Original Article

MUTAGENICITY, ANTIOXIDANT ACTIVITY AND NUTRITIONAL CONTENT OF LONG-LIFE TOMATO

MUTAGENICIDADE, ATIVIDADE ANTIOXIDANTE E CONTEÚDO NUTRICIONAL DE TOMATE LONGA VIDA

Regildo M. G. SILVA[1]; Célia C.M. FIGUEIREDO[2]; Amanda da C. GOMES[3]; Erika C.T. de MELLO PEIXOTO[4]; Paulo C. FERREIRA[2]; Luciana Pereira SILVA[5]

1. Professor, Doutor, Departamento de Ciências Biológicas, Universidade Estadual Paulista – UNESP, Assis, SP, Brasil. regildos@yahoo.com.br; 2. Nutricionista, Universidade Estadual Paulista - UNESP, Departamento de Ciências Biológicas - Laboratório de Fitoterápicos, Faculdade de Ciências e Letras de Assis, Assis, SP, Brasil; 3. Mestre em Biociências; 4. Professora, Doutora, Universidade Estadual do Norte do Paraná - UENP, Bandeirantes, PR, Brasil; 5. Professora, Doutora, Fundação Educacional do Município de Assis - FEMA, Assis, SP, Brasil.

ABSTRACT: The increasing tomato demand for the food market motivates improvements and the use of new biotechnologies in this fruit's production. The hybrid crop stands out for fruit production resistant to rot and postharvest wilt (long-life crops). Within this context, consumption of genetically modified food deserves attention regarding the safety and nutritional aspects due to the fact that inclusion and/or overexpression of genetic traits can cause harm to human health in the short or long term. In this scenario, this study aimed to evaluate genotoxicity and mutagenicity from different varieties of long-life tomatoes obtained by genetic breeding and also determines main bioactive compounds and antioxidant activity. The genotoxicity and mutagenicity were analyzed via the micronucleus test and the evaluation of chromosome aberrations in mice bone marrow respectively. We have also analyzed carotene, beta-carotene, lycopene, total phenol and flavonoid contents via spectrophotometry and antioxidant activity via DPPH radical scavenging assay. Considering the results obtained, it is possible to conclude that despite the absence of significant genotoxic activity among the evaluated samples, the antioxidant activity and the differences found in composition seems to be ruled by genetic factors, possibly due to the genetic breeding.

KEYWORDS: DPPH. *Solanum lycopersicum*. Micronucleus. Chromosomal aberration. Genetic breeding.

INTRODUCTION

Tomato is the *Solanum lycopersicum* fruit, native from South America and known to be a fruit rich in bioactive and nutritional compounds (FRIEDMAN 2002, 2013; FRUSCIANTE et al., 2007). The nature and concentration of such compounds are influenced by agricultural practices, environmental factors, variety and maturation (DAVIES; HOBSON, 1981; SANCHEZ-RODRIGUEZ et al., 2012). The fruit is grown worldwide and its global production has duplicated in the last 20 years. This was due to the culture expansion and consumption growth (Fao/Onu, 2001).

The increasing tomato demand for the food market motivates improvements and the use of new biotechnologies in this fruit's production. The use of hybrid seeds stands out for fruit production resistant to rot and postharvest wilt (long-life crops) (BENOR et al., 2008).

Nowadays there are three possibilities to create and/or obtain hybrid seeds from long-life tomato plants. The first one is based on conventional genetic breeding methods via superior parents' selection aiming to increase the frequency of favorable alleles providing greater firmness of fruit's pericarp (structural long life). The second one is also based on conventional genetic breeding methods, however using ripening mutants: individual containing simple mutant alleles with multiple effects (pleiotropic) capable to affect the fruit's ripening (***rin*** - "ripening inhibitor", ***nor*** - "non ripening", ***alc***- Alcobaça). And at last, the application of molecular biology techniques (transgenic cultivars) using homozygous transgenic orientation (sense and antisense), DNA translation and mRNA transcription interfering on ethylene production and the production and/or activity of enzymes involved in the normal tomato fruit ripening process (EZIN et al., 2010; FANTINI et al., 2013; MOORE, et al., 2010; WATANABE et al., 2015; CLAUSEN et al., 2011).

