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Increases the antioxidant capacity of passion fruit cultivated with two root systems after grafting

Aumento da capacidade antioxidante de maracujá cultivado com dois sistemas radiculares após enxerto

Aumento de la capacidad antioxidante del maracuyá cultivado con dos sistemas radiculares después del injerto

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Abstract

Aim of this study was to investigate the influence of the two methods of grafting on Passiflora *gibertti* in this antioxidant capacity. Experiment was conducted under field conditions in two agricultural years 2012/2013 and 2013/2014 following a randomized block design, being studied three treatments, plants not grafted, grafted plants by cleft grafting and grafted plants laterally and with maintenance of the root system. Fruit were harvested when they were physiologically ripe and taken to the laboratory for juice extraction and antioxidant compounds analysis. Total phenolic compounds, ascorbic acid, total flavonoids, Xanthoids contents and total antioxidant activity (DPPH) were evaluated. The use of two root systems showed higher contents of total phenolic compounds, flavonoids and ascorbic acid, however, had the total xanthoids contents decreased. It is concluded that the technique of grafting with the use of two root systems is favorable to the accumulation of higher levels of antioxidants.
Keywords: Ascorbic acid; Flavonoids; *Passiflora edulis* sims.; *Passiflora* gibertti; Phenols.

Resumo

O objetivo deste estudo foi investigar a influência de dois métodos de enxertia em *Passiflora gibertti* na capacidade antioxidante. O experimento foi conduzido em condições de campo em dois anos agrícolas 2012/2013 e 2013/2014 seguindo um delineamento de blocos casualizados, sendo estudados três tratamentos, plantas não enxertadas, plantas enxertadas por fissura e plantas enxertadas lateralmente e com manutenção do sistema radicular. As frutas foram colhidas quando estavam fisiologicamente maduras e levadas ao laboratório para extração do suco e análise de compostos antioxidantes. Foram avaliados compostos fenólicos totais, ácido ascórbico, flavonóides totais, conteúdo de xantóides e atividade antioxidante total (DPPH). O uso de dois sistemas radiculares apresentou maiores teores de compostos fenólicos totais, flavonóides e ácido ascórbico, porém, teve os teores de xantoides totais diminuídos. Concluiu-se que a técnica de enxertia com o uso de dois sistemas radiculares é favorável ao acúmulo de níveis mais elevados de antioxidantes.

Palavras-chave: Ácido ascórbico; Flavonoides; *Passiflora edulis* sims.; *Passiflora gibertti*; Fenóis.

Resumen
El objetivo de este estudio fue investigar la influencia de los dos métodos de injerto sobre *Passiflora gibertti* en la capacidad antioxidante. El experimento se realizó en condiciones de campo en dos años agrícolas 2012/2013 y 2013/2014 siguiendo un diseño de bloques aleatórios, estudiándose tres tratamientos, plantas no injertadas, plantas injertadas por hendidura y plantas injertadas lateralmente y con mantenimiento del sistema radicular. Los frutos se cosecharon cuando estaban fisiológicamente maduros y se llevaron al laboratorio para la extracción del jugo y el análisis de compuestos antioxidantes. Se evaluaron compuestos fenólicos totales, ácido ascórbico, flavonoides totales, contenido de xantoides y actividad antioxidante total (DPPH). El uso de dos sistemas de raíces mostró mayores contenidos de compuestos fenólicos totales, flavonoides y ácido ascórbico, sin embargo, el contenido total de xantoides disminuyó. Se concluye que la técnica de injerto con el uso de dos sistemas radiculares favorece la acumulación de niveles más altos de antioxidantes.
Palabras clave: Ácido ascórbico; Flavonoides; *Passiflora edulis* sims.; *Passiflora gibertti*; Fenoles.

1. Introduction

The cultivation of yellow passion fruit (*Passiflora edulis* Sims) has steadily decreased due to the attack of diseases, being the soil pathogens one of the main responsible for reduction of production in the State of São Paulo through the premature death of plants (Meletti & Bruckner, 2001).

