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Article

Low-temperature-resistant sugars in avocado rootstocks

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Abstract

In Mexico, due to the demand for fruit, orchards of avocado (*Persea americana* Mill.) variety 'Hass' of subtropical climate are erroneously established in cold areas, affecting their production; an alternative is to use cold-tolerant rootstocks of the Mexican race, tolerance related to the increase of sugars in areas of demand, which it shares through grafting with the 'Hass' variety; therefore, in 2021, the segregants of the 'rootstocks' duke 7, tepetl, aceitoso, and colecta 1 were evaluated at the College of Postgraduates; analyzing glucose, fructose, sucrose and starch contents in vegetative shoots at 1, 7, and 14 days of treatment; in chamber 1 (treatment) with luminosity of 380 µmol m^{-2} s^{-1} and temperatures with light of 15.61 °C, and darkness of 4.40 °C; and chamber 2 (control) with luminosity of 367 µmol m^{-2} s^{-1} and temperatures with light of 23.2 °C, and darkness of 19.29 °C, considering the factor of cold and low luminosity, it was observed that, chlorophyll in the leaves shows growth without photosynthetic deficiency in plants in both chambers; the glucose content of the 'Hass' variety varies according to the glucose content in the rootstock; the fructose content increases in grafted and non-grafted materials, acting as an osmoprotectant, the sucrose content increases in the grafted aceitoso material and the starch content is not affected; as a result, duke 7 and tepetl were the materials with the highest concentration of glucose and fructose under cold conditions.

Keywords:

cold tolerant, fructose, glucose, 'Hass', segregating.

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Introduction

The avocado varieties grown are the result of hybridization between races of the species (Knight, 2002). The 'Hass' variety is a hybrid of the Guatemalan race but with genes of the Mexican race and fruits with a large storage and transport capacity that favor its postharvest management characteristics (Crane *et al*., 2013). In 2020, avocado production in Mexico was 2.39 million tons (PROFECO, 2021). This production of avocado fruit involves a process that begins with the selection of the rootstock, which is responsible for providing the commercial variety with a root support and maintaining its genetic characteristics, without the variability obtained from the variety propagated by seed, since the success or failure of a plantation depends on it (Barrientos-Priego *et al*., 2000).

Avocado materials from subtropical areas, such as the 'Hass' variety, are more susceptible to low temperatures (Crane *et al*., 2013), so an alternative in cold conditions is the use of rootstocks of the Mexican race as a possible solution to the effects of cold (Lockard and Schneider, 1981). Lacono *et al*. (1998) mention that, together, the graft and the rootstock form characteristics in the plant, which are the result of the characteristics of each.

Affirming the influence of rootstock on the variety, Mickelbart *et al*. (2007) found significant differences in nutrient uptake because of different clonal rootstocks. Bergh (1992) mentions that the Mexican race has contributed genes that favor cold tolerance, because there is a concentration of soluble sugars that reduce the freezing point of the intracellular solution (Poirier *et al*., 2010).

Therefore, the increase or decrease in the concentration of sugars in the plant is an acclimatization response, such as the glucose content in the leaves of vegetative shoots considered tissues of demand, which can change its concentration due to environmental, biochemical, and physiological factors (Rolland *et al*., 2002), or the increase in fructose content that acts as an osmoprotectant against adverse environmental conditions due to heat, cold, or water stress (Marschall *et al*., 2019) and thus continue to use sucrose as the main source of energy to continue their biochemical processes (Hopkins and Huner, 2004).

Therefore, in this research, avocado segregants were selected from four materials of the Mexican race (duke-7, tepetl, aceitoso, and colecta 1), grafted and non-grafted with 'Hass', to evaluate, under a 14-day cold treatment, the concentration of glucose, fructose, sucrose, and starch as possible sugars involved in tolerance to low temperatures.

Materials and methods

We began with the sowing of seeds in September 2020 and later in February 2021, we performed the side-veneer grafting with a 'Hass' scion in the greenhouse area of the 'La Cruz' experimental center of the Salvador Sánchez Colin-CICTAMEX, SC Foundation, located in Coatepec Harinas, State of Mexico, at 18° 55' 10.4" north latitude, 99° 45' 39.7" west longitude, with an altitude of 2 100 m and we concluded with the transfer of the plant in May 2021 to the cold chambers located at the College of Postgraduates, Montecillo, Texcoco, State of Mexico, at 19° 27' 36.1" north longitude, 98° 54' 22.5" west latitude, with an altitude of 2 250 m.

