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Article

Carbohydrate content and season collection of cuttings from 'Roxo de Valinhos' fig tree

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Abstract

Choosing the best season collection of cuttings is essential to have success with propagation, due mainly to carbohydrate content, since is a source of energy for roots and shoots development. In literature, there are no papers that associate the collection season of cuttings with carbohydrate contents in figs. Therefore, the current study aimed to evaluate carbohydrate contents and season collection of cuttings to propagate 'Roxo de Valinhos' fig tree. The experiment was conducted in a greenhouse; cuttings were collected in the first week of the following months: June, July, August and September. The design was in completely randomized blocks, with four replicates of 30 cuttings collected per season. The following traits were evaluated: carbohydrate content; percentage of root cuttings, bud cuttings, cuttings without bud and root; number of buds, leaves and roots per cutting; length of the largest root and bud; bud diameter; bud and root dry mass; and root volume. Results indicated high carbohydrate content in cuttings that were collected in August; therefore, the best month to collect cuttings of 'Roxo de Valinhos' fig tree in the city of São Manuel, state of São Paulo. This result is due to high percentage of rooted cuttings and better development. Furthermore, carbohydrate content mainly affects root growth; besides that, reducing sugars are the most important feature in the development of cuttings.

Keywords: *Ficus carica*, cuttings, starch, sucrose, reducing sugars, correlation

Introduction

Brazil stood out as the world´s 8th largest producer of figs (*Ficus carica* L.) in 2013, with a production of 28 thousand tons (FAO, 2016). The state of São Paulo is the 2nd largest producer in Brazil; being the country´s largest producer of fresh figs. (IBGE, 2016). 'Roxo de Valinhos' is the most common type of cultivar found in the country (DALASTRA et al., 2009).

Although propagation techniques are crucial to a better quality of seedlings, but little explored; therefore, must be studied since fig trees are very rustic and vigorous plants. Furthermore, they are perennial plants and will produce over long period of time (TOMAZ et al., 2014), which is crucial for fruit trees. Fruits production depends indirectly on the production of good quality seedlings, which are responsible for establishment success in a field (ZACCHEO et al., 2013).

In fig tree farms, the most common propagation technique is the hardwood cutting during winter pruning (SOUSA et al., 2013); they are collected independently of the best season to propagate. Several factors affect the success of propagation methods; i.e. either external (management practice and climatic conditions) or internal (endogenous plant hormones and carbohydrates levels).

Carbohydrates are the result of photosynthesis process and is mainly produced

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by mature leaves (DANTAS et al., 2007). Before plants of temperate climate enter a period of rest, they store carbohydrates in their roots, primarily in the form of starch. At the end of the period, starch is hydrolysed and converted into soluble sugars and sucrose, then transported back to branches (BORBA et al., 2005).

The increase in transport of carbohydrates towards branches promote the sprouting (RICKES et al., 2016), as it will be used as a source of energy to build new tissues (VEYRES et al., 2008). Therefore, this fact allows us to say that seedling vegetative performance will be more vigorous, the greater the carbohydrate reserves in the cuttings.

Cuttings' collection time to propagate fig trees have already been studied by some authors. Kotz et al. (2011) reported that the best periods to collect were between June and August in Marechal Cândido Rondon, state of Paraná; as well as Nava et al. (2014), which observed more rooting cuttings in July in Dois Vizinhos, Paraná. These results are related to the experiments conditions, as temperature affects carbohydrates distribution within plants (Marafon et al., 2011).

Although these studies aimed to verify the best season to collect fig cuttings, they did not explore the amount of carbohydrates at collection time, with subsequent rooting and seedling development. Given all the above, the current study aimed to evaluate carbohydrate content of cuttings collected over different seasons to verify its relationship with the development of 'Roxo de Valinhos' fig trees.

Material and Methods

Experimental area characterization and material preparation

The experiment was conducted from June to December 2016 in a greenhouse, located at Department of Forest Science, School of Agriculture (FCA-UNESP), Botucatu, state of São Paulo, which is in the south-central region of São Paulo state (48°25'O; 22°51'S); climate type Cwa, according to Köppen classification.