The chemical control of these pathogens has not been shown to be efficient and an alternative to circumvent the problem of this disease is the use of the technique of grafting, because found that some sPTGies of the genus *Passiflora* have been shown to be resistant and tolerant to the disease, as is the case of the *Passiflora gibertti* NE Brown (Fischer et al., 2005).

Cavichioli et al. (2011) found that the method of cleft type grafting of *Passiflora edulis* on *P. gibertti* introduces a certain degree of incompatibility, due to lower plant development, as well as lower production of fruits and, consequently, lower production.

The use of multiple root systems is being used mainly to replace the conventional rootstocks for other more resistant Múller et al. (2002). In Japan it was possible to increase the production of pomeleiro using an additional root system (Nakajima et al., 1992). The greatest force of the plant with two root systems was also found by Girardi (2005) that discovered rejuvenation of the plant, as well as, greater productivity.

According to Lee (1994), generally, the rootstocks are known to influence the plant growth and fruit yield, however it was recognized that the quality of the fruits, as well as the flavor, firmness, shelf life, peel color, etc., can also be influenced by the rootstocks. One way to check this change is by the biochemical analysis of carbohydrates, proteins and lipids, but some more sPTGific analyzes, as total carotenoids, phenolic compounds (including the flavonoids and vitamin C may indicate the antioxidant capacity of a system, differentiating the food). However, there is little information about the influence of wild rootstocks in antioxidant capacity to the passion fruit (Salazar et al., 2016). In this sense, the objective of this work was to study the effect of grafting and the presence of two root systems in the antioxidant capacity of the passion fruit.

2. Methodology

2.1 Study area

The study was conducted at the experimental farm of Agência Paulista de Tecnologia dos Agronegócios – APTA, Presidente Prudente (SP), Brazil. The geographic coordinates are 22º69'65' south latitude and 51º36'86" west longitude and altitude of 430 m. The climate of the region is classified as Aw, according to the classification of Köeppen, with average annual temperatures around 25ºC.

2.2 Experimental design

The experimental experience was in randomized blocks with three treatments (plants not grafted - PNG, grafted plants by cleft grafting - PTG and grafted plants with maintenance of two root systems - PTR) in eight replicates, and each plot consisted of five plants.

2.3 Preparation of the seedlings

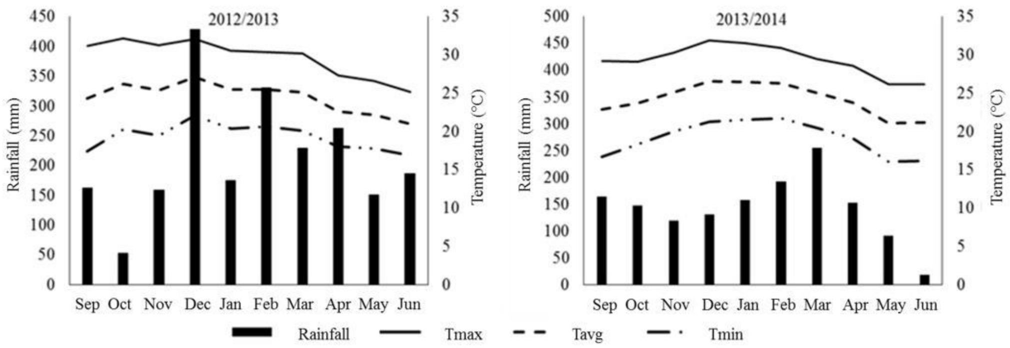
The seeds of *Passiflora edulis* Sims cv. Afruvec/Sul-Brasil and *P. gibertti* NE Brown were removed from ripe fruits collected in January 2012 and 2013 from the collection of germplasm of the APTA - Alta Sorocabana, being the sowing of two materials performed 30 days after the extraction in polystyrene trays of 200 cells filled with commercial substrate based on pine bark *Pinus*. After 30 days, the seedlings were transplanted into plastic bags with dimensions of 18 x 28 cm and filled with commercial substrate based on *Pinus* and enriched with slow-release fertilizer for six months, being planted only one individual per bag, with the exception of plants that the two root systems were kept.