Experimental design

A randomized complete block design was used with an arrangement of divided plots, where large plots represent the factor of temperature (factor A). Chamber 1 was the cold treatment, with an average temperature of 15.61 °C during daylight hours, with a light intensity of 380 µmol m^{-2} s^{-1} and an average temperature in darkness of 4.4 °C (data recorded with a datalogger); on the other hand, chamber 2, considered the control, kept an average temperature of 23.2 °C in light, with a light intensity of 367 µmol m^{-2} s^{-1} and an average temperature in darkness of 19.29 °C (data recorded with a datalogger). In the small plots (factor B), 20 avocado materials grafted with the 'Hass' variety and 20 non-grafted materials and factor C, which includes 10 plants of each material used (aceitoso, colecta 1, tepetl, and duke 7), using a total of 40 rootstocks in each chamber. The results between

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chambers and the results by material were compared using the LSD test in the SAS (Statistical Analysis System) version 9.4 program.

Plant growth measurement

At the beginning of the experiment, the height of each plant was measured in centimeters with the help of a ruler, marking the base of the stem at the level of the container substrate to ensure a correct second measurement at the end of the experiment.

Sample preparation

The leaves of the vegetative shoots of the plants were cut at 1, 7 and 14 days, considering that the perception of cold begins on day 1, and a possible start of acclimatization at 7 and 14 days; they were stored in aluminum sachets at -20 °C, the fresh weight (between 0.1 g and 0.13 g) was recorded with a Scientech, SA 120 electronic analytical balance. Samples were ground directly in 1.5 ml Eppendorf tubes with 500 µl of 80% ethanol, then centrifuged at 10 000 rpm for 10 min in a Dlab D3024 24-tube Eppendorf centrifuge, and the supernatant was extracted for placement in water vapor immersion for 1 h at 80 °C.

Glucose, fructose, and sucrose reading

A reaction mixture (Table 1) was prepared, and 200 µl was placed in each well of a reading plate, 2.5 µl of sample was added, and then 10 µl of each enzyme previously dissolved independently in 1.5 ml of reaction mixture (Table 2) was applied; finally, the samples were read with the help of a Thermo Scientific multiskan FC.

Table 1. Reaction mixture for 10 ml.

Hepes 0.5 M pH 8.0	KCl	MgCl	ATP	NAD^+	Hexokinase	H_2O
2 ml	2.5 ml	150 µl	8.5 mg	2.83 mg	10 µ	5.4 ml

Information from the College of Postgraduates (2021).

Table 2. Process for reading sugars.

	Enzyme	Amount (µl)	Incubation time (min)	Temperature (°C)
Reading 1	Hexokinase	10	Direct	room
Reading 2	G6PDH	10	40	35
Reading 3	PGI	10	40	35
Reading 4	Invertase	10	40	35

Information from the College of Postgraduates (2021).

Starch determination

One milliliter of dimethyl sulfoxide (DMSO) was added to the samples and they were homogenized with a vortex (Analog vortex Mixer) for 30 s, placed in a bain-marie for 30 min at boiling point; they were again homogenized, and the supernatant was extracted to be placed in two 2 ml Eppendorf tubes, placing 100 µl in each, and 450 µl with and without enzyme was added to each tube.

The 100 µl samples with and without enzyme were prepared at 5:00 pm and left overnight at 55 °C, left to cool at room temperature and centrifuged at 7 000 revolutions per minute for 5 min; from the fully identified 2 ml Eppendorf tubes, 10 µl of sample was extracted and placed in the holes (wells) of the plates, to add 200 µl of reaction mixture and proceed to the next process (Table 3).

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Table 3. Process for reading starch.

	Enzyme	Amount (µl)	Incubation time (min)	Temperature (°C)
Reading 1	Hexokinase	10	Direct	Room
Reading 2	Dehydrogenase	10	35	35

In starch, the standard curve was generated, calibrated to 20µl only for glucose

Source: information from the College of Postgraduates (2021).

