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Total Soluble Sugars in Root and Stem of Wichita and Western *Carya Illinoinensis* Varieties in Northern Mexico

Ernesto Concilco-Alberto[1], *Luis Manuel Valenzuela-Núñez[2], Anselmo González-Torres[1], Cristina García-De La Peña[2], Edwin Amir Briceño-Contreras[2], Juan Ramon Esparza Rivera[2], José Luis Reyes-Carrillo[1], Mario García-Carrillo[1]

[1] Postgrado en Ciencias en Producción Agropecuaria, Universidad Autónoma Agraria Antonio Narro Unidad Laguna, Periférico Raúl López Sánchez s/n, Colonia Valle Verde. C. P. 27054. Torreón, Coahuila, México

[2] Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango. Laboratorio de Biología y Ecología Forestal. Av. Universidad s/n. Fraccionamiento Filadelfia, C. P. 35010, Gómez Palacio, Durango, México

Abstract. The aim of the current study was to determine the monthly total soluble sugar concentration in the stem and root of the Wichita and Western walnut (*Carya illinoinensis*) varieties during a productive cycle in northern Mexico. Vegetable samples were obtained using a systematic sampling method, and processed by means of a spectrophotometric assay to determine the total soluble sugars (TSS) concentration in productive walnut trees. Data analysis was performed by means of a 2-way ANOVA. The results of the TSS concentration had differences throughout the months of an annual productive cycle, and were also different between the evaluated walnut varieties. The highest total soluble sugar concentration in stem and root were in the lowest temperature months, and it decreases in both tissues when the environmental temperature rises. The Wichita variety had a higher total soluble sugar concentration than the Western in every month during the productive cycle. Further research is required to assess the effect of other type of factors including environmental or agronomic over the dynamics of the total soluble sugars in productive walnut trees.

Key words: *Carya illinoinensis*, reservoir, non-structural carbohydrates, deciduous trees.

Introduction

Vegetable species produce compounds that are stored in their tissues as a reservoir for future use (Tixier, *et al*., 2018; Martínez-Trinidad, Plascencia-Escalante, & Islas-Rodríguez, 2013). Carbohydrates are the most important vegetable reservoir compounds (Davison, 2019; Hennion *et al*., 2019), since these compounds participate in most of the plant's fundamental functions (Pérez-Urria, 2009), and are essential for its survival (Davidson, 2019; Furze et al., 2019). These compounds can be found in either soluble or insoluble form (Davidson, 2019; Pallardy, 2008), and are classified according to their functions as either structural, or as non-structural (Davidson, 2019; Liu *et al*., 2018; Smith *et al*., 2018; Pallardy, 2008). Non-structural carbohydrates are an energetic and carbon source in plants (Davidson, 2019; Liu *et al*., 2018; Smith *et al*., 2018; Pallardy, 2008), and these compounds are mostly found as starch and soluble sugars (Furze *et al*., 2019; Jin *et al*., 2018; Smith *et al*., 2018).

Soluble sugars can be stored in different vegetable tissues (Davidson, 2019; Liu *et al*., 2018; Smith *et al*., 2018; Pallardy, 2008) during time periods when either their production is abundant (Briceño-Contreras *et al*., 2021; Magel, Eining, & Hampp, 2000), or their demand is lower than their synthesis (Pérez-Urria, 2009). It has been reported that the highest total soluble sugars (TSS) production and accumulation rate occurs prior to flowering (Briceño-Contreras *et al*., 2018), and these soluble sugars are used when either the plant's energetic demand overcomes the production, or the plant could be facing some stress level (Valenzuela-Núñez *et al*., 2019; Pérez-Urria *et*

* Corresponding Author's email: luisvn70@hotmail.com

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al., 2009). Plants can dispose of energy required for their growth, reproduction, defence against diseases, as well as to withstand adverse environmental conditions or any other type of stress (Aluko *et al*., 2021; Ok *et al*., 2021; Liu *et al*., 2018; Pinkard *et al*., 2018; Martínez-Trinidad, Plascencia-Escalante & Islas-Rodríguez, 2013).

