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Evaluation of Some Biochemical Properties in Different Tomato Genotypes Obtained From Tissue Culture Technique

Mahran Mokhtar El Nagar — Horticulture Department, Faculty of Agriculture, Research Park (Biotechnology Lab.), Benha University, Egypt.

Enas Mahmoud Mekawi — Agricultural Biochemistry Department, Faculty of Agriculture, Research Park (Biotechnology Lab.), Benha University, Egypt.

ABSTRACT

A laboratory study was conducted to develop a broadly applicable in vitro regeneration method for tomato. Therefore, ten different tomato genotypes were analyzed with regard to their efficiency for regeneration in vitro. Cotyledons were used in vitro from young seedlings as target tissue for regeneration of tomato. Healthy multiple shoot regeneration was obtained from explants of cotyledon cultured on MS medium containing 2.0 mg/l BAP and 0.1 mg/l IAA. The maximum root induction from the regenerated shoots was achieved on half the strength of MS medium supplemented with 0.1 mg/l NAA. Regenerated plants appeared morphologically normal and set flowers and fruits with seeds that could germinated normally. Results strongly showed that there were significant differences between tomato genotypes. Generally the highest values of all regeneration parameters were obtained from genotypes Pantano Romanesco and Strain B. Genotype Ponderosa Seligocia had the lowest regeneration parameters while other genotypes were moderate response.

The nutritional and antioxidant composition of regenerated ten tomato genotypes at full ripe stage was determined. The analysed components included ascorbic acid, phenolic compounds, total sugar, lycopene and β-carotene contents. Results indicated that the potential efficiency of biochemical compounds vary considerably with genotype. Genotype Strain B had the maximum content of ascorbic acid (19.3 mg/100 g fresh weight). Genotype Red Stone had the highest phenolic content of 34.4 mg/100 g fw at their full red ripe stage. The highest tomato genotype in present investigation of total sugar content was Impreal (4.5%). Petomech genotype was a promising genotype in terms of lycopene content (13.3 mg/100 g fw), while the yellow tomato genotype Pomodoro Banana Giallo had the highest content of β-carotene (4.6 mg/100 g fw). Overall, these tomato genotypes could contribute as sources of important nutritional and antioxidants related to the prevention of chronic diseases associated to oxidative stress, such as cancer and coronary artery disease.

KEYWORDS : Tomato, in vitro regeneration, antioxidant compounds, ascorbic acid, phenolics, carotenoids.

Introduction:

Tomato (*Solanum lycopersicum*) is one of the most important vegetables grown in Egypt and many other regions around the world (El Nagar, 2013; Verma *et al*., 2015). Egypt's population grows with an annual birth rate of 2.7%. The number of Egypt's population by the year 2025 will rise to 110 million. Keeping in mind the future demands of tomato for the Egyptian and human population, there is an immediate need to improve the production and productivity of tomato utilizing the advances in the areas of biotechnology and tissue culture (Rosati *et al*., 2000; Sarker *et al.,* 2009).

The conventional seed propagation method of new cultivar production and yield enhancement is less successful because of longer time requirement for cultivar development and several existing biotic and abiotic stress in normal field conditions. Moreover, the conventional method of tomato plant propagation using seeds is restricted by the short span of viability and low germination rate of seeds as well as the high price of hybrid seeds. *In vitro* plant regeneration is essential for the rapid multiplication of disease free planting materials shorten time period for cultivar development and is an imperative for the application of biotechnology tools to plant breeding and genetic improvement (Christou and Klee, 2004). It is also important for the conservation of genetically pure planting materials. Improvement of tomato variety in terms of increased antioxidant compounds, enhanced nutrient content and resistance to biotic and abiotic stress can be achieved through genetic manipulation using plant genetic engineering approach. An efficient *in vitro* regeneration protocol for tomato plants is a prerequisite for high production of transformed plants (Oceania *et al*., 2015). There are reports of successful tomato regeneration *in vitro* using different explants (Bhatia *et al*., 2004; Mamidala and Nanna, 2011; Chandra *et al*., 2013; El Nagar, 2013).

The direct and positive relationship between health and diet has now attracted the attention of plant breeders and biotechnologists who are directing their efforts to breed genotypes with high content of phytochemicals (El Nagar and Mekawi, 2014 a and b; El Nagar and Mekawi, 2015). The tomato fruits are considered as an important commercial and dietary vegetable crop conferring health benefits because of rich contents of ascorbic acid, sugars, phenolic compounds, lycopene and β-carotene (Ilahy *et al*., 2011; Sima *et al*., 2011; Oceania *et al*., 2015). The role of ascorbic acid in the prevention of diseases related to oxidative damage occurs due to its ability to neutralize the action of free radicals in the biological systems (Borguini and Torres, 2009). Phenolic compounds have been associated with the inhibition of atherosclerosis and cancer due to their ability to chelate metals, inhibit lipid peroxidation and scavenge free radicals (Guil-Guerrero and Rebolloso-Fuentes, 2009; Vallverdú-Queralt et al., 2011). Tomato antioxidants include carotenoids such as β-carotene, a precursor of vitamin A, and mainly lycopene, which is largely responsible for the red color of the fruit (Agrawal and Rao, 2000; Rao and Rao, 2007; Kotkov et al., 2011). These compounds may play an important role inhibiting reactive oxygen species responsible for many important diseases, through free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways (Clinton, 1998; Desai et al., 2008; Crozier et al., 2009).

