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'BRS Tospodoro': a high lycopene processing tomato cultivar adapted to organic cropping systems and with multiple resistance to pathogens

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ABSTRACT

'BRS Tospodoro' is a high lycopene tomato cultivar, which combines multiple disease resistance genes and desirable processing traits. This cultivar was found to be suitable for both conventional and organic crop systems. 'BRS Tospodoro' was obtained via backcross breeding using 'Viradoro' as recurrent parent and the inbred line 'CNPV 1306' as the donor of the *Pto* gene (resistance to *Pseudomonas syringae* pv. *tomato* race 0). 'BRS Tospodoro' has the *Mi1-2* gene that controls resistance to root-knot nematodes (*Meloidogyne incognita*, *M. javanica*, and *M. arenaria*) as well as tolerance to populations of the aphid *Macrosiphum euphorbiae* (vector of *Potyvirus* species), and to whiteflies (*Bemisia tabaci*). 'BRS Tospodoro' has also the *Sw-5b* gene, which controls resistance to major *Tospovirus* species (*Groundnut ringspot virus*, *Tomato chlorotic spot virus*, *Chrysanthemum stem necrosis virus*, and *Tomato spotted wilt virus*). This cultivar is also resistant to *Stemphylium solani* and *S. lycopersici* (*Sm* gene), *Fusarium oxysporum* f. sp. *lycopersici* race 1 (*I-1* gene) and *Verticillium dahliae* race 1 (*Ve* gene). 'BRS Tospodoro' presents uniform fruit ripening, with the first harvest being done around 110 to 120 days after plant emergence. The fruits are firm, square-shaped, with an average weight of 46 g (in organic cropping) to above 70 g (in conventional cropping). The soluble solids content is in the range between 4.6 and 4.8 °Brix. The external fruit color is uniform (gene *u*) and bright red ($L^* = 44.1$; $a^* = 33.9$; $b^* = 20.4$). The average lycopene content of mature fruits is 104 µg/g. This cultivar has the jointless locus (*j2*), which facilitates both manual and mechanical harvesting. 'BRS Tospodoro' has determinate growth habit (locus *sp*) with vigorous foliage, which provides good fruit protection from sunscald. 'BRS Tospodoro' can be cultivated in all the traditional processing tomato-producing areas of Brazil without the need of any technical adjustment. 'BRS Tospodoro' displayed very good adaptation to the South Brazilian Region and South Cone of South America (Argentina and Uruguay), where *P. syringae* pv. *tomato* and *Tospovirus* are serious economic problems for the crop. 'BRS Tospodoro' might also be cultivated in traditional processing tomato-producing areas (South-East, North-East and Central Brazil), if geminivirus control measures are adopted. Due to its vigorous foliage, 'BRS Tospodoro' requires careful monitoring of the environmental conditions that favors late blight (*Phytophthora infestans*) epidemics. 'BRS Tospodoro' has been evaluated under organic crop systems with excellent performance in field assays conducted in Brasília-DF, Brazil.

Keywords: *Solanum lycopersicum*, tomato, breeding, organic cropping.

RESUMO

'BRS Tospodoro': Cultivar de tomate para processamento com alto licopeno, adaptada aos sistemas de cultivo orgânico e com múltiplos genes de resistência a patógenos