The TSS in perennial deciduous trees is mobilised from the organ reservoirs to the specific demand sites to mainly provide energy during the winter and dormancy stage (Smith *et al*., 2018); to contribute to withstanding chilling temperatures; and also to contribute to dormancy breakage and promote bud growth during spring (Ok *et al*., 2021; Tixier *et al*., 2019). Total soluble sugars also participate in respiration and osmotic regulation (Briceño-Contreras *et al*., 2021; Hartann & Trombore, 2016), as well as in root (Valenzuela-Núñez *et al*., 2014), leaf and stem growth (Magel, Eining, & Hampp, 2000).

The TSS in fruit species is mobilised to all organs and tissues according to their energy requirements in order to be fully developed and functional (Moscardini *et al*., 2020; Liu *et al*., 1999). However, there are fruits in the developing stage, like coffee beans, in which the TSS is mobilised in a higher concentration from carbon reservoirs to fruits in formation. A plant's demand for non-structural carbohydrates for fruit production can be up to 30% of total plant carbon, as such energetic and carbon demands are much higher than for the formation of other organs and tissues such as branches and stems. It is possible that vegetative and reproductive pathways could be simultaneously active, which represents a much higher energy and carbon demand (Moscardini *et al*., 2020). Other additional situations where vegetable organisms mostly depend on the TSS reservoirs are when the plant is under stress, and also when the total carbohydrate production in the plant or tree is too low (Tixier *et al*., 2018).

Walnut tree [*Carya illinoinensis* (Wangenh.) K. Koch.] is a caducifolious fruit tree that is very important worldwide (Giuseppe, Fabrizio-Van der Watt, & Coetzer, 2018), whose commercially production is in arid and semi-arid climates in northern Mexico. This tree is originally from northern Mexico and the U.S. southeast, and these two countries are the most important world walnut producers (USDA, 2019). Walnut fruit production in Mexico is 164,308 $t \bullet year^{-1}$, mainly in northern regions (Valenzuela-Núñez *et al*., 2019; García-Hernández *et al*., 2009), and in the Comarca Lagunera (Coahuila, Mexico) this crop represents an annual income of up to 200 million pesos, with an annual regional production of 9,376.6 t (SIAP-SAGARPA, 2018). The dominant varieties in this region are Wichita and Western, which represent 33% and 50% of the crop area respectively, and 17% are composed of other varieties such as Barton, Mahan, Texas, Pawnee, Cheyenne and Criollas or hybrids (Orona-Castillo *et al*., 2013). Hence, it is highly important to elucidate the behaviour of plant reservoir compounds like soluble sugars in order to thoroughly understand the adaptive capacity of productive walnut tree varieties.

The effect of several factors including environmental conditions (Aluko *et al*., 2021), season of the year, plant or tree age, and species (Pinkard *et al*., 2018) on the total soluble sugar concentration has been evaluated. Furthermore, in several caducifolious species more fluctuations in TSS concentration have been reported than in perennial species (Lui *et al*., 2018; Hoch, Richter, & Körner, 2003). It is related to the available amounts of TSS in reservoirs to the tree defoliation grade (Yang *et al*., 2021), since the soluble sugars are used by the plant for osmotic regulation and phloem maintenance (Michelot-Antalik *et al*., 2019). Moreover, the soluble sugar concentration can be affected by the plant hydration level, as in sweet cherry (Vosnjak *et al*., 2021); on the other hand, TSS concentration in species like *C. illinoinensis* depends on the phenological crop stage (Valenzuela-Núñez *et al*., 2019). Thus, there is a lack of information regarding the dynamics of the total soluble sugar concentration in vegetable tissues, considering parameters such as variety and season of the year given that TSS concentration in plant organs depends on diverse factors (Briceño-Contreras *et al*., 2018).

Hence, the aim of the current study was to determine the total soluble sugar concentration in root and stem of two walnut Wichita and Western varieties produced in northern Mexico during a productive cycle.

Materials and Methods

The current study was run in an Experimental farm of the Universidad Autónoma Agraria Antonio Narro, in Torreón, Coahuila (México) with the coordinates 25° 33' 22" and 103° 22' 07". The site has a desertic dry climate with annual temperature and average rainfall of 21 °C and 230 mm. Farm irrigation was carried out by flooding, applying an annual average of 748 mm using underground water with a pH of 8.2, and electric conductivity of 1480 $\mu\Omega \bullet cm^{-1}$ (INEGI, 2012).