Levels of these phytochemicals can vary with genotype, stage of maturity and conditions during growth and post-harvest handling (Dumas *et al*., 2003; Radzevičius *et al*., 2009: Turhan and Vedat, 2009; Hdider *et al*., 2013). Thus it becomes pertinent to study these variations in different genotypes at full ripe stage to select the best for health benefits. The aim of the present work was to develop a broadly applicable *in vitro* regeneration method for tomato and to evaluate different genotypes of regenerated tomato, harvested at full ripe stage for their biochemical properties.

Materials and Methods:

The present experiment was conducted in the Biotechnology Laboratory, Research Park, and in the Vegetable farm, Faculty of Agriculture, Benha University, Egypt to evaluate the total phenolic compounds, total flavonoid compounds and carotenoids content in fruits obtained from *in vitro* regeneration of ten tomato genotypes.

Plant material:
Mature seeds of ten tomato genotypes were used to raise seedlings for the present study. Seeds of Strain B, Impreal, Pantano Romanesco, Red Stone, Roma, Marmande, Minibel, Petomech, Pomodoro Banana Giallo and Ponderosa Seligocia were obtained from the Preservation Germplasm Laboratory of the Department of Horticulture, Faculty of Agriculture, Benha University, Egypt. The first 8 genotypes had red fruits at ripening stage while the last three genotypes had yellow fruits.

Establishment of Aseptic Plant Cultures:
For establishing aseptic cultures of tomato growing *in vitro*, dry mature seeds were surface sterilized with sodium hypochlorite a common disinfectant for surfaces of plant tissue. Seeds of the tomato genotypes were immersed in a 2.5% sodium hypochlorite for 10 min which is present in commercial bleach solutions (Clorox). Then they were rinsed five times with sterile distilled water for 10 min each. During immersion and rinsing the solution was stirred on a shaker at 200 rpm under the laminar air flow hood. The sterilized seeds were placed into sterile tissue culture jars containing a half concentrated basal MS medium (Murashige and Skoog 1962) supplemented with 3.0% sucrose and solidified with 0.7% Oxoid-Agar. The medium was adjusted to pH 5.8 before autoclaving at 121°C and 1.2 kg cm-2 to 1.3kg cm-2 pressure for 20 min. All cultures were incubated at 25°C ± 1°C for 16 h photoperiod.

Regeneration of plants from cotyledons:
According to the standard procedure described by El Nagar 2013, cotyledons from ten days old seedlings were excised and used as explants. Each cotyledon was cut into three pieces with the part closest to the cotyledon termed "basal", the next "sub-apical" and the part at the tip of the cotyledon "apical". Only the "sub-apical" explants were used for the experiments. Explants induction media for all experiments contained 7 g/l agar and 30 g/l sucrose. Explants were sub-cultured every 2 weeks. The MS media were adjusted to 5.8 pH prior autoclaving at 121 ºC for 25 minutes. For induction and production of multiple shoots from cotyledon explants**,** MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l NAA were used. For root formation from the cut ends of regenerated excised shoots, half strengths of MS medium supplemented with 0.1 mg/l IAA was used. All media combinations contained 3% sucrose solidified with a 0.8% agar and with a pH of 5.8, adjusted before autoclaving. Cultures were maintained under a regime of 16 h photoperiod at 25±1ºC. Following the development of sufficient roots, plantlets were transferred to small pots containing sterilized soil. These plantlets were acclimated and then transferred to the greenhouse and maintained their till flowering and fruiting. The experiment was carried out with five replicates.

All tomatoes received similar water and fertilizer treatments. Fruits of ten tomato genotypes were harvested at the same time. Immediately after harvest, the fruits were placed in polyethylene bags and were then stored at - 20 °C until analyzed. Quantitative analysis was carried out for ascorbic acid, total phenolic, total sugars, lycopene and β-carotene contents.

Ascorbic acid content:
Ascorbic acid was quantitatively determined according to 2,6-dichlorophenolindophenol-dye method as described by Jones and Hughes (1983) with slight modifications. The ascorbic acid in 10 g of fresh sample was extracted with a 3% meta-phosphoric acid (v/v). The extract volume was made up to 100 ml, mixed and centrifuged at 3000 g for 15 min at room temperature. Ten milliliters were titrated against standard 2,6-dichlorophenolindophenol dye, which was already standardized against standard ascorbic acid. Results were expressed on mg /100 g fresh weight.

Total phenolic content:
Total phenols were extracted as described by Martinez-Valverde *et al*. (2002) on triplicate aliquots of homogenate juice (0.3 g). Briefly, 5 mL of 80% aqueous methanol and 50 µL of 37% HCl were added to each sample. The extraction was performed at 4°C, for 2 h, under constant shaking (300 rpm). Samples were centrifuged at 10000 × *g* for 15 min. The total phenols assay was performed by using the Folin-Ciocalteu reagent as described by Spanos and Wrolstad (1990) triplicate 50 µL aliquots of the supernatant. The absorbance was read at 750 nm using a spectrophotometer (SM 1600 UV-VIS, Abbota Corporation, New Jersey, USA). Results were expressed in mg gallic acid equivalent (GAE) /100 g fresh weight (fw).