'BRS Tospodoro' é uma cultivar de tomate para processamento industrial, rica no elemento antioxidante licopeno e adaptada a cultivos tanto convencionais quanto orgânicos. Esta cultivar é uma seleção derivada de um programa de retrocruzamentos entre a cultivar 'Viradoro' (usada como parental recorrente) e a linhagem 'CNPV 1306' (fonte doadora do gene *Pto* que controla a resistência à *Pseudomonas syringae* pv. *tomato* raça 0). 'BRS Tospodoro' apresenta ainda o gene *Mi1-2* que condiciona resistência a *Meloidogyne incognita*, *M. javanica* e *M. arenaria*, bem como tolerância para populações do pulgão *Macrosiphum euphorbiae* (vetor de *Potyvirus*) e da mosca-branca (*Bemisia tabaci*). 'BRS Tospodoro' também possui o gene *Sw-5b*, que confere resistência a quatro espécies de *Tospovirus* (*Groundnut ringspot virus*, *Tomato chlorotic spot virus*, *Chrysanthemum stem necrosis virus* e *Tomato spotted wilt virus*). Esta cultivar também é resistente aos fungos *Stemphylium solani* e *S. lycopersici* (gene *Sm*), *Fusarium oxysporum* f. sp. *lycopersici* raça 1 (gene *I-1*) e *Verticillium dahliae* raça 1 (gene *Ve*). Possui hábito de crescimento determinado (gene *sp*) com excelente cobertura dos frutos. A colheita dos frutos maduros é feita em torno dos 110-120 dias após o transplântio (mudas com 25 dias de idade). Os frutos são firmes, de formato quadrado-oblongo, maturação uniforme (gene *u*), com coloração externa vermelha escura, brilhante ($L^* = 44,1$; $a^* = 33,9$; $b^* = 20,4$). O teor médio de licopeno em frutos totalmente maduros é de 104 µg/g. O teor de sólidos totais variou entre 4,6 e 4,8 °Brix e o peso médio dos frutos varia de 46 g (em cultivo orgânico) acima de 70 g (em cultivos convencionais). 'BRS Tospodoro' apresenta o pedúnculo destituído de camada de abscisão (gene *j2*) e boa concentração de maturação de frutos. A cultivar mostrou excelente desempenho na Região Sul do Brasil e em cultivos no Cone Sul (Argentina e Uruguai), onde a infecção por *Tospovirus* e *P. syringae* pv. *tomato* são sérios problemas para a cultura. 'BRS Tospodoro' também é indicada para plantio nas principais regiões produtoras de tomate no Brasil (Sudeste, Nordeste e Centro-Oeste), desde que medidas preventivas de controle de geminivíroses sejam adotadas. Devido ao crescimento vigoroso de folhagem, 'BRS Tospodoro' requer monitoramento freqüente quando cultivado em condições ambientais que favoreçam a ocorrência de requeima (*Phytophthora infestans*). Esta nova cultivar mostrou excelente adaptação às condições de cultivo orgânico em ensaios conduzidos em Brasília-DF.

Palavras-chave: *Solanum lycopersicum*, tomate, melhoramento genético, cultivo orgânico.

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THE ORIGIN

'BRS Tospodoro' is a processing tomato (*Solanum lycopersicum* L.) cultivar, rich in the antioxidant lycopene and adapted to both conventional and organic crop systems. This cultivar displays multiple resistance genes to diseases and pests. 'BRS Tospodoro' was selected after self-pollinating plants of the fourth backcross generation during five cycles (BC_4F_5). The cultivar 'Viradoro' (Giordano *et al.*, 2000) was used as the recurrent parent and the inbred line 'CNPH 1306' was employed as the donor of the *Pto* gene (which confers resistance to *Pseudomonas syringae* pv. *tomato* race 0). Resistant plants were identified after inoculation via immersion of the leaves into a suspension (adjusted to 10^8 cfu/mL) of *P. syringae* pv. *tomato* isolate 'CNPH 31' (collected in Venda Nova dos Imigrantes, Espírito Santo State, Brazil). Ten plants free of symptoms were selected in each backcross generation. Throughout all the selection stages, the cultivar 'Viradoro' (susceptible) and the inbred line 'CNPH 1306' (resistant) were used as controls. The final evaluation was carried out with the *P. syringae* pv. *tomato* isolate 'CNPH 31' and also with the isolate 'CNPH 32' (collected in Patos de Minas, Minas Gerais State, Brazil), both classified as race 0. Comparative assays and progeny tests indicated that the *Pto* gene was incorporated in the homozygous condition in 'BRS Tospodoro' (Figure 1).

DESCRIPTION

'BRS Tospodoro' presents a determinate growth habit (gene *sp*) and a dense canopy, which offer protection for the fruits against sunscald damage. The ripe fruits are harvested around 110-120 days after the transplanting (using 25-day old plantlets). Therefore, 'BRS Tospodoro' displays vegetative and reproductive cycles similar to that of the cultivar 'Viradoro' (Giordano *et al.*, 2000). The average fruit weight ranged from 46 g (in organic cropping) to 70-85 g (in conventional cropping).

'BRS Tospodoro' does not contain abscission joint in the fruit pedicel (gene "jointless", *j2*), which increases the manual harvesting efficiency to around 25% and also facilitates mechanical harvesting (Boiteux *et al.*, 1995).