Samples of *C. illinoinensis* varieties Wichita and Western were obtained from adult trees (approx. 40 years old) using a systematic sampling procedure (Briceño-Contreras *et al*., 2018); two samples were taken from root and stem during each month of an annual productive year (from July 2016 to June 2017). Root and stem samples were obtained and processed after field sampling and transferred to the laboratory according to procedures published

by Briceño-Contreras *et al*. (2018). Walnut samples were stored in an ultrafreezer (Revco Value Plus® ThermoScientific® Waltham, United States) at -70 °C for a week, lyophilised afterwards for 7 days at 40 °C, and finally ground until obtaining a fine powder (Fritsch® Pulverisette 15®).

Total soluble sugar concentration was determined using the method published by Van Handel (1968). 10 mg of ground sample was placed in a 2 mL Eppendorf tube (MCT-200-C Clear Axygen Scientific® Schwerte, Germany), and mixed with 1000 µL of an extraction solution (70% methanol); after 10 minutes the mixture was centrifuged (Spectrafuge 16M® Labnet International, Edison, United States) at 10,000 rpm for 5 minutes at 4 °C. Then 500 µL of the mixture supernatant was extracted and mixed with 1000 µL of a solution of 50 mL of H_2SO_4 and 100 mg of anthrone (Sigma-Aldrich; Darmstadt, Germany). The mixture was boiled for 10 minutes and allowed to cool down at room temperature, and its absorbance was read at 625 nm using a spectrophotometer Genesys (Thermo scientific® Genesys 20), with each sample analysed in duplicate.

Shapiro-Wilk tests were run to prove the normality of the data set. Descriptive statistic parameters were obtained and a 2-way ANOVA ($p < 0.05$) was run to analyse the data of each organ (stem and root) among the varieties (Wichita and Western) during the months of a productive cycle (SPSS Inc® Trial Version, 2009).

Results

The total soluble sugar (TSS) concentration in the root of *C. illinoinensis* was significantly affected by the productive cycle month ($F = 5.823$, $p \leq 0.001$), variety ($F = 222.201$, $p \leq 0.001$), and the interaction of these two factors ($F = 8.557$, $p \leq 0.001$) (Table 2). The Wichita variety had the highest TSS concentration in February (38.19 mg•g^{-1} DM), whilst it had the lowest TSS concentration in July (35.87 mg•g^{-1} DM, Figure 1).

The total soluble sugar concentration in the stem of walnut trees was significantly affected by the productive cycle month ($F = 63.377$, $p \leq 0.001$), variety ($F = 194.778$, $p \leq 0.001$), and the interaction of these two factors ($F = 10.400$, $p \leq 0.001$) (Table 4). The Wichita variety had the highest TSS concentration in December (35.20 mg•g^{-1} DM), whilst the Western variety had the lowest soluble sugar concentration in July (20.75 mg•g^{-1} DM, Table 3, Figure 2).

Discussion

The behaviour of the total soluble sugar concentration in the root and stem of walnut (*C. illinoinensis*) trees indicate changes throughout the productive cycle, having the highest TSS concentration in both organs during the winter months. These results concur with those reported by Liu *et al*. (2018), who stated that soluble sugar concentration changes depend on the season of the year as an adaptive mechanism of trees to the conditions of each

Table 1

Results of Total soluble sugar concentration in the root of the Wichita and Western walnut (*C. illinoinensis*) tree during a productive cycle

MONTH	WICHITA	WESTERN
	Total soluble sugar, mg•g^{-1} DM	Total soluble sugar, mg•g^{-1} DM
July 2016	35.87±2.03	35.85±2.85
August 2016	37.27±0.69	34.87±0.96
September 2016	37.23±1.42	33.05±2.37
October 2016	36.34±2.11	34.37±0.53
November 2016	37.75±0.52	35.82±1.86
December 2016	37.19±1.26	36.65±0.64
January 2017	37.93±0.62	35.66±2.78
February 2017	38.19±0.72	34.75±0.96
March 2017	37.90±0.63	34.90±0.83
April 2017	37.60±0.90	33.04±0.56
May 2017	36.54±0.78	35.76±0.80
June 2017	36.36±0.70	35.62±1.65