Total sugar content:
Total sugar content of tomato fruits were determined according to method described by (Dubois *et al*., 1956).

Lycopene and β-carotene content:
The extraction of carotenoids was carried out according to the method described by Sadler *et al*. (1990) as modified by Perkins-Veazie *et al*. (2001). Carotenoids were extracted with 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone and 95% ethanol (1:1, v/v). Lycopene and β-carotene were separated by partition into hexane and directly assayed. HPLC column was C18-type column (Hypersil ODS C18) of 4.6 mm × 15 cm. Chromatographic analyses were carried out using a Y.L. HPLC, series YL-9100, equipped with a quaternary pump, an autosampler (YL9150), a degasser, and a YL-9160 spectrophotometric detector (Photo Diode Array detector–PDA), which was set at 455 nm.. The separation was performed by using a linear gradient of acetonitrile (A), hexane (B) and methanol (C) as follow: from 70% A, 7% B, 23% C to 70% A, 4% B, 26% C with a flow rate of 0.5 mL/ min. linear standard curves between 0.01 µg/mL and 10 µg/mL were created. The final results were expressed as mg/100 g fresh weight.

Experimental design and Statistical analysis:
Experiments were arranged in a completely randomized block design with 3 replications. Data were estimated as the mean and its standard error of the different traits. The calculations were done using Microsoft Excel 2010 program.

Results and Discussions:
***In vitro* regeneration of tomato:**
To establish the tissue cultures, aseptically grown seedlings were used. For the evaluation of the germination capacity, seeds of the tomato genotypes were observed *in vitro* (Figure 1). Differences were observed between genotypes after ten days of culture on germinating agar medium. The genotypes Pantano Romanesco, Strain B and Impreal showed the highest germination frequency (Table 1). Their germination rates were 90.0%, 89.0% and 80.0%, respectively. The lowest rate was observed in genotype Ponderosa Seligocia with 20.0% germination (Table 1). The other genotypes were moderate in their germination frequency 41.0% to 70.0% (Table 1).

To investigate the *de novo* shoot induction from cotyledons, regeneration rates of ten tomato genotypes were compared. The percentages of explants forming shoots, elongated shoots, rooted shoots as well as numbers of mature plants were recorded. Explants of all tomato genotypes studied regenerated shoots from cotyledons on shoot induction medium (Figure 1). Genotypes Strain B and Impreal regenerated shoots at highest rates of 70.0% or 65.0%, respectively, whereas from genotypes Minibel and Ponderosa Seligocia only 25.0% or 15.0% formed shoots, respectively (Table 1). Explants of all tomato genotypes initiated multiple shoots. Genotypes Impreal and Pantano Romanesco developed the highest number of shoots per explant of cotyledon with a mean value of 9.7 and 8.4 shoots, respectively. The lowest percentage of shoot formation was observed for genotype Ponderosa Seligocia (2.0 shoots per explant). Strain B following by Pantano Romanesco genotypes were the best of shoot elongation as well as rooted shoots (Table 1). Successfully rooted plants generally grew well under greenhouse conditions. Using our optimized procedure, we repeatedly regenerated mature tomato plants from 10 different tomato genotypes (Table 1). On average, explants developed in less than 6 months to mature, flowering, and fertile plants (Figure 1).

Many researches have previously reported that Zeatin stimulates the organogenesis of tomato cotyledons (Costa *et al*., 2000; Dorri and Altmann, 2001; Shahriari *et al*., 2006; Chandra *et al*., 2013) which quite agree with our results obtained on BAP (El Nagar, 2013). BAP is commonly available and less costly cytokinin than zeatin and sufficient shoots were obtained in the present study using BAP as a cytokine supplemented for *in vitro* regeneration.

It seems that all steps of the regeneration process were cultivar-dependent. The optimal medium for shoot regeneration is quite dependent on cultivars as reported by few researchers (Park *et al*., 2003; Bhatia *et al*., 2004; Shahriari *et al*.,2006; Mamidala and Nanna, 2011).

Cotyledons were able to produce shoots within 2-3 weeks and by the end of 4th week, the mean number of shoots per cotyledon explant was scored and used as a regeneration efficiency for all two genotypes (Table 1). These results were similar to the results obtained by Oceania et al., 2015. Considering the regeneration ability the combination of 2.0 mg/l BAP and 0.1 mg/l NAA was used for shoot regeneration (Fig. 1c). Shoots (3 cm) were rooted using half strength of agar solidified MS medium supplemented with 0.1 mg/l IAA. Results were in agreement with other researchers (Oktem *et al*., 1999; Romero *et al*., 2001; Sarker *et al*., 2009; Oceania *et al*., 2015).