Results and discussion

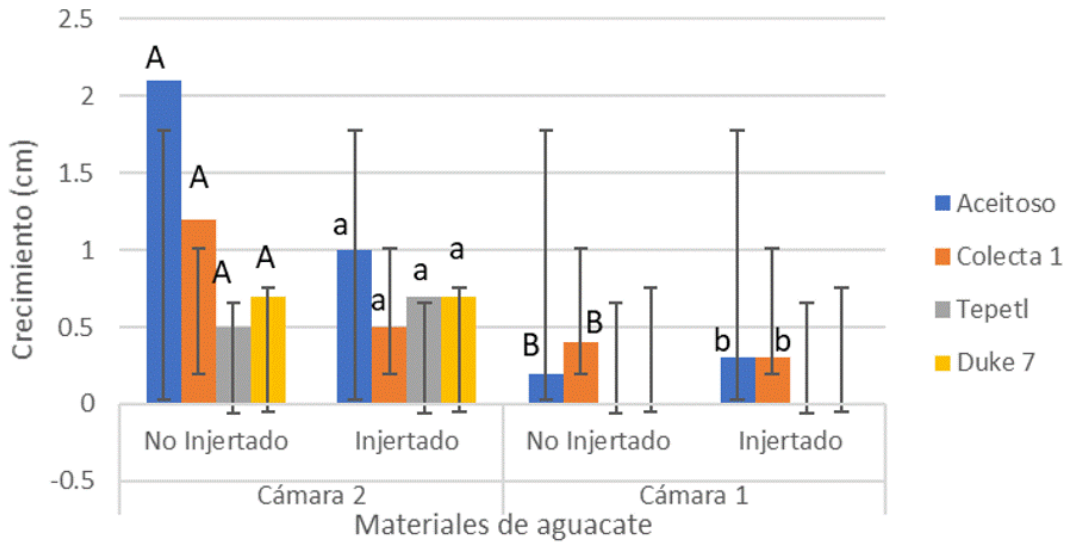
Plant growth

The growth results of the plants in chamber 2 (control) with temperatures with light of 23.20 °C, and darkness of 19.29 °C showed an increase in size at 14 days in the non-grafted and grafted materials; as mentioned by Lahav and Trochoulias (1982), the vegetative development of avocado plants takes place in the optimal temperature range of 20-30 °C, where there is a maximum net assimilation of CO_2 favoring the growth of the entire plant.

On the contrary, under the conditions of chamber 1 with temperatures with light of 15.61 °C, and darkness of 4.4 °C, little or no growth was recorded in the non-grafted and grafted materials; therefore, the effect of avocado tree growth and development is significantly reduced at temperatures below 10 °C (Whiley *et al*., 1990).

According to the comparison of means with a significance of α= 0.05, the growth of the non-grafted and grafted materials of chamber 2 (control), with values of 1.12 cm with a σ= 0.713 cm and 0.72 cm with a σ= 0.206 cm, respectively, were significantly higher than those obtained in the non-grafted and grafted materials of chamber 1 (cold treatment), with values of 0.3 cm in the non-grafted materials with a σ= 0.191 cm, and grafted materials with a σ= 0.173 cm (Figure 1).

Figure 1. Effect of cold treatment on the growth of non-grafted (A, B) and grafted (a, b) avocado plants 14 days after establishment in the chambers.



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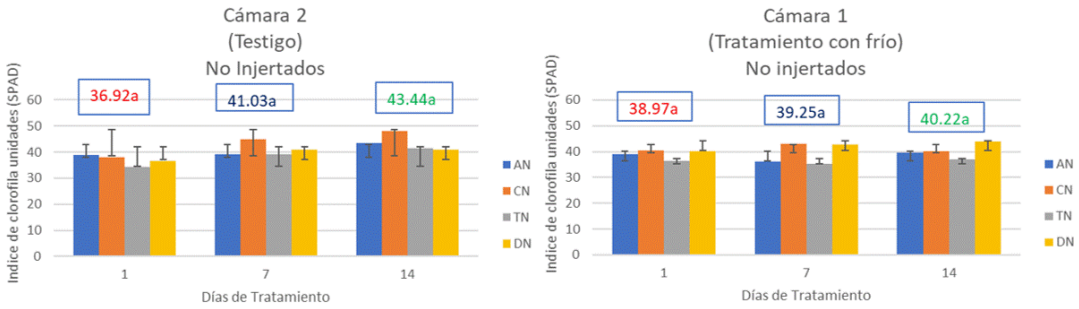
Chlorophyll content

Concerning the chlorophyll indices, (SPAD) units, of the non-grafted materials of chamber 2 (control), they show a general average reading of 40.46 units with a σ= 3.29 units, compared to 39.48 units with a σ= 0.65 units of the non-grafted materials of chamber 1 (cold treatment); according to Coelho *et al*. (2010), the range of 35.2 and 42.2 SPAD units is suitable for diagnosing a good level of N in potato (*Solanum tuberosum*).

Likewise, Taiz and Zeiger (2004) mention that the intensity of the green of the leaves is correlated with the chlorophyll content and the concentration of nitrogen; therefore, it can be mentioned that the chlorophyll content in the materials of both chambers is not affected by light intensity or cold treatment. According to the comparison of means with a significance α= 0.05, between the non-grafted materials of both chambers, there is no significant difference (Figure 2).