Branches of 'Roxo de Valinhos' fig tree were collected from São Manuel experimental orchard, which belongs to FCA-UNESP, during the first week of the following months: June, July, August and September. After collection, branches were wrapped in moistened paper and conditioned in thermal refractory. Subsequently, they were transported to the Department of Forest Sciences, where were sanitized in sodium hypochlorite 1% solution.

Cuttings were prepared using pruning shears with a standardized length of 20 cm (about five buds) and a mean diameter of 17 mm. A bevel cut was made at the base of the cuttings and a straight cut at the apex, which was made one centimetre above the first bud, while the base cut was performed one centimetre below the last bud.

Afterwards, cuttings were buried to 1/3 of their length in 290 cm^3 tubes, filled with medium grain of sand and kept on tables inside the greenhouse at a temperature of ≤30°C and >80% relative humidity, equipped with an intermittent nebulization irrigation system, flow rate by nozzle (7L/h^{-1}), automatically activated for 10 seconds by electromagnetic panel every 15 minutes from 9:00am to 4:00pm. All cuttings from each season collections remained in these conditions for 90 days.

In parallel, some of these cuttings were taken to the I Fruit Production Laboratory, Department of Horticulture (FCA-UNESP), where were conditioned in a drying oven at 60°C, with forced air circulation, until reaching constant mass. After drying, cuttings were ground in a mill to evaluate carbohydrate contents.

Traits evaluated

Carbohydrate contents were quantified for starch and reducing sugars (glucose + fructose); non-reducing sugar (sucrose); and total sugars, expressed as a percentage, according to Somogy method, adapted by Nelson (1944).

For seedlings development, cuttings were removed from the tubes after 90 days and taken to I Fruit Production Laboratory, Department of Horticulture (FCA-UNESP), where were washed in running water , dried in room temperature, and the following traits were evaluated: percentage of rooted cuttings, sprout cuttings and cuttings without sprouts and roots per season, counted individually by repetition; number of sprouted buds, leaves and roots per cutting, counted individually; length of the largest root and sprout, determined using a graduated ruler; sprout diameter, determined using a digital calliper; sprout and root fresh mass, measured using an analytical balance; sprout and root dry mass; and root volume.

For dry mass, all material was packed in paper bags and oven dried at 65°C, with forced air circulation, until reaching constant mass. Root volume was measured in a 100mL graduated beaker; therefore, roots were put into a beaker containing known volume of water; the respective volume was recorded in mL by using the water displacement method, then values were converted into cubic centimetres.

Experimental design and data analysis

The experimental design was a completely randomized design, with four treatments that corresponded each collection seasons of cuttings (June, July, August and September) and four replicates that consisted of 30 cuttings, i.e. 120 cuttings per season and a total of 480 cuttings were evaluated.

Data were submitted to analysis of variance and means were compared by Tukey test at 5% probability. Analyses were performed through the Computer Program System for Analysis of Variance - SISVAR (FERREIRA, 2011). The Pearson correlation test were performed between carbohydrate contents of the cuttings and other evaluated traits.

Results and Discussion

There was no significant effect of season collection of cuttings on non-reducing sugars content, percentage of sprouted cuttings and sprout diameter among all evaluated traits (Tables 1, 2 and 3).

Regarding to the carbohydrates types, starch presented the highest mean values, whilst the lowest means were observed in non-reducing sugars (Table 1). The lowest mean of non-reducing sugars in branches is since carbohydrate is easily translocated as non-reducing sugar within tissues, whereas reducing sugars are used as source of energy for biochemical reactions (VEYRES et al., 2008).

For reducing sugar contents, August presented the highest mean, while the lowest was in June. Cuttings collected in August also showed high total sugar content, but not statistically different from cuttings collected in July. When starch contents were evaluated, an inverse behaviour was observed in sugars, i.e. cuttings collected in June presented higher mean, but not statistically different from means observed in July and September (Table 1).