The two types of grafting (cleft grafting and grafting with maintenance of two root systems) of *P. edulis* on *P. gibertti* were performed 30 days after transplantation, using a scalpel and tape of grafting (parafilm), being held 15 cm above the base of the plant.

2.4 Cultural Treatments

The conduction system was the trellis with a flat wire, fixed on stakes of 2.8 m in height (more 0.5 m buried) spaced 5 meters; thus, the wire was suspended to 2 m in height. The spacing of the crop was 2.5 m between plants by 3.5 meters between rows. In the first year of cultivation, the planting of seedlings was performed in the month of September 2012 and were cut in July 2013 so that in September 2013 new seedlings could be transplanted and conducted until June 2014. The planting during this period occurred due to weather conditions, once that, it was the month in which there were significant amounts of precipitation, allowing the planting of seedlings without losses or need for irrigation (Figure 1).

Figure 1 - Temperature data and precipitation recorded during the period of experiments' performing (September/1012 toJune/2014). Presidente Prudente – São Paulo, Brazil.



Source: Authors.

Before planting it was performed the analysis of soil to verify the need for liming and fertilization of production. According to the results of the analysis of soil (Table 1) it was carried out the recommendations of fertilization and liming for culture (Piza Júnior, et al., 1996).

Table 1 - Chemical analysis of soil before pH correction and fertilization at planting in two agricultural years, APTA, Presidente Prudente – São Paulo, Brazil.

Year	pH	M.O.	P_{resine}	S	Al^{3+}	H+Al	K	Ca	Mg	SB	CTC	V%
	$CaCl_2$	g dm^{-3}	mg dm^{-3}		----------- mmol$_c$ dm^{-3} -----------							
2012	4.9	12.3	9.9	5.2	1	26.9	5.3	23.2	6.1	34.5	56.7	61.6
2013	5.2	11.4	6.9	5.2	0	19.1	4.5	19.0	7.2	61.6	49.9	61.5

Source: Laboratory of Analysis of Soil and plant tissue. Agronomy College - UNOESTE- University of Western of São Paulo, Presidente Prudente – São Paulo, Brazil.

The fertilization formation began after the establishment of the seedlings under the recommendation of [9] using ammonium sulphate during the initial development until it reaches the period of production, when it was performed fertilization with the formulated 20-05-20.

The seedlings were transplanted and conducted with a single branch of vegetation (primary industry) until it reaches the wire, being the side shoots removed. Upon reaching the wire, all branches were conducted to the same side, leaving it grow over the wire, and at this stage, the secondary branches issued were not eliminated.

2.5 Fruits analysis

Fruit were harvested, physiologically ripe, of each parcel to compose a composite sample. The fruits harvest always occurred in the month of March, because, it is the month in which all treatments had fruits. After the harvest, the fruits were brought directly to the laboratory to be sanitized and extracted the juice from their pulp, rubbing them against a sieve, to perform the analyzes of antioxidant compounds. The antioxidant potential of the fruits pulp was performed through the analysis of the following parameters: Contents of total phenols, total flavonoids, ascorbic acid, total carotenoids and determination of DPPH. antioxidant activity.

The determination of total phenols in the pulp of passion fruit was performed according to the Folin-Ciocalteau method (Singleton and Rossi, 1965), and the results expressed in g $100g^{-1}$ of sample in accordance with the calibration curve of gallic acid. The vitamin C content was determined through the content of ascorbic acid, in which 20 g of pulp were homogenized with 20 mL of oxalic acid to 1% and titrated with a solution of 2.6-dichlorophenol-indophenol (Carvalho et al., 1990) and the results expressed in mg $100g^{-1}$ of sample. The total flavonoids were determined according to method of Awad et al. (2000) and the results were expressed in mg $100g^{-1}$ of sample. The totals carotenes were determined by the Sims and Gamon (2002), and the results were expressed in µg g^{-1} of sample. The determination of antioxidant activity (DPPH) was performed according Brand-Williams et al. (1995) and the results were expressed in mg g^{-1} of sample.

The results were submitted to analysis of variance and their averages were compared by the Tukey test at 5% probability. To check which compound contributed to an antioxidant activity of the pulp of the fruits it was performed analysis of Pearson's linear correlation.