Nutritional characteristics and aspects of the fruits – The fruits of 'BRS Tospodoro' are firm, with uniform ripening (gene *u*) and oblong-squared shape (Figure 2). The soluble solid content ($^{\circ}$ Brix) in ripe fruits was estimated under open field conditions by using a digital refractometer (ATAGO model). In these assays, the Brix values varied from 4.6 to 4.8 $^{\circ}$ Brix. The values of colorimetric readings for the skin color at the stage of complete ripening were obtained by a colorimeter "Tristimulus" (Minolta Model CR-200b), with 8 mm of the measured diameter area of the equipment with diffuse illumination (illuminant D65, vision angle of 0° and chromatic space CIELAB). White ceramic tiles were employed to calibrate the system. The calibration values were: $L^* = 84.2$; a^*

$= 10.1$; and $b^* = 14.6$. 'BRS Tospodoro' displayed an external dark bright red color ($L^* = 44.1$; $a^* = 33.9$; $b^* = 20.4$). The ascorbic acid (vitamin C) content was determined by using the standard Tilmans titration method, which is based on the reduction of 2,6-sodium-dichlorophenolindophenol (DCFL) by the ascorbic acid (Pregnoletto & Pregnoletto, 1985). An aliquot of 10 mL of the standard ascorbic acid solution was added to an Erlenmeyer bottle containing 50 mL of an oxalic acid solution. The DCFL solution was titrated until obtaining a solution of persistent pink color for 15 seconds (standardization). The amount (in mg) of ascorbic acid in 100 mL of the samples was obtained through the following formula: $100 \times n' / [n/5 \times P]$. In which: n' = volume of DCFL (in mL) consumed in the titrating samples; V = sample volume used in the titrating method; n = volume of DCFL (in mL) consumed in the standardization procedure and P = weight of the sample (g). The average ascorbic acid content in fruits of the



Figure 1. Resistant response to *Pseudomonas syringae* pv. *tomato* (race 0) controlled by the presence of the *Pto* gene in the cultivar 'BRS Tospodoro' (right) and the susceptible control 'Viradoro' (left) [Resposta de resistência a *Pseudomonas syringae* pv. *tomato*, raça 0, conferidos pelo gene *Pto* presente na cultivar 'BRS Tospodoro' (direita) e ausente na cultivar suscetível 'Viradoro' (esquerda)]. Brasília, Embrapa Hortaliças, 2008.

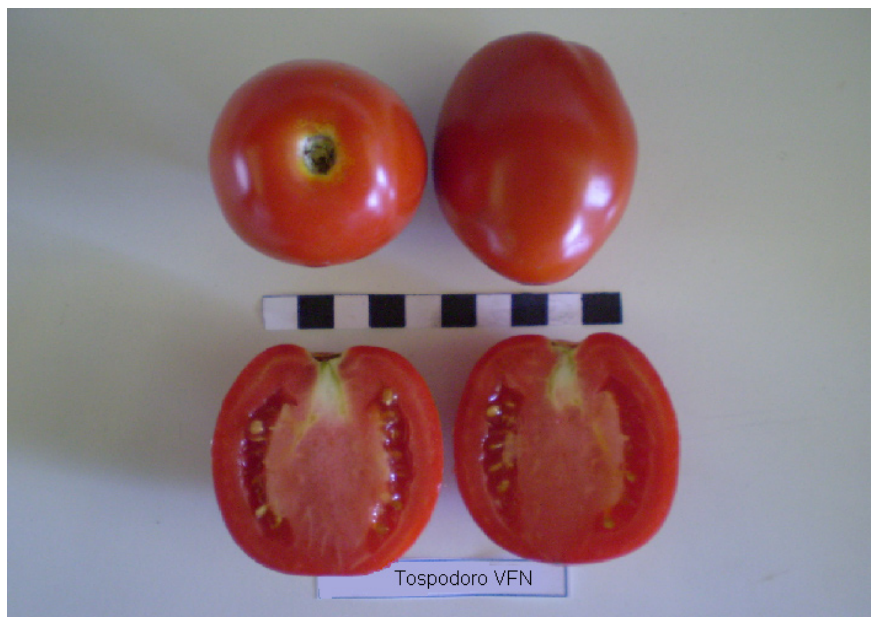


Figure 2. Fruit characteristics of the cultivar 'BRS Tospodoro' (características dos frutos da cultivar 'BRS Tospodoro'). Brasília, Embrapa Hortaliças, 2008.