Within this context, the consumption of genetically modified food deserves attention regarding safety and nutritional aspects due to the fact that the inclusion and/or overexpression of genetic traits can cause harm to human health in the short or long term (POLIVKOVÁ et al., 2010; PINHO et al., 2011). To ensure the safety of genetically modified products consumption there are safe and reliable biological tests that are capable to

Received: 07/01/16
Accepted: 02/05/16

assess potential harm to human health, being the preventive study of the DNA damage an important diagnostic tool (FERNANDES et al., 2010; SESTARI et al., 2014). The study of DNA damage induced by natural or synthetic substances is an essential area of genetic toxicology as long as chromosome mutation is an important event in carcinogenesis. (FAGUNDES et al, 2005; CARVALHO et al, 2011). The assays involving DNA analysis are known as genotoxicity and mutagenicity tests (PAYYVULÁ et al., 2012) and these are recommended by the international agencies and government institutions (SCOLASTICI et al., 2013). Determination of micronucleated cells and chromosome aberrations frequency has been used with success in monitoring populations exposed to natural or synthetic mutagenic agents, being considered of great value to evaluate the population's possibility of cancer development (AU et al., 2001; SHAMI; MOREIRA, 2004; GOSSMANN et al., 2010). *In vitro* chromosomal aberration experiments are greatly important to mutagenesis studies because the results obtained in *vitro* are commonly obtained also *in vivo* (TELLIER et al., 2011; ZHU et al., 2013; SHALABY; EL-BANNA, 2013). Another important aspect about chromosomal aberration test is the capacity to evaluate DNA damage caused by chronic exposure to mutagenic and clastogenic agents, mainly those incorporated on food (AGRAWAL AND KUMAR, 1999; RANVEER et al., 2013).

Considering last decade increase of long life tomato consumption and the rise of new varieties obtained mainly by genetic breeding, the safety of its use on both animal and human food can be assured by studies that involve the evaluation of its effects on genome, metabolism and physiology of the individual. Therefore, the objective of this study was to evaluate the genotoxicity and mutagenicity from different varieties of long life tomatoes obtained by genetic breeding.

MATERIAL AND METHODS

Plant Material

Three varieties of long life tomato were obtained from farmers of Assis/SP region in Brazil. These varieties were cultured under the same environmental conditions and identified by the Instituto Agronômico e Agência Paulista de Tecnologia de Agronegócios do Município de Assis: [Long life - structural - Santa Cruz Group (cultivar Débora Plus); Long life - mutant -TOM-559xFlorida7775 ($nor^{+}/nor^{A}go^{C+}/og^{C}$) and conventional tomato. Fruits from different varieties were selected, cleaned (immersed in Sodium Hypochloride 0.2% for 20 minutes) and stored in the average temperature of 4°C.

Extracts Preparation

Ten fruits of each tomato variety had their peel and pulp removed. The pulp obtained were frozen and then lyophilized. The dry extracts obtained were triturated and the resulting powder was used in the bioassays (mutagenicity), in the antioxidant activity and in the bioactive compounds determination.

Determination of Anti Mutagenic Activity

Animals and treatments

Male Swiss albino mice with 9-12 weeks old and weight 35-40 grams were obtained on the experimental animals' center (Centro Educacional de Patos de Minas - MG). Animals were kept in polyethylene boxesunder controlled environment (25±4 ºC, 55±5% humidity), with dark/bright control each 12 hour (from 7h00m a.m. to 7h00m p.m.). Food and water remained available *ad libitum*. Five animals were placed in each box and randomly distributed in five groups: 1) Animals receiving distilled water (negative control) by gavage; 2) Animals receiving Cyclophosphamide® 2.0mg for each kilogram of body weight (positive control) via intraperitoneal injection (ip); 3, 4 e 5). Animals treated daily via gavage with the extracts from different tomato varieties at concentrations of 100, 200 e 400mg respectively for each kilogram of body weight during 7 days. The animals were sacrificed via cervical dislocation 24 hours after one-week experimental period. This study was approved by the Animal Ethics Committee of UNIPAM, UEMG, Patos de Minas-MG, Brazil (Protocol no 88/07).

Choice criteria and Micronucleus Test in Mice Bone Marrow Cells

Genotoxic effects were evaluated in mice bone marrow cells via Micronucleus Test according to Schmid (1975). Immediately after sacrificing the animal a femur was removed and the bone marrow was collected and emulsionated until become a suspension. Then, the suspension was transferred to centrifuge tubes containing 2mL of fetal calf serum (FCS). The bone marrow suspension was centrifuged to 1000 rpm for 10 minutes and the supernatant was discarded. The pellet was resuspended in a drop of FCS. A small portion of this suspension was placed on slides. After 24 hours drying at room temperature, the slides were fixed

with absolute methanol during 5 minutes and stored at room temperature or directly stained for analysis. The slides were stained with Giemsa solution (Sigma Chemical Co., St. Louis, MO, USA) prepared in Phosphate Buffered Saline (0.06M; pH 6.8): potassium phosphate buffer (0.06 M; pH 6.8) (1:1). The slides were stained during 5 minutes, washed with distilled water to remove excess dye and dried at room temperature.