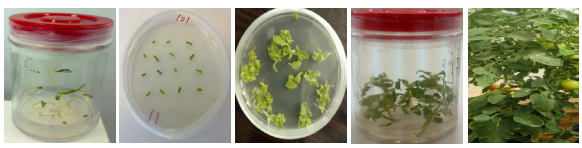

Figure 1: Plant regeneration from cotyledons of tomato cv. Pantano Romanesco.

(a) Seed germination (b) Explants of cotyledons on shoot induction medium (c) Formation of shoots from cotyledons explants 4 weeks after cultured on MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l NAA (d) Elongated shoots on shoot elongation medium (e) Normal regenerated tomato plants.

Table 1: Comparison of seed germination and regeneration frequencies of ten tomato genotypes during different stages of in vitro cultivation.

Genotype	Germination of seed (%)	Induced shoots (%)	Mean ± SE of shoots per explant	Elongated shoots (%)	Rooted shoots (%)	Mature plants (%)
Strain B	89	70	6.0±0.8	60	85	80
Impreal	80	65	9.7±1.3	40	70	66
Pantano Romanesco	90	58	8.4±0.2	50	85	82
Red Stone	70	50	4.3±0.4	30	45	41
Roma	67	45	7.1±0.2	35	35	70
Marmande	53	40	8.0±0.1	20	45	43
Minibel	50	25	3.6±0.3	32	60	46
Petomech	41	30	2.6±0.3	20	51	52
Pomodoro Banana Giallo	45	40	3.0±0.2	17	33	42
Ponderosa Seligocia	20	15	2.0±0.1	12	30	30

Biochemical compounds of different tomato genotypes:
Tomatoes and related tomato products are the major source of lycopene compounds; besides, they are considered an important source of carotenoids and vitamins in human diet (Rao, 2007; Desai *et al*., 2008). Therefore, considerable work has been conducted to increase their levels in tomatoes through biotechnological methods (Rosati *et al*., 2000). The amount of carotenes and their antioxidant activity as well as their biochemical composition are significantly influenced by tomato variety and maturity (Arias *et al*., 2000). The importance of genotype selection for high nutritional value is outlined first, followed by the optimization of environmental conditions and optimization of agricultural practices (Martine *et al*., 2008).

Ascorbic acid:
Ascorbic acid content was found to vary significantly among tomato genotypes harvested in ripe stage. The content on fresh weight varied from 10.2 to 19.3 mg/100 g fw (Table 2). Such variability in the total ascorbic acid content in the ripe stage was genotype dependant, in fact different patterns of change were evidenced for the studied tomato cultivar. According to our data (Table 2), tomato genotype Strain B had significantly highest amount (19.3 mg/100 g fw) of ascorbic acid compared to the other nine genotypes. The least amount of ascorbic acid was found in genotype Impreal and reached 10.2 mg/100 g fw. Many factors contribute to this variation and environmental growing conditions with cultivars genotype have been reported as having major effects on the ascorbic acid content (Simon, 1992; Peng *et al*., 2008). In addition, the variation can be ascribed to the antioxidant function of ascorbic acid in ripening cells which absorb high amount of oxygen as a result of increasing rate of cell respiration (Tunk *et al*. 1993). There are many researchers who evidenced different patterns of change in the ascorbic acid content for the studied tomato cultivar during ripening (Juroszek *et al*., 2009; Radzevičius *et al*., 2009; Caliman *et al*., 2010; Ilahy *et al*., 2011; Naz *et al*., 2011 and Hdider *et al*., 2013 (both in the order of 20 mg/100 g fw), but lower than the values found in (21.7-25.8 mg/100 g fw, Gupta *et al*., 2011; Kotkov *et al*., 2011) and (39-163 mg/100 g fw; Guil-Guerrero and Rebolloso-Fuentes, 2009; Sima *et al*., 2011).

Total phenolic content:
The total phenol content of ten tomato genotypes at full ripening stages of the fruit are presented in Table 2. Change in phenolic content was observed between the genotypes. Genotype Red Stone had the highest phenolic level 34.4 mg/100g fw in full ripe stage. The lowest phenolic content was obtained from genotype Strain B 10.4 mg/100g fw. Raffo *et al*. (2002) reported a gradual decline in the concentration of chlorogenic acid, one of the common phenols in tomatoes, during ripening of the greenhouse-grown cherry tomato cultivar 'Naomi' while Helyes and Lugasi (2006) reported that phenolic content of the tomato cultivar 'Lemance' remained unchanged during ripening. According to Ilahy *et al*. (2011) different patterns of change in phenolic content were observed between some high-pigment tomato cultivars. Although genetic control is the primary factor in determining the amount of phenols in fruits and vegetables, variations could also depend by ripening stages at the moment of harvesting, environmental factors (mainly light and temperature) (Dumas *et al*. 2003) and analytical methodology (Hdider *et al*., 2013). Moreover, the often contradictory results could be attributed to different pattern of changes in different classes of phenolic during tomato fruit ripening as was reported by Buta and Spaulding (1997) and Raffo *et al*. (2002).