Table 1 – Reducing sugar, non-reducing sugar, total sugars and starch of cuttings from 'Roxo de Valinhos' fig tree over different evaluation seasons followed by their means, coefficient of variation (CV), F test and low significant difference (LSD). Botucatu, 2016.

Season	Reducing sugar (%)	Non-reducing sugar (%)	Total sugars (%)	Starch (%)
June	1.66 d	2.26	4.04 b	16.61 a
July	4.14 b	2.07	6.32 ab	15.36 ab
August	4.79 a	3.34	8.31 a	11.68 b
September	2.53 c	1.73	4.35 b	12.97 ab
Mean	3.28	2.35	5.75	14.16
CV (%)	9.12	58.46	23.62	13.26
F test	92.30**	1.03NS	8.48**	5.68**
LSD	0.63	2.89	2.85	3.94

Equal letters in the column do not differ by Tukey test at 5% probability level.

NS = not significant; * = Significant at 5%; ** = significant at 1% by F test.

In cuttings, high-sugar with low-starch content result in a conversion of starch to sucrose (non-reducing sugar), glucose and fructose (reducing sugars). Waldie et al. (2010) stated that increased concentration of water-soluble carbohydrate in period of full dormancy is precisely due to the degradation of starch.

Therefore, the low-sugar content observed in June is because plants were at the beginning of the dormancy period, when most carbohydrates were stored in the form of starch, in which was hydrolysed and converted to soluble sugars that resulted in high means of reducing and total sugars between July and August. September is related to reduction period, since plants had already left dormancy; consequently, beginning issuing new branches by building new tissues from stored sugar.

In plants, high-carbohydrate content is important in the development during stress periods, vegetative dormancy, and source of energy for issuing new branches (BORBA et al., 2005).

Regarding to the percentage of rooted cuttings, August stood out with the highest mean (93.3%), but not statistically different from July (90.8%); while June was the lowest (61.7%), but did not differ significantly from September (65.0%). For percentage of cuttings without sprouts and roots, July performed the lowest mean; however, it did not differ significantly from June and August (Table 2). Considering this propagation method, the percentage of rooted cuttings is the most important feature, since it directly reflects on seedling production and indirectly on plant nursery business profit.

Like the current study, Ohland et al. (2009) studied fig cuttings in the absence of auxin and reported that August presented the highest percentage of rooted cuttings. While Nava et al. (2014) verified high means in July. Kotz et al. (2011) reported high percentage of rooted cuttings between June and August. However, Ramos et al. (2009) observed that September was the best season to collect cuttings.

In current study, the highest percentages of rooted cuttings coincided with the time they also presented high reducing and total sugar contents. Such result reinforces the importance of these compounds for roots and buds' development in cuttings. Furthermore, the use of carbohydrates occurs as a function of the water flow in plant organs, allowing to say that temperature plays a crucial role due to transpiration process (YAMAMOTO et al., 2010; MARAFON et al., 2011). With regards to cuttings methods and the best time of accomplishment, outcomes must consider different climatic conditions between regions, or even, from one year to another in the same region.

Table 2 - Percentages of rooted cuttings, sprouted cuttings and cuttings without buds and roots of 'Roxo de Valinhos' fig tree over different evaluation seasons followed by their means, coefficient of variation (CV), F test and low significant difference (LSD). Botucatu, 2016

Season	Rooted cuttings (%)	Sprouted cuttings (%)	Cuttings without buds and roots (%)
June	61.67 c	81.67	15.00 ab
July	90.83 ab	96.67	1.67 b
August	93.33 a	93.33	5.00 ab
September	65.00 bc	70.83	27.50 a
Mean	77.71	85.62	12.29
CV (%)	16.09	15.31	98.94
F test	7.10^{**}	3.20^{NS}	3.7^{*}
LSD	26.25	27.54	25.54

Equal letters in the column do not differ by Tukey test at 5% probability level.

^{NS} = not significant; * = Significant at 5%; ** = significant at 1% by F test.