**Data are presented as the mean ± SD (n = 16). DM = Matter Dry.*

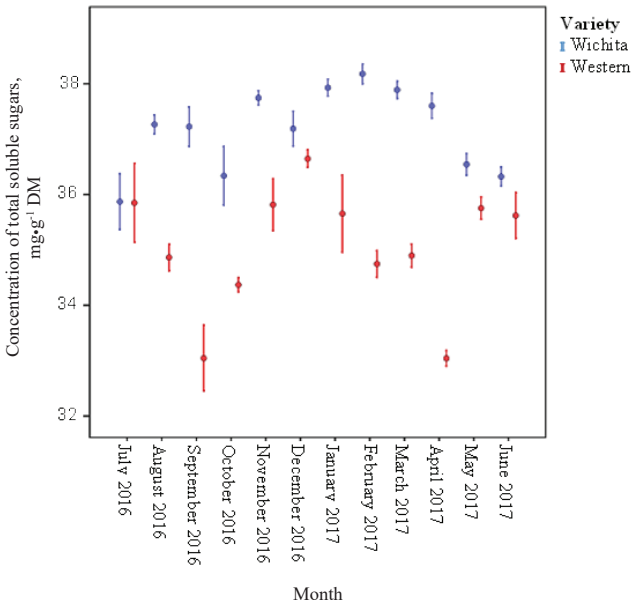


Figure 1. Mean results (n = 16) of total soluble sugar concentration in the root of the Wichita and Western walnut (*C. illinoinensis*) varieties during a productive cycle. Vertical bars show the standard error mean.

Table 2

Two-way ANOVA of the results of total soluble sugar concentration in the root of Wichita and Western walnut (*C. illinoinensis*) varieties during a productive cycle (Factors Variety and Month)

Origen	Sum of Squares type III	Degrees of freedom	Square mean	F	p
Adjusted model	759.567[a]	23	33.025	16.539	0.000
Intersection	500459.354	1	500459.354	250627.934	0.000
Variety	443.696	1	443.696	222.201	0.000
Month	127.912	11	11.628	5.823	0.000
Variety * Month	187.960	11	17.087	8.557	0.000

Table 3

Results of total soluble sugar concentration in the stem of Wichita and Western walnut (*C. illinoinensis*) trees during a productive cycle

MONTH	WICHITA	WESTERN
	Total soluble sugar, mg•g^{-1} DM	Total soluble sugar, mg•g^{-1} DM
July 2016	24.35±4.02	20.75±3.62
August 2016	35.16±0.78	25.01±5.40
September 2016	34.21±1.74	25.88±3.61
October 2016	33.14±1.35	28.39±4.30
November 2016	34.05±0.95	32.95±2.15
December 2016	35.20±1.03	34.86±1.25
January 2017	34.11±0.85	29.53±3.26
February 2017	32.37±1.86	29.43±1.46
March 2017	29.82±2.36	28.72±2.44
April 2017	26.10±2.93	23.66±4.33
May 2017	22.88±2.28	21.74±4.93
June 2017	30.62±3.06	21.93±2.11

**Data are presented as the mean ± SD (n = 16). DM = Matter Dry.*

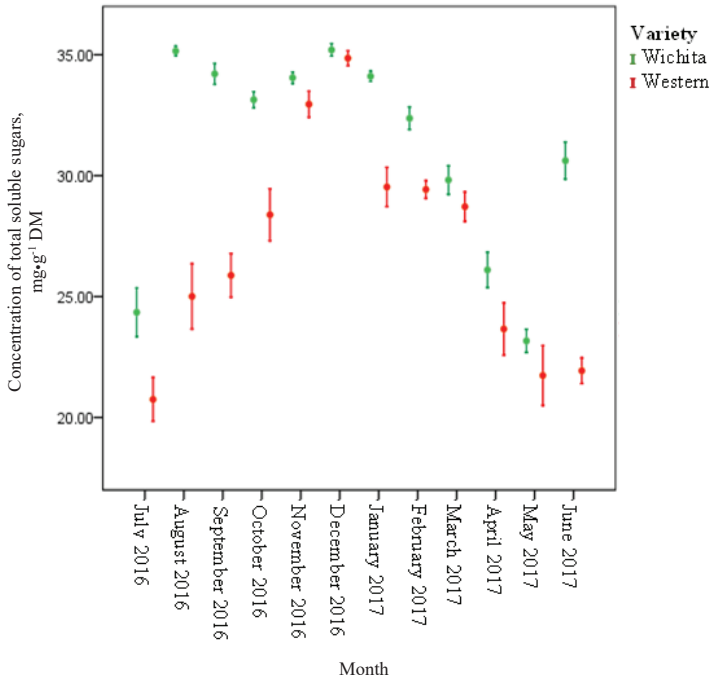


Figure 2. Mean results (n = 16) of total soluble sugar concentration in the stem of Wichita and Western walnut (*C. illinoinensis*) trees during a productive cycle. Vertical bars show the standard error mean.

