malundo, T.M.M., et al., 1995; Petersen, K.K., et al., 1998) show that the organoleptic properties in tomatoes are influenced primarily by carbohydrates and organic acids.

Despite the influence of genotype, the chemical composition of fruits is under the direct influence of environmental factors (Venter, F., 1977). Changes in light

intensity, temperature and relative humidity in the cultivation of tomatoes in protected environment can affect the photo-asimilats and consequently leads to changes in the chemical composition (Bakker, J.C., 1995).

According to our results, all genotypes in our study can be considered as genotypes with high content of total sugars. The high sugar content in tomato fruit is of great importance for the flavor of the tomatoes (Kader, A.A., 1986). Since cherry tomatoes are cultivars intended for fresh consummation, sugar content is very important trait. Tests for sugar content during tomato fruit development show that their content progressively increases during maturation of the fruit, with sugar accumulation is most intense with the first appearance of yellow pigment in the walls of the fruit (Beltran, E.G. and Macklin, K.E., 1962). The results for the content of sugars in our study were significantly different and in line with those reported by Jongen (2002), where total sugars varied from 2.19-3.55%, and those reports by Turhan, A., 2009 (1.67-3.73%).

Titratable acidity of the fruit follows the same dynamics of accumulation in the fruit as sugar content. In our experiment genotype Ch 7/2 had highest value for titratable acidity (0,42 %) followed by genotypes Ch 1/5 (0.39 %) and 1/4 (0.33 %). Genotype 9/2 had lowest value for this parameter of 0.26 %. Other authors (Turhan, A., 2009; Salunkhe, D.K., and Desai, B.B., 1984) report similar values for total acid content. Total acids content in the fruit of tomato reaches its peak at the moment of first occurrence of yellow pigmentation, followed by progressive reduction of acidity as ripening continues (Winsor, G.W., et al., 1962). After harvest, the decline of the content of acids and soluble substances are associated with the decline in the quality of tomatoes (Zapata, P.J. F., 2008) and affects the perception of consumers about the quality of the fruit.

Between the genotypes in our studies, significant variation was observed with parameter dry matter content. The content of dry matter varied from 3.64% to 7.07%. The percentage of dry matter can vary within a broad framework and is in direct correlation with the genotype (Jongen, W., 2002). Petro-Turza, M., 1987, notes that the average dry matter content of fresh tomatoes should be at least 5%. In accordance with this, genotype Ch 1/5 and Ch 9/2 meet this criterion. Among other things Rodica, S., et al., 2008, indicate that dry matter content is an essential parameter for assessing the quality of fresh tomato. DePascale, S., et al., 2001, states that the high content of dry matter in tomato fruit is essential in the processing industry, because it increases the quality of the final products.

When 1/3 segment of a cotyledon was used as an explant source, genotype Ch 1/5 showed largest potential for callus formation on MS3 and MS4 media. This result is consistent with those of Chaudhry, Z., et al., (2004), where the largest percentage of callus formation was obtained on medium with added concentration of hormones of 2 $mg \cdot l^{-1}$ IAA + 2 $mg \cdot l^{-1}$ BAP or 2 $mg \cdot l^{-1}$ NAA + 4 $mg \cdot l^{-1}$ KIN.

Shoot formation was successful on media with various concentrations of IAA, NAA and BAP. Genotype Ch1/5 showed highest percentage of shoot formation on MS2 media, where apical buds were used as an explant source (Figure 2).

Davis, D.G., et al., (2004) noted regeneration from hypocotyl on a media which with much higher concentrations of IAA (1.0 $mg \cdot l^{-1}$) and BAP (7.0 $mg \cdot l^{-1}$). Jatoi, S.A., et al., (1997) reports for regeneration from leaf explants on media with even higher concentrations of IAA (1.5 $mg \cdot l^{-1}$) and BAP (8.0 $mg \cdot l^{-1}$) than the concentration we used.

In general, from all the cultured explant sources from cherry tomato-apical buds, hypocotyls and 1/3 segment of a cotyledon, the apical buds demonstrated highest potential for regeneration and organogenesis. Both of the tested cherry tomato genotypes Ch 1/4 and Ch 1/5 demonstrated high shoot formation potential of 83.33% on MS3 and 80.00% on MS2 media respectively.