Regarding to the number of sprouted buds, June presented the lowest mean, which also had the lowest mean of reducing and total sugar contents. For leaf number, root number and length of the largest sprouted buds, August and September presented the highest means, while the lowest were in June and July (Table 3). In a different way, Nava et al. (2014) found that leaf number of fig cuttings was greater in July than August; besides that, Ohland et al. (2009) observed that number of sprouted buds were higher in June than May, July, August and September.

Regarding to the number of sprouted buds, higher means result in vigorous cuttings; however, such trait is not an important feature on fig trees, since seedling carries only one branch. However, the largest number of leaves is an important trait, because it is directly linked to photosynthetic capacity in plants. Moreover, high number of roots is also an important trait for seedling establishment in a field. Although cuttings collected in September presented more leaves and roots, they also presented low percentage of rooting.

For length of largest root and root volume, August presented the highest means. The roots of the cuttings collected in June were smaller, but not statistically different from those collected in July. Whilst June, July and September did not present any significant difference in root volume (Table 3). Regarding to the largest length of the root, high root volume promotes better seedling establishment in a field after transplanting, since it increases plants survival rate.

For sprout dry mass, August presented the highest mean; however, June had the lowest one, but did not differ significantly from July. For root dry mass, the highest mean was also observed in August; however, not statistically significant within seasons (Table 3). Ramos et al. (2008) reported that root dry mass was greater in September, followed by October and August. As for previous traits, the highest dry mass of sprouts and roots were obtained in cuttings with high-sugar and low-starch content at collection time.

Table 3 - Number of sprouted buds, leaves and roots per cutting; sprout length and diameter; root length and volume; sprout and root dry mass from cuttings of 'Roxo de Valinhos' fig tree over different evaluation seasons followed by their coefficient of variation (CV), F test and low significant difference (LSD). Botucatu, 2016.

Season	Sprouted bud number	Leaf number	Root number	Sprout Length (cm)	Sprout diameter (mm)	Root length (cm)	Root volume (cm^3)	Sprout dry mass (g)	Root dry mass (g)
Jun	1.61 b	3.98 b	9.82 b	2.30 b	3.80	2.92 c	0.67 b	0.79 c	0.03 b
Jul	2.81 a	3.72 b	18.25 b	1.79 b	4.22	4.52 bc	0.94 b	1.14 bc	0.08 b
Aug	2.50 a	8.39 a	32.78 a	3.64 a	4.67	9.63 a	2.48 a	2.82 a	0.29 a
Sept	3.17 a	8.31 a	29.50 a	3.16 a	4.17	5.60 b	0.71 b	1.93 b	0.08 b
Mean	2.52	6.10	22.59	2.72	4.21	5.67	1.20	1.67	0.12
CV (%)	14.00	19.34	18.44	11.14	14.74	17.81	37.98	23.03	28.77
F test	14.33^{**}	19.43^{**}	25.62^{**}	30.24^{**}	1.32^{NS}	32.08^{**}	14.15^{**}	22.04^{**}	47.07^{**}
MSD	0.74	2.48	8.75	0.64	1.3	2.12	0.96	0.81	0.07

Equal letters in the column do not differ by Tukey test at 5% probability level. ^{NS} = not significant; * = Significant at 5%; ** = significant at 1% by F test.

Regarding to Pearson correlation test performed between carbohydrates content and cuttings developmental traits, a positive and significant correlation was observed among reducing sugars and percentage of rooted cuttings; root number; root length; root volume; sprout and root dry mass. The correlation with higher magnitudes, i.e. greater than 0.7, were among reducing sugars and percentage of root cuttings; root length and root dry mass (Table 8).

In correlation studies, it is important to consider magnitude, direction (positive or negative) and significance. According to Nogueira et al. (2012), a positive correlation coefficient indicates that as one variable increases the other also increases, whereas negative correlations means that as one variable increases in detriment of other.

For non-reducing sugars, a positive correlation was observed in carbohydrates with root volume and root dry mass, but none of them was greater than 0.7. Showing a greater effect of reducing sugars on vegetative development of cuttings. For total sugars, there was a positive correlation with percentage of rooted cuttings; percentage of sprouted cuttings; root number; root length; root volume; sprout and root dry mass. Besides that, the highest correlations were between total sugars with root volume and root dry mass (Table 8).