The slides were examined on an optic microscope (Olympus, Japão) under 1000x zoom. According to Krishnae Hayashi (2000) the micronucleus count criterion was essentially based on diameter, shape and color. To evaluate induction on MN formation we consider the micronucleated polychromatic erythrocytes (MNPCE) scored in 2000 PCE for each mouse.

Chromosome Aberration Test

Bone marrow cells were collected from femur via 2.2% sodium citrate solution injection and centrifuged at 1000 rpm about 10 minutes. After centrifuge, cells were treated with 5 mL hypotonic KCl solution (0.075 M) pre-heated for 20 minutes at 37ºC , and ressuspended twice in 5 mL cold Carnoy fixer (methanol – glacial acetic acid, 3:1, v/v) for 20 minutes at room temperature. After final centrifuge, cells were carefully mixed using the tip of a Pasteur pipette and dropped 3-4 cm high in wet and clean slides. These were stained with Giemsa 10% for 20 minutes. Aberrations score and analysis were done as described by Preston et al., (1987). Chromosomal aberrations were scored under 1000x magnification in fifty metaphases for each mouse using an optical microscope (Olympus, Japão). Chromosome aberrations total profiles were analyzed observing breakage, ring and chromosomal gaps formation. Only well spread metaphases were randomly analyzed (42±1 chromosomes). Chromosomal damage was assessed by comparing treated groups with positive and negative control groups.

Statistical Analysis

Results were expressed as average ± standard deviation (DP) to compare MNPCE and PCE frequencies between treated and control groups. Data were statistically analyzed by the Mann-Whitney nonparametric U-test, with significance level α=0.05, using SPSS 12.0 software (SPSS, Chicago, IL) to micronucleus assay statistical analysis. Chromosome aberrations results were analyzed and expressed as average ± standard deviation. Average percentage difference between treated and control groups were evaluated with chi-square test (χ^2) ($p \leq 0.05$).

DPPH radical scavenging

1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma Co., USA) scavenging activity was determined by Blois method (1958). Dry extract of each sample was dissolved in ethanol (75%) at different concentrations (100, 250, 500 and 1000 µg mL^{-1}) and mixed with 5 mL DPPH solution ($1,5x10^{-4}$M). After 30 minutes resting in the dark at room temperature, the extract was submitted to UV-Vis spectrophotometer at 517nm wavelength. Analyzes were performed in triplicate and gallic acid was used as control. Calculation of percentage inhibition of DDPH (I%) was performed according to the formula: %AA= [(Acontrol - Asample) / Acontrol] x 100.

Determination of Bioactive Compounds

Aceto-hexane extract preparation

Samples from different tomato varieties were peeled, had their pulp removedand were divided in 20 grams portions. Then, these portions were crushed and incubated with 20 mL acetone and 60 mL hexane heated at 60°C. Subsequently, they were placed in a container protected from light and stored at -18ºC mean temperature for 24 hours. After this period, the extracts were filtered under vacuum and used in different concentrations.

Carotenoid, betacarotene and lycopene quantification

Carotenoid, betacarotene and lycopene quantification was determined according to the methodology described by Zscheile and Porter (1947) based in spectrophotometric analysis. Aceto-hexane extract (80 mL) from different tomato samples was transferred to a 250 mL separatory funnel. After phase separation, basal immiscible was eluted, followed by washing twice with distilled water and eluting the supernatant. Then was added 20mL of methyl alcohol 90% eluting basal immiscible. After complete phase separation, procedure was repeated with further additions of 20 mL of potassium hydroxide 20% and 20 mL of methyl alcohol 90%.