Table 1. Results from the morphological characteristics of the fruit in open field conditions

Genotype	Fruit colour	Friut shape	Friut weight(g)	Friut width(cm)	Friut length (cm)	Fruit index length/width	Thickness of pericarp (cm)	Number of locules
Ch1/4	red	round	27.32b	4.12bc	3.60bc	0.87b	0.32b	2.10b
Ch1/5	red	round	16.14c	4.44b	4.34ab	0.97a	0.31b	2.30b
Ch7/2	red	round	28.92b	4.19bc	3.86bc	0.92ab	0.31b	2.20b
Ch9/2	red	round	14.81c	3.37c	3.24c	0.96a	0.32b	2.10b

Table 2. Results from the morphological characteristics of the fruit in protected environment

Genotype	Fruit colour	Friut shape	Friut weight(g)	Friut width(cm)	Friut length (cm)	Fruit index length/width	Thickness of pericarp(cm)	Number of locules
Ch1/4	red	round	25.70bc	3.69c	3.51bc	0.95a	0.34ab	2.30b
Ch1/5	red	round	31.56b	4.41b	3.99b	0.91a	0.33ab	2.20b
Ch7/2	red	round	20.44c	3.65c	3.44bc	0.94a	0.30b	2.00b
Ch9/2	red	round	14.80d	3.28c	3.04c	0.92a	0.25b	2.10b

Table 3. Biochemical characteristics of cherry tomato fruits

Genotype	Total sugars (%)	Titratable acids (%)	Dry matter (%)
Ch1/4	3.10ab	0.33b	4.80c
Ch1/5	3.20a	0.39ab	7.07a
Ch7/2	2.84b	0.42a	3.64d
Ch9/2	3.26a	0.26c	5.12b

Table 4. Callus induction and regeneration percentage of different cherry tomato lines

Genotype	Media	Apical buds			Hypocotyls			1/3 Cotyledons		
		Rooting %	Shoots %	Callus %	Rooting %	Shoots %	Callus %	Rooting %	Shoots %	Callus %
Ch1/4	MS1	5.26	5.26	0.00	0.00	0.00	26.31	0.00	0.00	13.04
	MS2	0.00	11.76	5.88	5.88	0.00	5.88	5.26	0.00	31.58
	MS3	0.00	83.33	0.00	0.00	0.00	33.33	0.00	0.00	3.57
	MS4	0.00	20.00	20.00	0.00	0.00	20.00	3.70	0.00	7.41
Ch1/5	MS1	0.00	10.00	0.00	0.00	20.00	0.00	0.00	0.00	3.33
	MS2	0.00	80.00	10.00	0.00	0.00	30.00	10.00	0.00	20.00
	MS3	25.00	10.00	0.00	0.00	25.00	0.00	0.00	0.00	54.54
	MS4	0.00	66.67	0.00	0.00	0.00	33.33	0.00	0.00	30.77

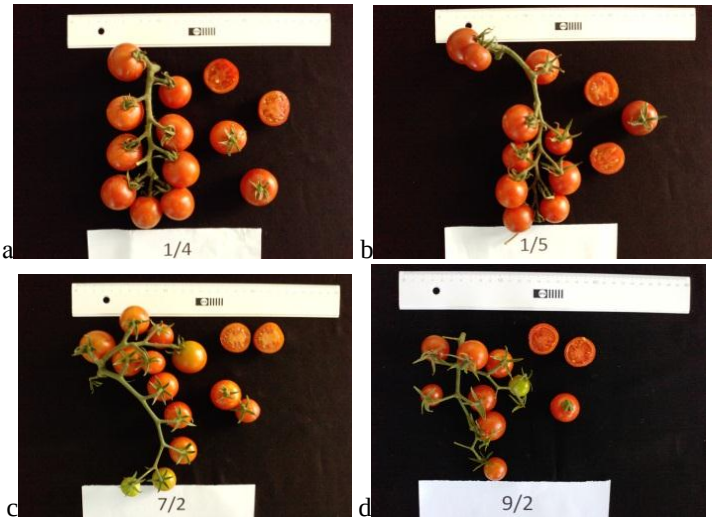

Figure 1. Genotypes fruit cluster a) Ch1/4, b) Ch1/5, c) Ch7/2 and d) Ch 9/2

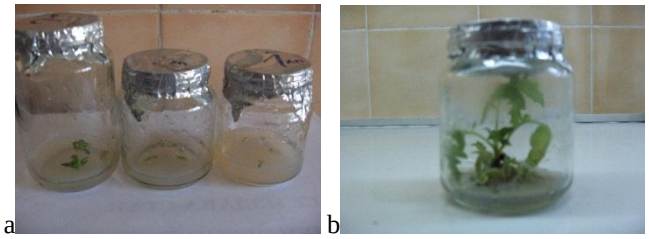

Figure 2. Development of initial explants of cherry tomato a) initial apical buds on MS medium, b) development of plantlet from apical bud on MS2 medium at Ch1/5 genotype

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