The correlation among sugars with sprout and root developmental traits of cuttings is due to the action of plant regulators can be inactivated by combining sugars from sucrose, glucose and fructose hydrolysis; consequently, changing the balance of the active molecules of auxin and cytokinin (Agulló-Antón et al., 2011), showing a strong interaction between carbohydrates and hormone levels in plants.

For starch contents, negative correlations were observed with percentage of cuttings without sprout and root; sprouted bud number; leaf number. However, there was a positive correlation with percentage of sprouted cuttings (Table 8). Although there was a positive correlation between starch with percentage of sprouted cuttings, the correlation with sprouted bud number was negative; therefore, showing that high starch content results in lower number of sprouted buds, as well as lower number of leaves and roots. According to Veyres et al. (2008) starch is carbohydrate reserve, whereas water-soluble carbohydrates are effectively used as a source of energy in biochemical processes, such as building plant tissues.

Table 4 - Pearson correlation coefficients between different carbohydrate types and vegetative developmental traits of fig cuttings ‘Roxo de Valinhos’. Botucatu, 2016.

	PRC	PSC	PCWSR	SBN	LN	RN	SL	SD	RL	RV	SDM	RDM
RS	0.75^{**}	0.48^{ns}	-0.48^{ns}	0.35^{ns}	0.24^{ns}	0.53^{*}	0.23^{ns}	0.39^{ns}	0.71^{**}	0.66^{**}	0.55^{*}	0.72^{**}
NRS	0.30^{ns}	0.31^{ns}	-0.26^{ns}	-0.10^{ns}	0.13^{ns}	0.27^{ns}	0.32^{ns}	0.29^{ns}	0.34^{ns}	0.51^{*}	0.38^{ns}	0.50^{*}
TS	0.66^{**}	0.50^{*}	-0.47^{ns}	0.15^{ns}	0.23^{ns}	0.51^{*}	0.36^{ns}	0.43^{ns}	0.66^{**}	0.75^{**}	0.59^{*}	0.78^{**}
S	0.49^{ns}	0.62^{**}	-0.65^{**}	-0.60^{*}	-0.57^{*}	-0.38^{ns}	-0.48^{ns}	0.13^{ns}	0.02^{ns}	0.15^{ns}	-0.23^{ns}	0.10^{ns}

NS = not significant; * = Significant at 5%; ** = significant at 1% by F test.

RS: reducing sugar. NRS: non-reducing sugar. TS: Total sugar. S: starch. PRC: percentage of rooted cuttings. PSC: percentage of sprouted cuttings. PCWSR: percentage of cuttings without sprout and root. SBN: sprouted bud number. LN: leaf number. RN: root number. SL: sprout length. BD: sprout diameter. RL: root length. RV: root volume. SDM: sprout dry mass. RDM: root dry mass.

It is believed that carbohydrates do not act directly in rooting, but they are source of energy and carbon for synthesis of substances essential for root formation, such as plant hormones (SOUZA; PEREIRA, 2007). Such assertion is also supported by Hartmann et al. (2011), who stated the importance of carbohydrates for rooting is because auxin needs a source of carbon for biosynthesis of nucleic acids and proteins; therefore, requiring energy and carbon for roots formation.

In general, by means of correlations between carbohydrate levels and developmental traits of cuttings from ‘Roxo de Valinhos’ fig tree, especially percentage of root cuttings, root volume and root dry mass due to the high magnitude of coefficients; therefore, carbohydrates present in the cuttings act more effectively in increasing root developmental traits than sprouts at the season of collection.

Conclusions

August is the best month for cuttings to be collected to produce ‘Roxo de Valinhos’ seedlings in São Manuel, state of São Paulo, since cuttings have high carbohydrate contents and, consequently, a high percentage of rooting and better vegetative development.

Carbohydrate content has a high correlation with root developmental traits in detriment to sprouts, besides that, reducing sugars have a significant effect on cuttings development compared to non-reducing sugars.

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