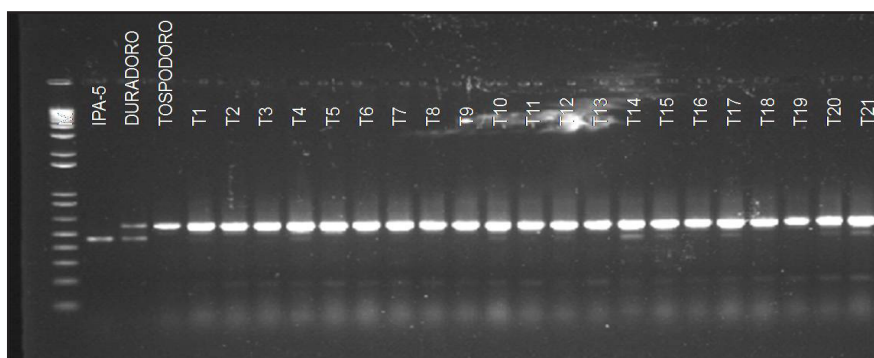


Figure 3. Marker-assisted selection using the Sw-5-2 primer pair (Dianese *et al.*, 2010). 'IPA-5' (homozygous susceptible cultivar), 'Duradoro' (heterozygous resistant hybrid), and 'BRS Tospodoro' (homozygous resistant for the Sw-5 locus). Samples from T1 to T21 are the electrophoretic profiles obtained from DNA extracted of individual 'BRS Tospodoro' plants randomly chosen for this marker-assisted analysis [seleção assistida com o par de 'primers' Sw-5-2 (Dianese *et al.*, 2010). 'IPA-5' (cultivar homocigota suscetível), 'Duradoro' (híbrido heterocigoto resistente) e 'BRS Tospodoro' (homocigoto para o locus Sw-5). Amostras T1 até T21 representam o perfil eletroforético do DNA extraído de plantas de 'BRS Tospodoro' colhidas ao acaso para análise via marcadores]. Brasília, Embrapa Hortaliças, 2008.

cultivar 'BRS Tospodoro' was 27 mg in 100 mL of juice (filtered). Currently, the daily recommended intake of vitamin C is 60 mg/day (Carr & Frei, 1999).

Analysis of amount of lycopene and determination of carotenoid types in mature fruits – Evaluation was done through the extraction of total carotenoids with ketone and followed by "High Performance Liquid Chromatography" (HPLC) analysis in C18 polymeric column. A mixture of acetonitrile: methanol: ethyl acetate was employed as a movable phase

(Rodriguez-Amaya, 2001). The total amount of carotenoids was obtained from the data of sample weight and absorbance (450 nm) in petroleum ether through the formula: $[(Abs_{450nm} \times 1.000,000 \times 100) / (2592 \times 100) / \text{weight}(g)]$. The determination of the carotenoid profile of 'BRS Tospodoro' was done by the characteristic absorption spectrum and retention time for each pigment. The fruit carotenoid amount was estimated by the peak percentages in the chromatograms. The results indicated an average mean of lycopene of 104.3 $\mu\text{g/g}$ in the fully

mature fruits. This lycopene amount of 'BRS Tospodoro' was also confirmed via colorimetric analysis (Carvalho *et al.*, 2005). Lycopene confers the typical red color of the tomato fruit and presents high antioxidant activity. This molecule has an outstanding nutraceutical action, which helps to prevent cardiovascular problems as well as prostate and ovarian cancer (Rao & Agawal, 2000; Smidt & Burke, 2004).

Fruit viscosity – Samples containing 1.3 kg of fruits were put into a microwave oven (frequency of 2450 megacycles and input power of 950 W) for 12 minutes in order to inactivate the enzymes. After staying in the microwave oven, the samples were weighed again and then, with the use of distilled water, their original weight was re-established. The samples were grinded in a multiprocessor (National MJC 13) and the pulp passed by a strainer with holes of 0.8 mm of diameter. After that, the air was taken out from the samples by a vacuum pump for about 2 minutes. The viscosity was measured using a Bostwick viscosimeter (consistometer). The viscosity results were reported as the distance traveled (cm) in 30 sec at 25°C (Barret *et al.*, 1998). The viscosity means obtained for 'BRS Tospodoro' was 13 cm in 30', indicating that the fruits are adequate for the production of items like "Ketchup".