Total sugar:
The total sugar (TS) content is the most important characteristic of tomatoes taste (Rodica *et al*., 2008). High sugars are required for best flavor (Kader, 1986). Sugar content of ripe tomatoes on the average is 3% (Jones and Scott, 1984), but in the other tomatoes genotypes, the average amount of total sugar is 4.37% (Viškelis *et al*., 2005). In this study, the TS content of full ripe tomato fruit ranged from 3.8 in genotype Strain B to 4.9% in genotype Impreal (Table 2). Other researches showed that the amount of total sugar little varied in different cultivars and ranged from 4.01 to 4.17% (Viškelis *et al*., 2007). Radzevičius *et al*., 2009 reported that total sugar content had a little variation too. It varied from 4.32% to 5.03%. On the other hand, Petro-Turza (1987) found that the total sugar content of ripe tomato between 1.7 and 4.7%. Moreover, Jongen (2002) found that total sugar content of fresh tomato fruit was varied from 2.19 to 3.55%. In another study with different fresh tomato cultivar (Marglobe), findings showed that total sugar content varied between 0.54 and 3.44 % of fresh weight (Melkamu *et al*., 2008). In addition, Turhan and Seniz, (2009) reported that TS content was found to be lower than 2.19% in nine genotypes.

Table 2: Determination of ascorbic acid and total phenolic content of ripe tomato fruits.

Genotype	Ascorbic acid (mg/100g fw)	Total Phenolic (mg/100g fw)	Total sugar (%)
Strain B	19.3±0.6	10.4±0.4	3.8
Impreal	10.2±0.4	17.9±3.5	4.9
Pantano Romanesco	11.8±0.6	27.0±0.4	4.8
Red Stone	11.7±0.7	34.4±4.4	4.5
Roma	10.6±0.3	19.6±2.7	4.3
Marmande	14.8±0.8	22.7±0.3	4.5
Minibel	12.4±0.5	11.6±0.3	4.6
Petomech	12.9±0.9	21.3±0.3	4.1
Pomodoro Banana Giallo	16.6±0.9	14.7±1.3	4.0
Ponderosa Seligocia	16.3±0.4	19.1±0.2	3.9

Carotenoids content:
Based on the data of our investigation (Figure 2), the highest amount of lycopene was established in genotypes Petomech (13.3 mg/100 g fw) and Red Stone (12.0 mg/100 g fw). The least amount of lycopene was established in the fruits of genotypes Ponderosa Seligocia (0.6 mg/100 g fw) and Pomodoro Banana Giallo (0.9 mg/100 g fw), which had yellow colour at full ripe stage. Lycopene is the most abundant carotene in red tomato fruit, accounting for up to 90% of the total amount of carotenoids. Typical red pigmented tomato fruit also contains lesser amount of β-carotene and other carotenoids (Radzevičius *et al*., 2009). Normalized values of lycopene content in different tomato cultivars in California ranged from 8.4 to 17.2 mg/100 g fw, i. e. there was 100% difference from lowest to highest (Barrett and An-

thon, 2001). According to Viškelis *et al*., 2007, the highest amount of lycopene (over 10 mg 100/g fw) was found in cultivar 'Rutuliai' and was 1.6 time higher comparing with hybrid 'Admiro' and 2 times higher comparing with hybrid 'Kassa'. In particular, at the red-ripe stage, lycopene values varied from 96.9 mg/ kg fw in 'Donald' to 232.9 mg/ kg fw in 'HLY 18'. Thus, in high-lycopene tomato cultivars the amount of lycopene was 1.26- to 2.4-fold higher than 'Donald'(Lenucci *et al*., 2006; Hdider *et al*., 2013). The differences between high-lycopene and ordinary tomato cultivars have been attributed to different growing conditions and cultivars (Dumas *et al*. 2003; Ilahy *et al*. 2009; 2010). It has been also reported that high-lycopene tomato cultivars derive from spontaneous mutants characterized by deeply pigmented fruits due to their exaggerated light responsiveness with respect to wild-type plants (Mustilli *et al*. 1999; Atanassova *et al*. 2007).

Our studies showed that the yellow tomato genotypes Pomodoro Banana Giallo and Ponderosa Seligocia presented the highest amount of β-carotene (4.6 and 4.1 mg/100 g fw) (Figure 2). The least amount of β-carotene was established in genotype Pantano Romanesco and reached 0.5 mg/100 g fw. β-carotene occurs in tomato fruits and varies in amounts of 0.23–2.83 mg/100 g fw (Stommel, 1992; Agarwal and Rao, 2000). Lenucci *et al*. (2006) reported that β-carotene accumulation was cv-dependent. According to Radzevičius *et al*., 2009 most of investigated cultivars had a similar amount of β-carotene, which varied from 1.43 to 1.70 mg/ 100 g fw. In addition, Hdider *et al*., 2013 determined the amount of β-carotene in 7 tomato genotypes at full ripe stage and they found that β-carotene ranged from 5.8 to 19.8 mg/100 g fw. Biacs *et al*. (1987) found that β-carotene approached its maximum carotene content decreased markedly during ripening. Level in yellow coloured fruits of the processing cultivar'Ventura' and then declined. The contrasting results are probably due to the influence of varietals factors on carotenogenesis in tomato fruits.