Figure 2. Reading of SPAD units of non-grafted materials of aceitoso (AN); colecta 1 (CN); tepetl (TN); and duke 7 (DN), comparing the same treatment day number between the two chambers.



The materials established in chamber 1 (cold treatment) did not present alterations in the maturation of the evaluated leaves, coinciding with the results of chamber 2 and with Gianquinto *et al*. (2003), who mention the positive correlation of the SPAD indices with the availability of nitrogen in the leaves; it can be said that the process of leaf maturation goes through different morphological stages, from their emergence to their senescence, from demanding to being sources of nutrients, as well as their response to environmental variables (Dickson *et al*., 2000), indicating that the low-temperature factor does not affect nitrogen content at the demand stage.

For the grafted materials of chamber 2 (control), with an average of 38.35 units and a σ= 3.11 units, compared to the grafted materials of chamber 1 (cold treatment), with an average of 39.07 units and a σ= 1.59, it can be mentioned that, according to the comparison of means with a significance of α= 0.05, there are no significant differences in the chlorophyll content between the grafted materials of both chambers (Figure 3).

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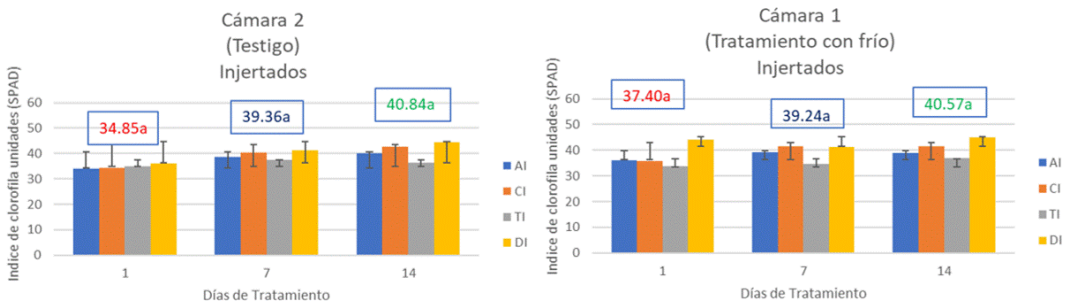
Figure 3. Reading of SPAD units of grafted materials of aceitoso (AI); colecta 1 (CI); tepetl (TI); and duke 7 (DI), comparing the means (LSD) of the same treatment day number between both chambers. The number above each set of bars of the rootstocks is the average value across all rootstocks.



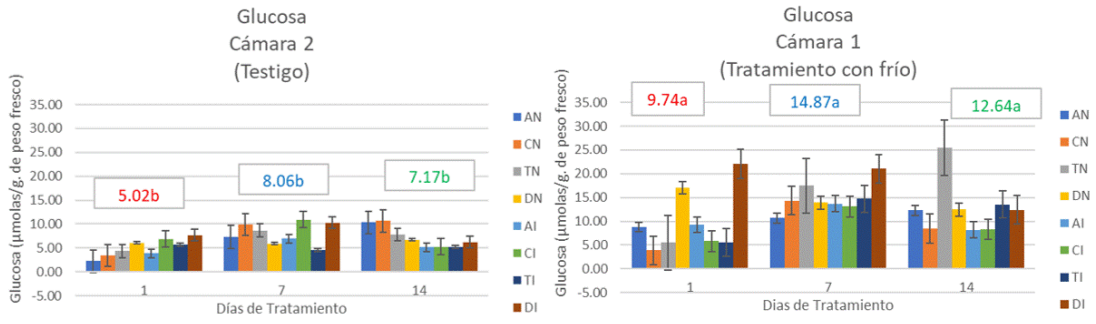
Sugar content

Glucose

Analyses carried out on leaves of young shoots, which, according to Liu *et al*. (2002), should be leaves less than 20 days after shooting, show that the concentration of glucose in the leaves of vegetative shoots increases under the conditions of chamber 1 (cold treatment), because glucose is used in the synthesis of compounds such as organic acids, amino acids, and lipids (Geigenberger *et al*., 2005), building blocks for biomass accumulation and plant development; therefore, the glucose content in the leaves of vegetative shoots as demand tissues is affected by environmental, biochemical, and physiological factors that together determine the amount of photosynthates that can be unloaded into the demand tissues (Rolland *et al*., 2002).