Resistance to diseases – Besides the gene *Pto* (qualitative resistance to *P. syringae* pv. *tomato* race 0), 'BRS Tospodoro' contains the locus Sw-5 in homozygous condition. Such locus contains the gene Sw-5-b, which controls a resistant reaction to distinct species of the *Tospovirus* complex found in South America (Boiteux & Giordano, 1993; Dianese *et al.*, 2010), including *Groundnut ringspot virus*, *Tomato chlorotic spot virus*, *Chrysanthemum stem necrosis virus*, and *Tomato spotted wilt virus*. The resistance evaluation to *Tospovirus* was carried out as described by Giordano *et al.* (2000) and confirmed via molecular markers specific for this genomic region (Dianese *et al.*, 2010). The marker-assisted analysis confirmed that 'BRS Tospodoro' does not segregate for the locus Sw-5 (Figure 3). The selection for resistance to the root-knot

Table 1. Yield and number of fruits per plant, mean fruit weight of ‘BRS Tospodoro’ and commercial tomato cultivars, advanced lines and experimental hybrids in an organic production system (produção, número de frutos por planta, peso médio de fruto e produtividade da cultivar ‘BRS Tospodoro’ e de cultivares comerciais de tomate, linhagens avançadas e híbridos experimentais em sistema orgânico de produção). Brasília, Embrapa Hortaliças, 2008.

Cultivar	Mean fruit weight (g)	Number fruits /plant	Yield/plant (kg)	Yield (t/ha)
HEI - 013	54.42 a*	56.15**	3.01	70.99 a*
BRS Tospodoro	46.22 b	64.85	3.00	70.59 a
HEI - 014	51.20 b	57.74	2.96	66.28 a
HEI - 012	53.79 a	53.74	2.89	66.09 a
HEI - 015	48.71 b	56.92	2.76	61.80 a
HEI - 016	47.34 b	55.97	2.62	61.78 a
LAI - 023	45.14 b	58.98	2.65	58.03 a
HEI - 018	49.87 b	51.52	2.58	57.68 a
LAI - 082	50.72 b	50.58	2.56	54.19 b
LAI - 002	57.58 a	40.55	2.33	53.60 b
Viradoro	48.41 b	48.03	2.33	53.54 b
Nemadoro	61.99 a	35.37	2.19	51.63 b
HEI - 017	50.38 b	40.70	2.03	47.83 b
Caline IPA-6	57.04 a	34.47	1.97	46.51 b
CV(%)	10.31	42.41	55.68	17.84

*Means followed by the same letters (in the columns) are not significant different (Scott-Knott test, 5%) (médias seguidas pela mesma letra na coluna não diferem entre si pelo teste de Scott-Knott, 5%).

nematodes was carried out essentially as described by Charchar *et al.* (2003) and via molecular markers. The presence of gene *Mi1-2* demonstrates simultaneous resistance to “root-knot nematodes” (*Meloidogyne incognita*, *M. javanica*, and *M. arenaria*); tolerance to populations of the aphid *Macrosiphum euphorbiae* (vector of *Potyvirus*) (Rossi *et al.*, 1998) as well as tolerance to *Bemisia tabaci* biotypes (Nombela *et al.*, 2003). ‘BRS Tospodoro’ displayed resistant reaction to *Fusarium oxysporum* f. sp. *lycopersici* race 1 (gene *I-1*), *Stemphylium solani* and *S. lycopersici* (gene *Sm*) and *Verticillium dahliae* race 1 (gene *Ve*). The selection of resistant plants to these three fungal diseases was carried out as described by Santos (1997).