After this process, 0.5 mL of sample was diluted with hexane until the final volume of 10 mL. Spectrophotometric measurements were performed at a range of absorbance between 0.1nm and 0.6nm wich is considered reliable. Total carotenoids, lycopene and betacarotene levels were obtained throught the formulas:

$$Carotenoids\,(\mu g/g) = \frac{\log\left(\frac{I_0}{I}\right)(487nm)x\frac{dilution\,1}{s.w.}x\frac{0,5}{dilution\,2}x10^5}{131x1xs.w.}$$

s.w. is the sample weight

$$Lycopene(\%) = \frac{\frac{\log\left(\frac{I_0}{I}\right)(502nm)}{\log\left(\frac{I_0}{I}\right)(487nm)}x181 - 42}{237}x100$$

$$Betacarotene\,(\mu g/100g) = \frac{[100 - Lycopene\,(\%)]\,x\,Carotenoids\,(\mu g/g)}{100}$$

Content of total phenol and flavonoid

Phenol content from different tomato varieties were analyzed according to Folin-Ciocalteu method modified by Singleton and Rossi (1965). Dry extract was diluted in ethyl alcohol at 100, 250, 500 and 1000 µg mL^{-1} concentrations to all tomato samples. For each 0.5 mL of extract in different concentrations were added 5 mL of distilled water and 0.25 mL of Folin-Ciocalteau reagent. After 3 minutes was added 1mL of Na_2CO_3 10% solution and the mixture was stored for 1 hour. Analyzes were performed in triplicate and the absorbance was measured at 725 nm. Total phenol levels were determined by interpolating the absorbance of samples against a calibration curve built with a gallic acid standard and expressed as milligram of GAE (gallic acid equivalents) per gram of extract.

RESULTS

Micronucleus Test, chromosomal aberrations and mitotic index

Groups treated with different concentrations of different tomato varieties shown higher frequency (MNPCE±SD) in the concentration of 400 mg Kg^{-1} bw (TC=7.00±3.21; SCDP=7.20±3.29 and TOMF=6.20±3.65). The groups treated did not shown significant difference between each other, but differ from groups that received water (1.60±0.89) and Cyclophosphamide (52.04±19.44) (Table 1).

Table 1. Micronucleated polychromatic erythrocytes (MNPCE) frequency and chromosomal aberrations in Swiss albino mice (n=5, A_1 to A_5) treated with different concentration of extract (100, 200 and 400 $mgKg^{-1}$ of body weight (bw)) of the tomato varieties (TC= conventional tomato; SC_{DP}= long-life – structural and TOMF= long-life - mutant-TOM-559xFlorida7775), negative control group (NC) treated with water and positive control group (PC) treated with Cyclophosphamide®, 2000 Polychromatic erythrocytes (EPC) per mice.

Treatment	($mgKg^{-1}$ bw)	MNPCE±SD[a]	Structural aberrations			Chromosomal aberrations %± SD[b]	Mitotic Index[b]
			Chromosomal Breakage	Gaps	Rings		
NC	0	1.60±0.89a	4	1	1	2.4±0.99a	10.38±0.52a
TC	100	2.80±1.41b	4	3	1	3.2±0.17a	9.17±1.67a
	200	3.60±1.80b	6	2	2	4.0±0.11a	9.88±0.93a
	400	7.00±3.21b	6	3	3	8.8±1.12b	9.95±1.52a
SC_{DP}	100	2.80±1.67b	6	3	2	4.4±0.93a	8.77±1.43a
	200	3.60±1.79b	5	3	4	4.8±0.89a	8.85±1.72a
	400	7.20±3.29b	7	3	4	5.6±1.23b	9.08±0.44a
TOMF	100	5.40±3.03b	4	3	2	3.6±1.21a	7.93±1.07a
	200	7.00±3.27b	5	4	4	5.2±1.45b	7.03±1.48a
	400	6.20±3.65b	6	4	6	6.4±1.65b	6.21±1.31b
PC	2	52.4±19.44c	121	26	8	62.0±11.22c	4.82±0.81b

[a]Means sharing the same letter in a column do not differ significantly by Mann-Whitney and U-test (α=0.05); [b]Means sharing the same letter in a column do not differ significantly by χ^2 ($p \leq 0.05$).

Chromosomal aberrations test has shown chromosomal breakage, gaps and rings formation. Treatment with different tomato varieties in the following concentrations: SC_{DP}200(4.8±0.89) and 400(5.6±1.23); TOMF 200(5.2±1.45) and 400(6.4±1.65) mg kg^{-1} bw has shown significant differences when compared with water group (2.4±0.99) while other concentrations did not shown difference when compared with water group. Otherwise all treated groups and water group statistically differ from group treated with Cyclophosphamide. For the mitotic index only the

treated group TOMF with the concentration of 400 mg kg^{-1} bw (6.21±1.31) did not differ significantly from the PC group (4.82±0.81), the others concentrations and treatment groups showed no significant difference compared to the NC group (10.38±0.52) (Table 1).