In accordance with the above, the glucose concentration in conditions of chamber 1 (cold treatment) presents average values of 9.74 µmoles g^{-1} fresh weight for day 1, 14.87 µmoles g^{-1} fresh weight on day 7, and 12.64 at 14 days, compared to the control, with average values of 5.02 µmoles g^{-1} fresh weight for day 1, 8.06 µmoles g^{-1} fresh weight on day 7, and 7.17 µmoles g^{-1} fresh weight at 14 days; it can be mentioned that, according to the comparison of means with a significance of $\alpha = 0.05$, the 3 average values of the cold treatment are significantly higher than those of the control (Figure 4).

Figure 4. Effect of cold treatment on the glucose content in young leaves of materials of aceitoso (AN and AI); colecta 1 (CN and CI); tepetl (TN and TI); and duke 7 (DN and DI), comparing the same treatment day number between both chambers. The number above each set of bars of the rootstocks is the average value across all rootstocks. The comparison of means (LSD) is between chambers on the same day of treatment.



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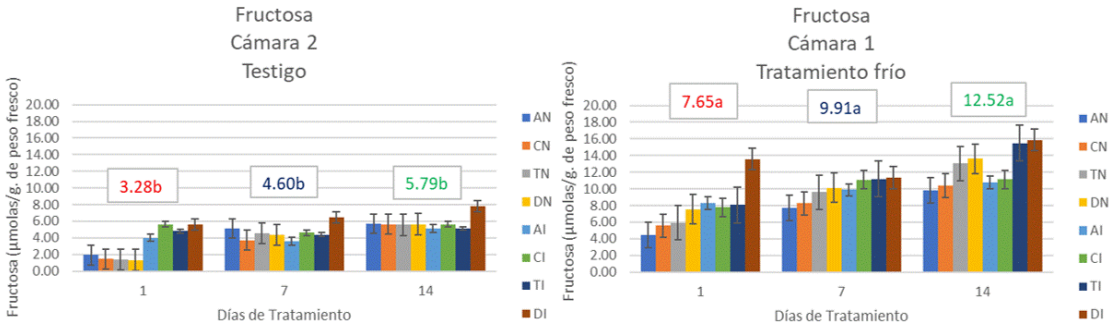
Fructose

The fructose content in the leaves of vegetative shoots is related to the carbon reserve of higher plants (Marschall *et al*., 2019); these reserves are the fructose content of the materials established in chamber 2 (control); however, under stressful conditions due to cold, these carbohydrates are associated with tolerance of different types of stress, acting as osmoprotectants against adverse environmental conditions due to heat, cold or water stress (Marschall *et al*., 2019).

With average values of the materials established in chamber 1 (cold treatment) of 7.65 µmoles g^{-1} fresh weight for day 1, 9.91 µmoles g^{-1} fresh weight for day 7, and with 12.52 µmoles g^{-1} fresh weight for the 14 days, compared with chamber 2 (control) values of 3.28 µmoles g^{-1} fresh weight for day 1, 4.6 µmoles g^{-1} fresh weight for day 7, and 5.79 µmoles g^{-1} fresh weight at 14 days; it can be mentioned that, when comparing the means, with a significance of α= 0.05, the plants in chamber 1 (cold treatment) increased their fructose content in the leaves of the vegetative shoots from day 1 of treatment compared to the average values of the chamber 2 (control) (Figure 5).

Figure 5. Effect of cold treatment on the fructose content of young leaves of materials of aceitoso (AN and AI); colecta 1 (CN and CI); tepetl (TN and TI); and duke 7 (DN and DI), comparing the same treatment day number between both chambers. The number above each set of bars of the rootstocks is the average value across all rootstocks.



Sucrose

The transport of photoassimilates to sinks is carried out in the form of sucrose, which is one of the most mobile biochemical compounds, depending on the species of plant and the type of loading and unloading in the phloem (via symplast or apoplast) (Minchin and Lacointe, 2005); therefore, the effect of low temperatures may or may not affect its mobility in the young leaves of the vegetative shoots; sucrose is used by the plant as the main source of biochemical energy, when it is synthesized in the cytosol from phosphorylated glucose and fructose (Hopkins and Huner, 2004), by the symplast route, sucrose travels through ducts (plasmodesmata) and via the apoplast, sucrose can be incorporated by a specific transporter or can be hydrolyzed by a cell wall invertase (Padilla and Martínez, 2007).

According to the characteristics of sucrose and the average values of the materials in chamber 1 (cold treatment) of 0.32 µmoles g^{-1} fresh weight at day 1, 0.43 µmoles g^{-1} fresh weight at 7 days, and 0.58 µmoles g^{-1} fresh weight at 14 days, and when compared with the average values of chamber 2 (control) of 0.15 for day 1, 0.21 µmoles g^{-1} fresh weight at 7 days and 0.41 µmoles g^{-1} fresh weight at 14 days; it can be mentioned that, according to the comparison of means with a significance of α= 0.05, the results of days 1 and 7 of the cold treatment were significantly higher than those of the control and for the results of day 14, there is no significant difference (Figure 6).