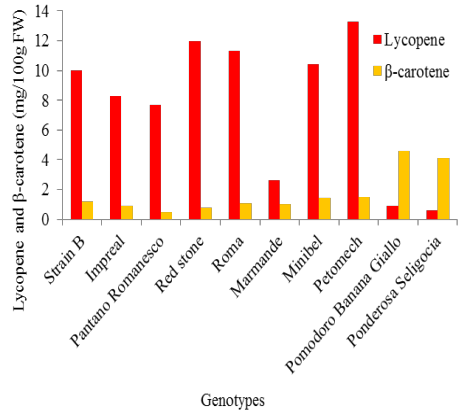

Figure 2: Lycopene and β-carotene content in different tomato genotypes at full ripe stage.

Conclusion:

The present study demonstrates a simple and promising protocol for *in vitro* plantlet regeneration of tomato from cotyledon explants. The use of BAP in combination with IAA favored plant regeneration. This protocol can be applied for the conservation and multiplication of genetically pure and disease free genotypes and also for transgenic experiments for enhancing nutrional values in *Solanum lycopersicum*. Significant variation in ascorbic acid, total phenols, total sugar, lycopene and β-carotene contents between tomato genotypes at full ripe stage, indicates that the potential efficiency of antioxidants vary considerably with genotype.