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3. Results and Discussion

The contents of total phenols and carotenoids exhibited interaction between treatments and the year of cultivation. Whereas the antioxidant capacity (DPPH) was influenced only by years of cultivation (Table 2).

Table 2 - Contents of total phenols (g equivalent of gallic acid 100 g^{-1} of juice), carotenoids (µg of total carotenoids g^{-1} of juice) and antioxidant activity (DPPH µm Trolox g^{-1} of Juice) in passion fruit fruits *Passiflora edulis* Sims from plants not grafted (PNG), grafted conventionally (PTG) under P. *gibertti* NE Brown and with maintenance of two root systems (PTR) in two years of cultivation.

Treatments	**Phenols**	**Carotenoids**	**DPPH**
PNG	0.05 ± 0.001 b	30.30 ± 6.19 ab	0.53 ± 0,16
PTG	0.08 ± 0.002 a	38.90 ± 2.11 a	0.53 ± 0,12
PTR	0.07 ± 0.001 ab	27.11 ± 4.25 b	0.49 ± 0,11
F treat	11.17**	3.75*	1.07^{ns}
Year	**Phenols**	**Carotenoids**	**DPPH**
1st	0.09 ± 0.001 a	40.07 ± 4.50 a	0.58 ± 0.13 a
2nd	0.03 ± 0.004 b	24.14 ± 6.24 b	0.44 ± 0.08 b
F year	37.61**	19.16**	11.26**
F TxY	4.79**	5.86*	0.83^{ns}
C.V.(%)	54.95	43.89	31.02

^{ns} – not significant by F test at 5% probability, **significant at 1% probability by F test * significant at 5% probability by F test. Averages followed by the same letters at the column do not differ significantly among themselves, by the Tukey test at 5%. Source: Authors.

Similar contents (0.05 g $100g^{-1}$) of total phenols in *Passiflora edulis* Sims was found for Santos et al. (2021) when studying the phytochemical compounds and antioxidant activity of the pulp of two brazilian passion fruit species.

The not grafted plants produced fruits with lower content of total phenols in the first year, while in the second year no difference was observed (Table 2), according Padgett and Morrison (1990), the grafting can improve the resistance of crops to biotic stress, besides being an important tool to improve the fruit quality (Martinez-Rodriguez et al., 2008). Still, according Fernández-García et al. (2004), fruit obtained from grafted plants may show better quality than those obtained from non-grafted plants as a function of the rootstock. Recent studies have shown that the environmental conditions and cultivation techniques can influence the content of phenolic compounds and minerals in eggplant (Hanson et al., 2006). In addition, positive relation was found between the use of grafted plants on different rootstocks and improvement in the parameters of quality of production and of fruits, including phytochemical content, such as phenolic compounds (Giorni et al., 2005).

The levels of total carotenoids were higher in fruits from grafted plants conventionally in the first year of cultivation (Table 3). In the second year of cultivation, the fruits which showed lower levels of carotenoids were those produced by plants with two root systems (Table 3). The presence of these pigments is an indication for the consumer to evaluate the point of ripening and fruit quality (Freire et al., 2014). Values higher (139,9 and 251 µg g^{-1}) was found by Reis et al. (2018) when they studied the antioxidant potencial and physicochemical characterization of yellow, purple and orange passion fruit.

Table 3 - Unfolding of the interaction between the treatments and the year of cultivation on the contents of total phenols (g equivalent of gallic acid 100 g^{-1} of juice) and carotenoids (µg of total carotenoids g^{-1} of juice) in passion fruits *Passiflora edulis* Sims from not grafted plants (PNG), grafted conventionally (PTG) under *P. gibertti* NE Brown and with maintenance of two root systems (PRD) in two years of cultivation.