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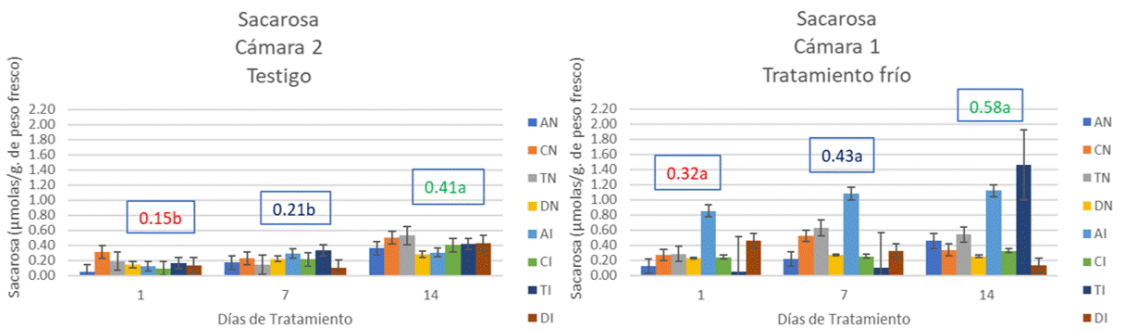
Figure 6. Effect of cold treatment on the sucrose content in young leaves of materials of aceitoso (AN and AI); colecta 1 (CN and CI); tepetl (TN and TI); and duke 7 (DN and DI), comparing (LSD) the same treatment day number between both chambers. The number above each set of bars of the rootstocks is the average value across all rootstocks.

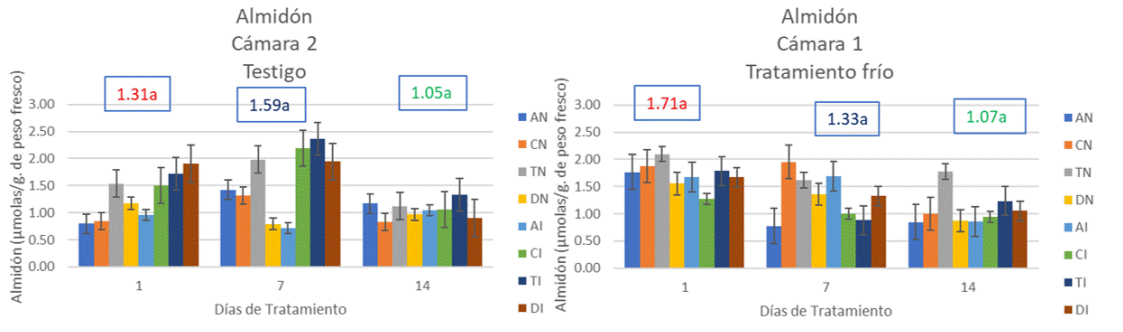


Starch

Starch is composed of amylopectin (85-70%) and amylose (15-30%), respectively (Mclauchlan *et al*., 2001); its metabolism in sinks (seeds, roots, tubers, developing leaves) is associated with the ratio of starch/sucrose synthesis at the source, export efficiency, sucrose transport, and sink priority and potency (Minchin and Lacointe, 2005). Young leaves from avocado vegetative shoots (sinks) showed a low concentration in starch content in both chambers.

Baguma *et al*. (2003) mention that the leaves are tissues that accumulate transient starch; therefore, the samples of the avocado materials under the conditions of chamber 1 (cold treatment) recorded average values of 1.71 µmoles g^{-1} fresh weight for day 1, 1.33 µmoles g^{-1} fresh weight at 7 days, and 1.07 µmoles g^{-1} fresh weight at 14 days; on the other hand, in chamber 2 (control), the mean values were 1.31 µmoles g^{-1} fresh weight for day 1, 1.59 µmoles g^{-1} fresh weight at 7 days, and 1.05 µmoles g^{-1} fresh weight at 14 days; therefore, according to the comparison of means with a significance of α= 0.05, it can be mentioned that, there are no significant differences between the materials in chamber 1 (cold treatment) and the control (Figure 7).

Figure 7. Effect of cold treatment on the starch content in young leaves of materials of aceitoso (AN and AI); colecta 1 (CN and CI); tepetl (TN and TI); and duke 7 (DN and DI), comparing the same treatment day number between both chambers. The number above each set of bars of the rootstocks is the average value across all rootstocks.