Table 4

Two-way ANOVA of the results of Total soluble sugar concentration in the stem of Wichita and Western walnut (*C. illinoinensis*) varieties during a productive cycle (Factors - Variety and Month)

Origin	Sum of Squares type III	Degrees of freedom	Square mean	F	p
Adjusted model	8429.945[a]	23	366.519	43.753	0.000
Intersection	322130.773	1	322130.773	38454.473	0.000
Variety	1631.644	1	1631.644	194.778	0.000
Month	5839.981	11	530.907	63.377	0.000
Variety * Month	958.321	11	87.120	10.400	0.000

specific season. Caducifolious species have larger fluctuations in TSS concentration than deciduous species, which is attributed to the regular defoliation occurring in caducifolious trees; thus, these trees use diverse resources including water, light, the energy and carbon reservoir, and nutrients in a more efficient way than perennial trees. Furthermore, the obtained results in the current study agree with Valenzuela-Núñez *et al*. (2019), who reported a higher TSS in walnut tree root during their dormancy stage (43.55 mg g^{-1} DM) when trees are defoliated; meanwhile TSS concentration decreases by up to 66% during the production months (December and January). On the other hand, Moscardini *et al*. (2020) reported that TSS concentrations in organs of deciduous species do not have the same behaviour every year; this could depend on the amount of photosynthetic organs such as leaves throughout the seasons of the year. Yang *et al.* (2021) also reported changes in TSS concentration of *Abies alba* during an annual productive cycle, stating that this tree species reacts to defoliation, making adaptive changes such as increases in reservoir compound concentration to assure plant survival. Moreover, Briceño-Contreras *et al*. (2021) stated that prior to flower formation, TSS accumulation increases as an adaptive mechanism of the tree in order to use the carbohydrate reservoirs during stages when there is low carbohydrate production, and these compounds are required to maintain the tree's basal metabolism. Vosnjak *et al*. (2021) registered that fluctuations in

TSS concentration in *Prunus avium* L., organs were not similar in different productive cycles, which was attributed to factors such as water availability and tree photosynthetic disorders, resulting in photosynthesis inhibition, and thereby affecting carbohydrate synthesis and accumulation. These researchers concluded that TSS concentration in fruit trees is mainly determined by fruit formation, which concurs with the findings reported in the current study, since TSS concentration in both evaluated walnut organs was significantly different during the months of fruit production compared to the rest of the year. Besides, the changes in TSS concentration found in the current study throughout the productive cycle months concur with Ok *et al*. (2021), who reported variations in TSS concentration in *Diospyros kaki* Thuns., evaluated from November to April, with the highest soluble sugar concentration being during the months of lowest temperatures. Moreover, Turhan & Ergin (2012) reported the highest TSS concentration during the tree acclimation months, which is mostly due to plant adaptive mechanisms that lead to the tree's adaptation to the cold winter temperature; and these researchers also reported that TSS concentration increases during dormancy breakage when the environmental temperature rises.

The TSS concentration in walnut stem and root in the current study were higher in the Wichita variety than in the Western in every month of the evaluated productive cycle, which is in line with Briceño-Contreras *et al*. (2021), who reported that starch content in Wichita walnut was higher than in the Western variety, attributing this to the difference in adaptive capacity and nutritional requirements between these two walnut varieties.

Ok *et al*. (2021) reported differences in TSS between Taishuu and Romans varieties of sweet kaki (*Diospyros kaki* Thumb.); they mentioned that these two varieties have different TSS in every month during a productive cycle, having the highest soluble sugar concentration during the lowest temperature months, which could result from differences in adaptive capacity to winter conditions for each species and variety.

Conclusions

The results of the total soluble sugar (TSS) concentration in walnut tree stem and root had differences throughout the months of a productive cycle, and were also different between the evaluated walnut varieties (Western and Wichita). The highest TSS concentration in stem and root was obtained in the months of lowest temperatures (winter), and it decreases when the environmental temperature rises. The Wichita walnut variety had a higher total soluble sugar concentration than the Western in every month during the productive cycle. Further research is required to assess the effect of other type of factors, including environmental or agronomic, over the dynamics of the total soluble sugars in productive walnut trees.

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