Treatments	Phenols		Carotenoids	
	Year 1	Year 2	Year 1	Year 2
PNG	0.06 ± 0.014 bA	0.04 ± 0.003 aB	22.36 ± 5.31 abA	27.55 ± 5.57 aA
PTG	0.13 ± 0.056 aA	0.04 ± 0.005 aB	24.29 ± 0.73 aA	23.80 ± 2.80 aA
PTR	0.10 ± 0.066 abA	0.04 ± 0.003 aA	18.39 ± 3.45 bA	17.45 ± 4.69 bA

Ns - not significant by F test at 5% probability. Averages followed by the same letters at the column do not differ significantly among themselves, by the Tukey test at 5%. Source: Authors.

The carotenoids are pigments responsible for the coloration of several plant products by assigning the colors ranging from yellow to red (Silva & Mercadante, 2002). The passion fruit is an important source of carotenoids which when added in the human diet can act in two ways, the first is as a precursor of vitamin A (Zeraik & Yariwake, 2010) and the second acting as an antioxidant by preventing many degenerative diseases and several types of cancer (Barbosa-Filho et al., 2008).

The plants with two roots showed higher content of ascorbic acid only in the first year of cultivation, while the grafted plants conventionally produced fruits with lower contents. In the second year of cultivation, there was no change in the levels of ascorbic acid (Table 4).

Salazar et al. (2016) when characterizing fruits of grafted yellow passion fruit observed that the levels of ascorbic acid found in fruits of all combinations of *P. edulis* with rootstocks and *P. edulis* Sims(from seed) exceeded the value of 20 mg of ascorbic acid 100 g^{-1} of pulp. Similar values were observed in our study because, Santos et al. (2009), these levels of ascorbic acid are commonly found in yellow passion fruit. How was verified for Melo et al. (2020) studying physical-chemical characterization of yellow passion fruit produced in different cultivation found 37.14 mg ascorbic acid 100^{-1} g fresh weight in passion fruit produced of conventional system with grafting and 43.02 mg ascorbic acid 100^{-1} g fresh weight in passion fruit produced of conventional system without grafting.

In relation to the content of flavonoids, plants with two roots produced fruits with higher levels of flavonoids when compared to fruits produced by plants, being approximately 50% lower in the first year and approximately 70% lower in the second year. The plants grafted conventionally produced fruits with intermediaries of flavonoids (Table 4). The contents observed were less than 0,16 mg. mL^{-1} observed by Zeraik and Yariwake (2010) in *P. edulis*.

Table 4 - Contents of flavonoids (mg equivalent of quercetin $100g^{-1}$ of Juice), ascorbic acid (AA - mg of ascorbic acid 100^{-1} of Juice) in passion-fruit *Passiflora edulis* Sims from not grafted plants(PNG), grafted conventionally (PTG) under *P. gibertti* NE Brown and with maintenance of two root systems (PRD) in two years of cultivation.

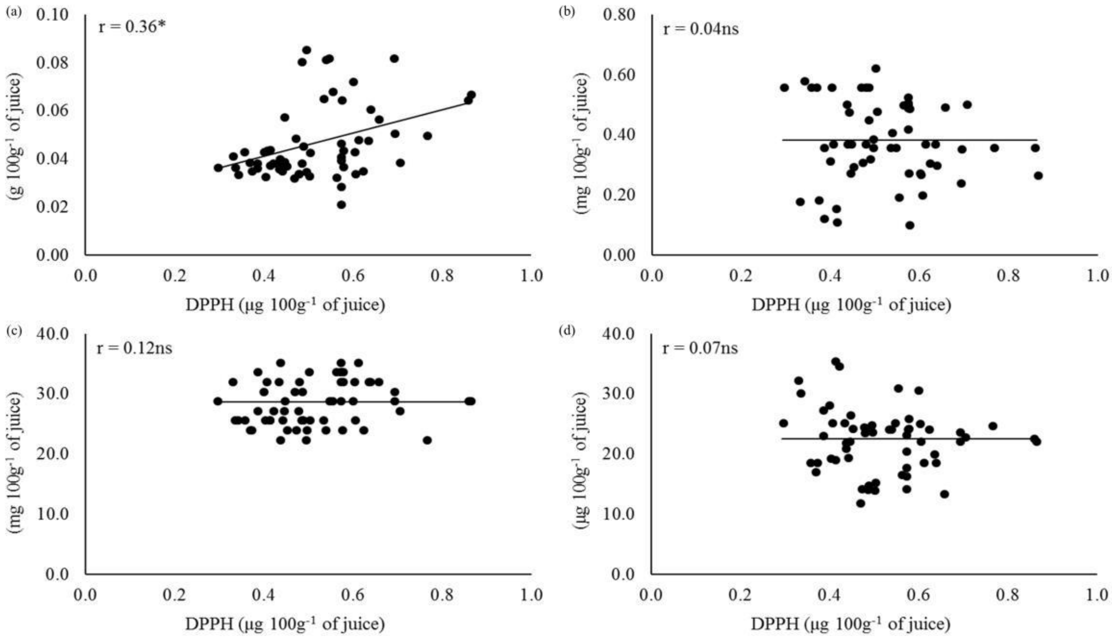
Treatments	**Ascorbic acid**		**Flavonoids**	
	Year 1	**Year 2**	**Year 1**	**Year 2**
PNG	28.22 ± 2.43 b	29.35 ± 3.86	0.27 ± 0.05 c	0.16 ± 0.06 c
PTG	25.44 ± 2.38 c	29.03 ± 4.30	0.36 ± 0.04 b	0.37 ± 0.05 b
PTR	31.93 ± 3.38 a	28.38 ± 3.33	0.49 ± 0.03 a	0.56 ± 0.03 a
F	19.75**	0.20^{ns}	61.46**	105.14**
C.V. (%)	8.07	12.05	11.8	14.01