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Mexican race avocado materials evaluated

Aceitoso

The sugar content in the aceitoso material under temperature and light conditions of chamber 1 (Table 4), recorded an average glucose content (AcG) significantly higher in the non-grafted plants on days 1 and 7 and for the grafted plants on days 1, 7 and 14, compared to chamber 2 (control). Nonetheless, for fructose (AcF), the non-grafted and grafted plants in the three samples taken at 1, 7, and 14 days were significantly higher than those of the control. For sucrose (AcS), the averages in non-grafted plants were significantly higher on day 1 and for grafted plants on days 1, 7, and 14; for starch (AcSt), in non-grafted plants, the sample on day 1 was significantly higher and in grafted plants, it was significantly higher on days 1 and 7.

Table 4. Average values of glucose (AcG), fructose (AcF), sucrose (AcS), and starch (AcSt) contents of the non-grafted and grafted aceitoso material.

Material	Variable	Chamber 2 (control)			Chamber 1 (treatment)		
		1 day	7 days	14 days	1 day	7 days	14 days
Aceitoso non-grafted (AN)	AcG (µmoles g^{-1})	2.22b	7.3b	10.31a	8.82a	10.65a	12.29a
	AcF (µmoles g^{-1})	1.95b	5.14b	5.69b	4.46a	7.7a	9.83a
	AcS (µmoles g^{-1})	0.05b	0.17b	0.36a	0.12a	0.22b	0.46a
	AcSt (µmoles g^{-1})	0.8b	1.42a	1.17a	1.77a	0.78b	0.85b
Aceitoso grafted (AI)	AcG (µmoles g^{-1})	3.86b	6.92b	5.2b	9.28a	13.71a	8.2a
	AcF (µmoles g^{-1})	4.02b	3.59b	5.13b	8.28a	9.9a	10.79a
	AcS (µmoles g^{-1})	0.12b	0.29b	0.31b	0.85a	1.08a	1.12a
	AcSt (µmoles g^{-1})	0.96b	0.71b	1.05a	1.68a	1.69a	0.86b

Means with the same letter in rows and day of sampling are not significantly different (LSD test).

Colecta 1

The sugar content in the colecta 1 material (Table 5), under the temperature and low luminosity conditions of chamber 1, had an average glucose content (AcG) significantly higher in the result of day 7 in the non-grafted plants and for the grafted plants, a significantly higher difference only in the sample taken at 14 days; fructose (AcF) is significantly higher in both non-grafted plants and grafted plants; sucrose (AcS) for non-grafted plants is significantly higher only in the sample on day 7, and for grafted plants, it was significantly higher in the sample on day 1; finally, starch (AcSt) showed a significant difference in the sample on day 1 in the non-grafted plants and for the grafted materials, it is significantly higher in the sample on day 7.

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Table 5. Average values of glucose (AcG), fructose (AcF), sucrose (AcS), and starch (AcSt) contents of the non-grafted and grafted Colecta 1 material.

Material	Variable	Chamber 2 (control)			Chamber 1 (treatment)		
		1 day	7 days	14 days	1 day	7 days	14 days
Colecta 1 non-grafted (CN)	AcG (µmoles g^{-1})	3.46a	9.96b	10.65a	3.87a	14.34a	8.44a
	AcF (µmoles g^{-1})	1.49b	3.72b	5.65b	5.56a	8.25a	10.39a
	AcS (µmoles g^{-1})	0.31a	0.23b	0.51a	0.27a	0.53a	0.34b
	AcSt (µmoles g^{-1})	0.85b	1.32a	0.84a	1.88a	1.95a	1a
Colecta 1 grafted (CI)	AcG (µmoles g^{-1})	6.88a	10.92a	5.25b	5.79a	13.11a	8.3a
	AcF (µmoles g^{-1})	5.64b	4.61b	5.65b	7.79a	11.09a	11.14a
	AcS (µmoles g^{-1})	0.1b	0.21a	0.4a	0.24a	0.25a	0.33a
	AcSt (µmoles g^{-1})	1.51a	2.19a	1.06a	1.27a	1b	0.94a

Means with the same letter in rows and day of sampling are not significantly different (LSD test).

Tepetl

The sugar content in the tepetl material, under the temperature and low luminosity conditions of chamber 1, registered an average glucose content (AcG) significantly higher in the results of day 7 and 14 in the non-grafted plants, compared to the control; for plants grafted with 'Hass', they only showed a significant difference in the samples taken on days 7 and 14; fructose (AcF) was significantly higher in the three samples carried out in both non-grafted and grafted plants; sucrose (AcS) was significantly higher for non-grafted plants in the sample on day 7 and for grafted plants, it is significantly higher in the sample on day 14; finally, the starch content (AcSt) did not show a significant difference compared to the control in both non-grafted and grafted plants (Table 6).