REFERENCES

Agarwal, S. and Rao, A. V. (2000). Tomato lycopene and its role in human health and chronic diseases. Canadian Med Ass J. 163, 739–744. | Arias, R.; Lee, T. C. and Logendra, L. (2000). Correlation of lycopene measured by HPLC with the L*, a*, b* colour readings of a hydroponic tomato and the relationship of maturity with colour and lycopene content . J Agric Food Chem. 48, 1697–1702. | Atanassova, B.; Stoeva-Popova, P. and Balacheva, E. (2007). Cumulating useful traits in processing tomato. Acta Horticulturae (ISHS) 758, 27-36. | Barrett, D. M. and Anthon, G. (2001). Lycopene content of California-grown tomato Varieties. Acta Horticult. 542, 165–173. | Bhatia, P.; Ashwanth, N.; Senaratna, T. and Midmore, D. (2004). Tissue culture studies of tomato (Lycopersicon esculentum). Plant Cell Tissue Org. Cult. 78: 1-21. | Biacs, P.A.; Daood, H.G.; Czinkotai, B.; Hajdu, F. and Kisskutz, N. (1987). Effect of Titavit treatments on the dynamics of tomato fruit ripeness. Acta Horticult. 220, 433-438. | Borguini, R. and Torres, E. (2009). Tomatoes and tomato products as dietary sources of antioxidants. Food Rev. Intern. 25, 313–325. | Buta, J.G. and Spaulding, D.W. (1997). Endogenous levels of phenolics in tomato fruit during growth and maturation. J Plant Growth Reg. 16, 43-46. | Caliman, F.R.B.; Derly, J.H.; Stringheta, P.C.; Paulo, C.R.; Gisele, R.M. and Everardo, C.M. (2010). Quality of tomatoes grown under a protected environment and field conditions. IDESIA (Chile), 28(2) 75-82. | Chandra, I.;Singh, P.;Bhattacharya, A.;Singh, P.; Javed, S. and Singhamahapatra, A. (2013). In vitro callus induction, regeneration and micropropagation of Solanum lycopersicum. Int. J. Curr. Microbiol. Applied Sci., 2: 192-197. | Christou, P. and Klee, H. (2004). Handbook of plant biotechnology. John Wiley & Sons Ltd. 1, West Sussex, England. PP 738. | Clinton, S.K. (1998). Lycopene; chemistry, biology and implications for human health and diseases. Nutr. Rev. 56, 35–51. | Costa, M.G.C.; Noguera, F.T.S. and Cecon, P.R. (2000). Influence of the antibiotic timentin on regeneration of tomato plant. (Lycopersicon esculentum). J. Plant Cell Rep. 19: 327-332. | Crozier, A.; Jaganath, I.B. and Clifford, M.N. (2009). Dietary phenolics: chemistry, bioavailability and effects on health. Nat. Prod. Rep. 26, 1001–1043. | Desai, A. G.; Qazi, G. N. and Ganju, R. K. (2008). Medicinal plants and cancer Chemoprevention. Current Drug Metabolism. 9, 581–591. | Drorri, E. and Altman, A. (2001). Transformation of tomato with the beta A gene to confer osmotic stress tolerance (glycine-betaine production). Plant Sci. 49: 152-153. | Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Robers, P.K. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Anal Chem. 28:350-356. | Dumas, Y.; Dadomo, M.; Lucca, G.D.; Grolier, P. and Di Lucca, G. (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J Sci Food Agric. 83, 369-382. | El Nagar, M. M. (2013). Genetic engineering for increasing salinity tolerance in tomato. Australian Journal of Basic &Applied Science. Aust J Basic Appl Sci. 7 (1):433-440. | EL Nagar, M.M. and Mekawi, E. (2014 a). Comparison of different genotypes of fennel (Foeniculum vulgare Mill.) in terms of chemical compounds extracted from seeds and in the callus induced from tissue culture. Curr Sci Int. 3 (4): 445-453. | EL Nagar, M.M. and Mekawi, E. (2014 b). Comparison of different genotypes of rocket (Eruca sativa) in terms of chemical compounds extracted from seeds and in the callus induced from tissue culture. Middle East J. Agric. Res. 3 (4): 1074-1082. | EL Nagar, M.M. and Mekawi, E. (2015). Regeneration of pepper (Capsicum annuum L.) and evaluation of antioxidant compounds at various ripening stages. Ind J Res. 4(8): 348-353. | Guil-Guerrero, J.L. and Rebolloso-Fuentes, M.M. (2009). Nutrient composition and antioxidant activity of eight tomato (Lycopersicon esculentum) varieties. J. Food Comp. Anal. 22, 123-129. | Gupta, A.; Kawatra, A. and Sehgal, S. (2011). Physical-chemical properties and nutritional evaluation of newly developed tomato genotypes. African J food Sci Technol. 2 (7): 167-172. | Hdider, C.; Ilah, R. and Tlili, I. (2013). Effect of the stage of maturity on the antioxidant content and antioxidant activity of high-pigment tomato cultivars grown in Italy. Food, 7 (1): 1-7. | Helyes, L. and Lugasi, A. (2006). Formation of certain compounds having technological and nutritional importance in tomato fruit during maturation. Acta Alimentaria 35 (2): 183-193. | Ilahy, R.; Hdider, C. and Tlili, I. (2009). Bioactive compounds and antioxidant activity of tomato high lycopene content advanced breeding lines. In: Daami-Remadi M (Ed). Afric J Plant Sci Biotechnol. 3 (Special Issue 1): 1-6 | Ilahy, R.; Hdider, C. and Tlili, I. (2010). Assessing agronomic characteristics lycopene and total phenolic contents in pulp and skin factions of different tomato varieties. Afric J Plant Sci Biotechnol. 4 (SI 2): 64-67. | Ilahy, R.; Hdider, C.; Lenucci, M.S.; Tlili, I. and Dalessandro, G. (2011). Antioxidant activity and bioactive compound changes during fruit ripening of high lycopene tomato cultivars. J Food Comp Anal. 24 (4-5): 588-595. | Jones, E. and Hughes, R. E. (1983). Foliar ascorbic acid in some angiosperms. Phytochem. 22: 2493–2499. | Jones, R.A.; Scott, S.J. (1984). Genetic potential to improve tomato flavor in commercial F1 hybrids. J. Am. Soc. Hortic. Sci. 109:318-321. | Jongen, W. (2002). Fruit and vegetables processing. Wood head publishing in food sci. and technol. Wageninngen University, Netherlands, 350 pp. | Juroszek, P.; Lumpkin, H.M.; Yang, R.Y.; Ledesma, D.R. and Ma, C. H. (2009). Fruit quality and bioactive compounds with antioxidant activity of tomatoes grown on-farm:comparison of organic and conventional management systems. J. Agric. Food Chem. 57, 1188–1194. | Kader, A.A. (1986). Effect of post harvest handling procedures on tomato quality. Acta Hortic. 