ns – not significant by F test at 5% probability, **significant at 1% probability by F test * significant at 5% probability by F test. Averages followed by the same letters at the column do not differ significantly among themselves, by the Tukey test at 5%. Source: Authors.

The increase of secondary metabolites of plants, such as phenolic compounds, is a response to some kind of stress to which the plant was submitted by changing their contents according to the environment in which they are grown (Treutter, 2005). The rootstock studied is a non-domesticated species, which presents greater rusticity, providing increased tolerance or resistance to the pathogens attack. The tolerance or acquired resistance in these cases occurs through the production of several phytochemicals, among them, the phenolic compounds.

The antioxidant activity of phenolic compounds is mainly due to its reducing properties and its chemical structure. These characteristics play an important role in neutralizing or sequestration of free radicals and chelating agent of transition metals, acting both at the stage of initiation as in spreading of the oxidative process (Chun et al., 2005). In Figure 2 it is observed the positive correlation between the antioxidant activity and the content of phenolic compounds in the fruits of yellow passion fruit. Among the phytochemicals, the polyphenols may be responsible for the health benefits as in fighting free radicals and inactivation of other pro-oxidants, which act as protectors of the oxidative deterioration (Veberic et al., 2006).

Figure 2 - Correlation between the bioactive compounds (A) total phenolic compounds, (B) total flavonoids, (C) ascorbic acid and (D) total carotenoids and antioxidant activity in fruits of yellow passion fruit (*Passiflora edulis* Sims).



Source: Authors.

Total phenolic compounds are the main phytochemicals responsible for the antioxidant activity of fruits and vegetables, it one is remarkable since the fruit is rich in compounds that act on free radical scavenging activity (Carvalho et al., 2018**).**

Antioxidant potential of *Passiflora* spp is strongly influenced by factors such as variety, developmental stages, cultivation system, analytical method and the composition of phenolic compounds (Reis et al., 2018; Carvalho et al., 2018).

Silva et al. (2020) studyng the physicochemical quality, bioactive compounds and *in vitro* antioxidant activity of a new variety of passion fruit cv. BRSSertão Forte (*Passiflora cincinnata* Mast.) also them correlation results suggest that the bioactive compounds of passion fruit contribute strongly to the antioxidant activity, presenting significant relationships between most of the studied variables.

4. Conclusion

The presence of two root systems in the passion provides an increase in the levels of bioactive compounds like flavonoids, ascorbic acid and total phenols of the fruits.

Among the phytochemicals studied, what contributes the most to the antioxidant activity was the total phenolic compounds. This fact could be stimulate to use more methods or technology to potentiality the fruit´s characteristics to the foods quality will be better.

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