Content of carotenoids, betacarotene, lycopene, total phenol and flavonoid and antioxidant activity.

Among different tomato varieties evaluated, the extract obtained from TOMF variety presented higher pigmentation in 1000 mgmL^{-1} concentration (carotenoids: 59.26±2.36 µg g^{-1}; betacarotene: 34.83±1.76 µg 100g^{-1} and lycopene 41.21±2.09%) (Table 2). A quantitative analyses of the evaluated pigment revealed their presence in the three varieties. The higher concentration was found in the TOMF variety, followed by SC_{DP} and the TC. Regarding betacarotene, TOMF variety has shown higher concentration.

Results from the antioxidant DPPH test demonstrated higher scavenging activity in the 1000mg mL^{-1} concentration. Regarding to the varieties, results were: TC=24.06±2.27%, SC_{DP}= 15.12±2.16% and TOMF=14.09±1.98% (Table 2). Higher concentrations of total phenol compounds were founded on the following samples: SC_{DP} (31.12±0.98); and TOMF (36.98±0.67) in the 1000mg mL^{-1} concentration. Regarding the flavonoid quantification, the SC_{DP} variety shown (21.02±1.29) mgEq/g, and TOMF with (27.18±2.11) mg Eq/g of extract also in 1000mg mL^{-1} concentration.

Table 2. Tomato extract samples total carotenoid, betacarotene, lycopene content, antioxidant activity (%AA) and total phenol and flavonoid determination of extract of tomato varieties (TC= conventional tomato; SC_{DP}=long-life-structural and TOMF= long-life-mutant-TOM-559xFlorida7775) in different concentrations (100, 250, 500 e 1000 µg mL^{-1}).

Variety	Extract (mg mL^{-1})	Carotenoids (µg g^{-1})	Betacarotene (µg 100g^{-1})	Lycopene (%)	Total Phenols[a]	Total Flavonoids[b]	%AA[c]
TC	100	11.26±0.97	9.37±0.98	16.76±1.17	5.62±0.32	1.22±0.09	10.09±1.09
	250	19.23±0.99	14.89±0.78	22.54±1.32	6.17±0.43	3.23±0.11	11.54±1.02
	500	22.14±0.95	12.84±1.87	41.98±1.98	10.22±0.98	6.98±0.17	20.15±1.98
	1000	28.98±1.17	14.75±1.56	49.09±1.78	25.98±0.67	12.89±1.23	24.06±2.27
SC_{DP}	100	29.17±0.76	22.76±1.12	21.87±1.02	6.45±0.19	3.12±0.78	9.13±1.23
	250	33.12±0.98	25.01±1.43	24.47±1.09	10.12±0.32	4.98±0.91	10.23±1.98
	500	38.16±1.13	24.37±1.56	36.12±1.67	23.89±0.47	11.78±1.18	13.42±2.02
	1000	42.17±1.21	25.25±1.19	40.11±1.45	31.12±0.98	21.02±1.29	15.12±2.16
TOMF	100	29.34±0.43	21.68±1.76	26.09±1.23	8.19±0.78	2.89±0.09	8.17±1.10
	250	39.74±0.92	28.35±1.89	28.65±1.90	19.23±0.19	9.78±1,17	10.12±1.93
	500	47.27±1.34	31.49±1.19	33.56±1.98	25.48±0.87	17.14±2.09	11.23±1.99
	1000	59.26±2.36	34.83±1.76	41.21±2.09	36.98±0.67	27.18±2.11	14.09±1.98

[a]Values of total phenols levels (mg of gallic acid equivalent/g of extract); [b]Total flavonoids levels in quercetin equivalent per mg/g of extract; [c]Percentage values of DPPH radical scavenging activity.

DISCUSSION

In general, genetically modified plants have been the shorter, economical and durable solution to find sustainable agriculture. Even so, the usual crossing schemes and selection of genotypes need to be increasingly improved in order to make them more direct and less random in the process of obtaining individuals with suitable characteristics (VEASEY et al., 2011). The adoption of any agricultural technology involves costs and benefits both for farmers and for consumers. Therefore, determine them and identify them is essential to support a good legislation (GALVÃO; BORCHGRAVE, 2004).