Technical recommendations for cultivation in Brazil – ‘BRS Tospodoro’ demonstrated adaptation to the Southern Region of Brazil, where the infection by *Tospovirus* and *P. syringae* pv. *tomato* are serious problems for the crop. ‘BRS Tospodoro’ might also be indicated for planting in the major processing tomato-

producing regions in Brazil (such as the Northeast, Southeast and Mid-West), only if control measures to prevent *Begomovirus* infection are established. ‘BRS Tospodoro’ is susceptible to this group of pathogens. Due to its vigorous foliage growth, ‘BRS Tospodoro’ requires constant monitoring when cultivated in environmental conditions favorable to late blight (*Phytophthora infestans*) epidemics. ‘BRS Tospodoro’ also presented excellent adaptation to organic production systems. ‘BRS Tospodoro’ ranked among the tomato cultivars for processing with the highest yields in the organic system of production in comparative field assays (carried out in Brasília-DF) (Nassur *et al.*, 2007). ‘BRS Tospodoro’ yields were around 70 t/ha (65 fruits/plant) totalizing approximately 3 kg of fruits/plant (average fruit weight = 46 grams), surpassing several hybrids F1 as well as the cultivars ‘Viradoro’, ‘Nemadoro’, and ‘IPA-6’ (Table 1). Yields of around 100 t/ha were obtained in conventional crop systems. The combination of several characteristics of interest (including a

number of dominant resistance genes) makes the ‘BRS Tospodoro’ a promising parental inbred line for use in breeding programs aiming to develop F₁ tomato hybrids.

The evaluation of ‘BRS Tospodoro’ in Uruguay – This cultivar also demonstrated high productivity and resistance to *Tospovirus* isolates in experimental fields conducted in Argentina and Uruguay. This cultivar was evaluated under conventional crop conditions in Uruguay, in 2006-2007 (González *et al.*, 2007). The prevalent environmental conditions were of pluviometric precipitations at the end of the cycle. Under such conditions, ‘BRS Tospodoro’ presented a productivity of 57 t/ha (Table 2), soluble solid content between 4.3 and 4.7 °B and average fruit weight of 80 g. In these field assays, the performance of ‘BRS Tospodoro’ did not demonstrate significant differences in productivity in relation to the cultivars commercially-used in Uruguay. The high levels of field resistance of ‘BRS Tospodoro’ to *Tospovirus* were also confirmed in this assay. The susceptible hybrid ‘Red Summer’ presented 11.1% of infected plants, whereas ‘BRS Tospodoro’ demonstrated all plants free of symptoms. In the second year of evaluation, with a more favorable environmental condition, ‘BRS Tospodoro’ yields were 92.4 t/ha, average fruit weight of 85.3 g and soluble solid content around 4.13 °B (González *et al.*, 2008). ‘BRS Tospodoro’ was also evaluated by small farmers in the South of Uruguay, demonstrating excellent adaptation. The foliage sanity, the size and firmness of the fruits as well as the “jointless” trait were the most relevant characteristics considered by the farmers of the family-based crop systems in Uruguay.

AVAILABILITY OF SEEDS

Embrapa Vegetable Crops will keep the genetic seeds in stock and has carried out multiplication of the basic seeds.

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Table 2. Yield and mean fruit weight of 'BRS Tospodoro' compared with commercial cultivars employed under conventional cropping system (2006-2007). Planting density of 29,300 plants/ha [produtividade e peso médio de frutos da cultivar 'BRS Tospodoro' e um conjunto de cultivares comerciais utilizadas no Uruguai em condições de plantio convencionais. Densidade de plantio de 29.300 plantas/ha. INIA), Las Brujas, Canelones, Uruguai].

Cultivar	Yield (t/ha)	Mean fruit weight (g)
Cuyano	61.3a*	87.5 **
HMX 3860	61.2a	78.4
H 6803	60.0a	64.8
Loica	58.6a	75.2
BRS Tospodoro	57.2a	80.8
H 9997	57.0a	86.1
HMX 3861	55.1a	71.2
IPA 6	54.9a	56.8
Rio Grande	54.3a	108.8
York	54.0a	71.0
Fortune	50.0a	70.9
Donald	49.5a	45.8
Red Summer	45.3b	84.6
HMX 2853	41.1b	84.7
H 9663	40.8b	102.0
UG 86	38.9b	110.8
H 5803	36.7b	116.8
H 8204	29.2b	64.2
CV (%)	17.15	19.1

*Means followed by the same letters (in the column) are not significant different (Scott-Knott test, 5%); **Samples of 30 fruits (médias seguidas pela mesma letra na coluna não diferem entre si pelo teste de Scott-Knott, 5%; amostra de 30 frutos).

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