Table 6 . Average values of glucose (AcG), fructose (AcF), sucrose (AcS), and starch (AcSt) contents of the non-grafted and grafted tepetl material.

Material	Variable	Chamber 2 (control)			Chamber 1 (treatment)		
		1 day	7 days	14 days	1 day	7 days	14 days
Tepetl non-grafted (TN)	AcG (µmoles g^{-1})	4.32a	8.67b	7.82b	5.49a	17.48a	25.47a
	AcF (µmoles g^{-1})	1.38b	4.54b	5.57b	5.9a	9.62a	13.04a
	AcS (µmoles g^{-1})	0.19a	0.15b	0.53a	0.29a	0.63a	0.54a
	AcSt (µmoles g^{-1})	1.54a	1.98a	1.12a	2.1a	1.62a	1.77a
Tepetl grafted (TI)	AcG (µmoles g^{-1})	5.74a	4.51b	5.23b	5.58a	14.71a	13.53a

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Material	Variable	Chamber 2 (control)			Chamber 1 (treatment)		
		1 day	7 days	14 days	1 day	7 days	14 days
	AcF (µmoles g^{-1})	4.8b	4.4b	5.15b	8.08a	11.2a	15.48a
	AcS (µmoles g^{-1})	0.17a	0.33a	0.42b	0.05b	0.1b	1.46a
	AcSt (µmoles g^{-1})	1.72a	2.36a	1.33a	1.78a	0.88b	1.24a

Means with the same letter in rows and day of sampling are not significantly different (LSD test).

Duke 7

The sugar content in this material, under the conditions of temperature and low luminosity of chamber 1, showed an average glucose content (AcG) significantly higher in the samples of days 1, 7, and 14 in the non-grafted and grafted plants, compared to the control; in the case of the fructose content (AcF), it is significantly higher in the samplings carried out on day 1, 7, and 14 in the non-grafted plants and in the grafted ones. In relation to the sucrose content (AcS), for the non-grafted plants, it is significantly higher only in the sample of day 1, and for the grafted plants, it is significantly higher only in the samples of day 1 and 7; finally, the average content of starch (AcSt) in the non-grafted plants only showed significant difference in the sample of day 7, and in the grafted materials, no significant differences were recorded in the three samples (Table 7).

Table 7. Average values of glucose (AcG), fructose (AcF), sucrose (AcS), and starch (AcSt) contents of the non-grafted and grafted duke 7 material.

Material	Variable	Chamber 2 (control)			Chamber 1 (treatment)		
		1 day	7 days	14 days	1 day	7 days	14 days
Duke 7 non-grafted (DN)	AcG (µmoles g^{-1})	6.02b	5.93b	6.7b	17.03a	13.93a	12.48a
	AcF (µmoles g^{-1})	1.33b	4.36b	5.66b	7.52a	10.15a	13.63a
	AcS (µmoles g^{-1})	0.14b	0.22a	0.29a	0.23a	0.27a	0.25a
	AcSt (µmoles g^{-1})	1.18a	0.79b	0.97a	1.55a	1.37a	0.88a
Duke 7 grafted (DI)	AcG (µmoles g^{-1})	7.67b	10.3b	6.24b	22.02a	21.03a	12.41a
	AcF (µmoles g^{-1})	5.59b	6.46b	7.81b	13.59a	11.36a	15.88a
	AcS (µmoles g^{-1})	0.14b	0.1b	0.43a	0.46a	0.32a	0.13b
	AcSt (µmoles g^{-1})	1.91a	1.94a	0.9a	1.67a	1.33a	1.05a

Means with the same letter in rows and day of sampling are not significantly different (LSD test).

Conclusions

Low temperature stress affects the sugar content, significantly increasing the concentration of glucose and fructose in the vegetative shoots of avocado materials of the Mexican race (aceitoso, colecta 1, tepetl and duke 7) grafted and not grafted with the 'Hass' variety, being associated with cold tolerance as a natural osmoprotectant.

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Considering that the materials with the highest concentration of glucose and fructose under cold conditions were duke 7 and tepetl, they are recommended for establishment as rootstocks of the 'Hass' variety in areas with temperatures ranging between 15.61 °C average during the day and 4.4 °C average at night.

The materials that showed growth under cold conditions were aceitoso and colecta 1, grafted and non-grafted; nevertheless, the growth of plants without an increase in the concentration of sugars (glucose and fructose) in vegetative shoots makes them more susceptible to cold damage.

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