Micronucleated Polychromatic Erythrocytes (MNPCE) results suggest there is no significant difference between groups treated with different concentrations of the extracts (Conventional tomato, structural long-life, mutant long-life), negative (water) and positive (Cyclophosphamide) control groups. However, in the treatment with the extract from mutant long life variety, a concentration increase will possibly lead to a higher MNPCE frequency in concentrations higher than 400mg Kg^{-1} because it is possible to observe a slight increase in MNPCE frequency.

This results can be explained considering studies realized by Fenech et al. (2002), who demonstrated that products in feed diet such as

agricultural pesticides, antioxidants, dyes, flavorings and preservatives are associated with the occurrence of DNA damage, but this evidence can only be found in chronic, continuous use and with a high concentration of a compound containing these substances. Moreover, studies carried out by Ho and Steinbrecher (1998); Brigulla et al., (2010); El-Nahas et al., (2011) and Oraby et al., (2015) showed that there are health risks related to the ingestion of diets containing genetically modified components, both by classic genetic engineering and transgenesis.

Similar results were observed in the evaluation of chromosome aberrations. But treatment with lower concentrations of different tomato varieties extracts showed no statistically significant difference when compared with negative control (water). The significant difference found between treatment with 400mg kg^{-1} may be due presence of agricultural pesticides in pulp tissue of tomato, regardless of genetic breeding. The Pan American Health Organization data (2009) show that even with the agricultural pesticides and chemical improvement applied to these pesticides, the tomato plant and its fruits still have significant influence on consumption of pesticides and interference with human health, depending mainly of the production region.

As regards chemical compounds in different varieties of tomato, these resulted in a considerable variation in their levels of certain components which demonstrates dose-dependence. Furthermore, antioxidant activity was higher for the conventional tomato variety. This is can be explained by its faster ripening metabolic stage when compared to the long life variety. These compound variations are similar to those found in other fruits containing these compounds and thus can contribute to dietary composition and demonstrate the need to assess nutritional quality. (SHAMI; MOREIRA, 2004; GOMES, 2007; ALMEIDA ET AL., 2011; ROCHA; SILVA, 2011; VIEIRA et al., 2011).

Numerous studies have shown that both genetic and environmental factors can regulate the biosynthesis of tomato's bioactive compounds. (PRUDENTE et al., 2010; CARLI et al., 2011; BARBAGALLO et al., 2013; MAZZUCATO et al., 2013; IGLESIAS et al., 2014). Therefore, it is possible to conclude that despite the absence of significant genotoxic activity among evaluated samples, the differences in composition and in antioxidant bioactivity observed in the tomato samples cultured under the same environmental conditions seems to be ruled by genetic factors, possibly due to genetic breeding.

ACKNOWLEDGEMENTS

Authors acknowledge with thanks the support from Centro Universitário de Patos de Minas, Minas Gerais, Brazil for providing experimental animals, technical support and infrastructure for mutagenicity tests.

RESUMO: O aumento da demanda na produção de tomate para o mercado alimentício vem incentivando transformações e implementações de novas biotecnologias na produção desse fruto, destacando-se a utilização cultivares híbridas que produzem frutos com maior resistência ao fenecimento e apodrecimento após colheita (cultivares do tipo longa vida). Dentro deste contexto, sabe-se que o consumo de alimentos oriundos de melhoramento genético necessita de atenção no aspecto de segurança alimentar e poder nutricional, pois a inclusão e/ou super expressão de características genéticas de interesse pode acarretar a curto ou em longo prazo danos à saúde humana. Neste cenário, o presente estudo teve como objetivo avaliar a genotoxicidade e mutagenicidade de diferentes variedades de tomates do tipo "longa vida" obtidos por melhoramento genético, assim como determinar seus principais compostos bioativos e atividade antioxidante. A genotoxicidade e mutagenicidade foram analisados por meio do teste do micronúcleo e pela avaliação de aberrações cromossômicas em medula óssea de camundongos. Foram determinados caroteno, betacaroteno, licopeno e o conteúdo de polifenóis e flavonoides totais por meio espectrofotométrico e atividade antioxidante pelo método do sequestro do radical DPPH. Diante dos resultados obtidos foi possível concluir que apesar da ausência de atividade genotóxica significativa entre as amostras avaliadas, as diferenças na composição e bioatividade antioxidante observadas no presente estudo, parecem ser governados por fatores genéticos, possivelmente provenientes do melhoramento genético realizado.

PALAVRAS-CHAVE: DPPH. *Solanum lycopersicum*. Micronúcleos. Aberrações cromossômicas. Melhoramento genético.

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