190: 209-221. | Kotkov, Z.; Lachman, J.; Hejtmnkov, A. and Hejtmnkov, K. (2011). Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. LWT - Food Sci. & Technol. 44, 1703-1710. | Lenucci, M.S.; Cadinu, D.; Taurino, M.; Piro, G. and Dalessandro, G. (2006). Antioxidant composition in cherry and high-pigment tomato cultivars. J. Agric. Food Chem.54(7): 2606-2613. | Mamidala, P. and Nanna, R.S. (2011). Effect of genotype, explant source and medium on in vitro regeneration of tomato. Int. J. Genet. Mol. Biol. 3: 45-50. | Martine, D.; Ehret, D. L. and Papadopoulos, A. P. (2008). Tomato (Solanum lycopersicum) health components: from the seed to the consumer. Phytochem Rev.7(2): 231–250. | Martinez-Valverde, I.; Periago, M.J.; Provan, G. and Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicon esculentum). J Sci Food Agric. 82, 323-330. | Melkamu, M.; Seyoum, T. and Woldetsadik, K. (2008). Effects of pre-and post harvest treatments on changes in sugar content of tomato. Afr. J. Biotechnol. 7(8): 1139-1144. | Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497. | Mustilli, A.C.; Fenzi, F.; Ciliento, R.; Alfano, F. and Bowler, C. (1999). Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. Plant Cell, 11, 145-157. | Naz, F.; Haq, I.; Asghar, S.; Shah, A.S. and Rahman, R. (2011). Studies on growth, yield and nutritional composition of different tomato cultivars in Battal Valley of District Mansehra, Khyber Pakhtunkhwa, Pakistan. Sarhad J Agric. 27(4): 569-571. | Oceania, C.; Doni, T.; Tikendra, L. and Nongdam, P.(2015). Establishment of efficient in vitro culture and plantlet generation of tomato (Lycopersicon esculentum Mill.) and development of synthetic seeds. J Plant Sci. 10 (1): 15-24. | Oktem, H.A.; Bulbul, Y.; Oktem, E. and Yucel, M. (1999). Regeneration and Agrobacterium-mediated transformation studies in tomato (Lycopersicon esculentum Miller). J. Bot. 23: 345-348. | Park, S.H.; Morris, J.L.; Park, J.E.; Hirschi, K.D. and Smith, R.H. (2003). Efficient and genotype-independent Agrobacterium-mediated tomato transformation. J. Plant Physiol. pp: 200-257. | Peng, Y.; Zhang, Y. and Ye, J. (2008). Determination of phenolic compounds and ascorbic acid in different fractions of tomato by capillary electrophoresis with electrochemical detection. J Agric Food Chem. 56, 1838–1844. | Perkins-Veazie, P.; Collins, J.K.; Pair, S.D. and Roberts, W. (2001). Lycopene content differs among red-fleshed watermelon cultivars. J Sci Food Agric. 81, 983-987. | Petro-Turzo, M. (1987). Flavor of tomato and tomato products. Food Rev. Int. 2(3): 309-351. | Radzevičius, A.; Karklellene, R.; Bobinas, Č. And Viškelis, P. (2009). Nutrition quality of different tomato cultivars. Zemdirbyste-Agric. 96 (3): 67–75. | Raffo, A.; Cherubino, L.; Vincenzo, F.; Ambrozino, P.; Salucci, M.; Gennaro, L.; Bugianesi, R.; Giuffrida, F. and Quaglia, G. (2002). Nutritional value of cherry tomatoes (Lycopersicon esculentum cv. Naomi F1) harvested at different ripening stages. J Agric Food Chem. 50, 6550-6556. | Rao, A. V. and Rao, L. G. (2007). Carotenoids and human health. Pharmacological Res. 55, 207–216. | Rodıca, S.; Apahıdean, S.; Apahıdean, A.; Manıtiu, M.; Paulette, L. (2008). Yield, physical and chemical characteristics of greenhouse tomato grown on soil and organic substratum. 43rd Croatian and 3rd Int. Symposium on Agric. Opatija. Croatia. pp. 439-443. | Romero, J.P.; Houlne, G.; Canas, L.; Schantz, R. and Chamarro, J. (2001). Enhanced regeneration of tomato and pepper seedling explants for Agrobacterium-mediated transformation. J. Plant Cell Tiss. Org. Cult. 67: 173-180. | Rosati, C.; Aquilani, R. and Dharmapuri, S. (2000). Metabolic engineering of beta carotene and lycopene content in tomato fruit. Plant J. 24, 413–420. | Sadler, G.; Davis, J. and Dezman, D. (1990). Rapid extraction of lycopene and beta carotene from reconstituted tomato paste and pink grapefruit homogenates. J Food Sci. 55, 1460-1461. | Sarker, R.H.; Islam, K. and Hoque, M.I. (2009). In vitro regeneration and Agrobacterium-mediated genetic transformation of tomato (Lycopersicon esculentum Mill.). Plant Tissue Cult.& Biotech. 19(1): 101-111. | Shahriari, F.; Hashem, H. and Hosseini, B. (2006). Factors influencing regeneration and genetic transformation of three elite cultivars of tomato (Lycopersicon esculentum L.). Pakistan J Biol Sci. 9(15): 2729-2733. | Sima, R.; Maniutiu, D.; Apahidean, A.; Apahidean, M.; Lazar, V.; Sima, N. and Ficior, D. (2011). The evaluation of yield potential and quality of fruits at tomato hybrids cultivated in greenhouse. UASVM Horticulture, 68 (1): 273-287. | Simon, J. A. (1992). Vitamin C and cardiovascular disease: a review. J Am College Nut. 11, 107–125. | Stommel, J. R. (1992). Tomato nutritional quality: genetic improvement of carotenoid Content. Agro-food Industry Hi-Tech. 3, 7–11. | Tunk, R.; Seniz, V.; Ozdemir, N. and Suzen, M.A. (1993). Changes in chlorophyll, carotenoid and lycopene content of tomatoes in relation to temperature. Acta Hortic. (ISHS) 398, 856-862. | Turhan, A. and Vedat, S. (2009). Estimation of certain chemical constituents of fruits of selected tomato genotypes grown in Turkey. African J Agric Res. 4 (10): 1086-1092. | Vallverdú-Queralt, A.; Medina-Remón, A.; Martínez-Huélamo, M.; Jáuregui, O.; Andres-Lacueva, C. and Lamuela-Raventos, R.M. (2011). Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. J. Agric. Food Chem. 59, 3994–4001. | Verma,S.; Sharma, A.; Kumar, R.; Kaur, C.; Arora, A.; Shah, R. and Nain, L. (2015). Improvement of antioxidant and defense properties of tomato (var. Pusa Rohini) by application of bioaugmented compost. Saudi J Biol Sci. 22, 256–264. | Viškelis P.; Jankauskienė, J. and Bobinaitė, R. (2007). Influence of ripeness on tomato fruit quality. Sodininkystė ir daržininkystė. 26 (4): 278–288. | Viškelis, P.; Vilkauskaitė, G.; Noreika, R. K. (2005). Chemical composition, functional properties and consumption of tomatoes. Sodininkystė ir daržininkystė. 24(4): 182–192. |