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EVALUATION OF THE SUGAR AND STARCH CONTENT IN THE LEAVES OF SOME MEDITERRANEAN WOODY SHRUBS GROWING IN DIFFERENT CONDITIONS

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The study was carried out in 2011–2013 in Botanical garden of Slovak University of Agriculture in Nitra, Slovak Republic. three species of Mediterranean evergreen shrubs *Pittosporum tobira* L, *Trachycarpos fortunei* (HOOK) H.WENDL and *Laurus nobilis* L. were planted in two types of plantings, plants planted in the ground and plants planted in pots which is removed during the winter time (10.11–25.03) in side the greenhouse average temperature was 8 °C during the winter time. The sample of young, one year leaves had been taken in end of January when temperature was (-3 °C) at 9 am. The study showed that starch and total sugar affected by planting type, planting in greenhouse led to increase starch and total sugar significantly comparing with planting outside during the winter time. The best result was obtained in *Laurus nobilis* which Showed supremacy in the entire studied characteristic, lowest value of starch and total sugar were found in *Trachycarpos fortunei*.

Keywords: Mediterranean woody shrubs, growing condition, sugar, starch

Introduction

Many plant species introduced by humans to areas outside their natural ranges. Mediterranean woody plants are very popular in central Europe countries in landscape and garden architecture as the composition element, also for oil, tea and as medical plants. Hot, dry summers and mild to cool, wet winters are the characters of the Mediterranean climate, the identification of common morphological, life history and reproductive traits found among different plant species assemblages living under similar environmental conditions, have often been interpreted as a consequence of adaptive processes (Verdu et al., 2003). The climatic factors include rainfall and water, light, temperature, relative humidity, air, and wind. They are a biotic components, including topography and soil, of the environmental factors that influence plant growth and development. In general, plants survive within a temperature range of 0 °C to 50 °C. Enzyme activity and the rate of most chemical reactions generally increase with rise in temperature. Up to a certain point, there is doubling of enzymatic reaction with every 10 °C temperature increase. Temperature effects at different levels of organization, biochemical, physiological, morphological, and agronomic and systems – are considered, the changes in the enzyme activities were followed during fall, winter, and spring in relation to the changes in starch content and frost hardiness. Starch levels were negatively correlated with hardiness whereas most soluble sugars were positively correlated in grapevines (Jones et al., 1999). Low temperature lead to increase of the raffinose family oligosaccharides (RFO) proportion in total carbohydrate in *Olea europaea* (Rejškova et al., 2007). Nowadays we have much information about Mediterranean woody plants. Main aim of this study is to determine the effect of low temperature on sugar and starch contents of selected Mediterranean ever green shrubs grow under Slovak republic climate condition.

Material and methods

The study was carried out in 2011–2013 year at Botanical garden of Slovak University of Agriculture in Nitra, Slovak Republic. Three species of Mediterranean evergreen shrubs were planted in two kinds of planting, plant planted in the ground and plant planted in pots which is removed during the winter time inside the greenhouse average temperature was 8 °C, as shown in the (Table 1). Air temperature and rainfall were received from dates of metrological station of Botanical garden during study period as shown in (Table 2). The sample of young (one year) leaves was taken in 5. 3. 2013 at 9.00 am and the average temperature was -3 °C. Total sugar was determined according to Somogyi (in Michlik et al., 1978 and Frederick, 1989). In this procedure the 1 ml samples were combined either with 10 mg substrate and 1 ml citrate buffer (0.1 M, pH 5.0) or with 1 ml substrate solubilized in 0.1 M citrate buffer (pH 5.0) at 1% (w/v) in 50 ml *Folin tubes*. A control for each sample was prepared with substrate and buffer. Tubes were incubated at 40 °C for 24 h. After incubation, 2 ml of copper reagent,

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consisting of 4 parts KNa tartarate: Na_2CO_3 : Na_2SO_4 : $NaHCO_3$ (1 : 2 : 12 : 1.3) and 1 part Cu-$SO_4 \cdot 5H_2O$: Na_2SO_4 (1 : 9), was added to each tube. Both copper reagents must be prepared by boiling to completely dissolve the components; they can then be stored at room temperature. They were mixed together just prior to use. After 1 ml of sample was added to the appropriate control tubes, all tubes were boiled for 10 min in a water bath. The tubes were then cooled completely, 2 ml of arsenomolybdate reagent (25 g ammonium molybdate in 450 ml H_2O + 21 ml H_2SO_4 + 3 g $Na_2HASO_4 \cdot 7H_2O$ dissolved in 25 ml H_2O) was added to each tube, and the tubes were shaken thoroughly before adjusting the final volume to 25 ml with water. Individual samples were filtered through filter paper, and colorimetric measurements were determined by transmitted light at 500 nm in a spectrophotometer. The results of sugar and starch content in leaves are expressed in% of dry weight (d. w). The starch content was determined according to the polarimetric method of Ewers (Michalik et al, 1978). A portion of 5 g of a homogenised sample is weighed in a 100 ml Kohlrausch volumetric flask and its content is mixed with 25 ml of 1.124% HCl solution. After addition of another 25 ml of 1.124% HCl solution, the suspension is heated on a boiling water bath for 15 min (after 3 min the content of a volumetric flask is mixed to avoid coagulation). Once the hydrolysis is finished, 20 ml of 1.124% HCl solution is added. After fast cooling (using a stream of flowing water), clarification using 5 mL of Carrez I (30% $ZnSO_4$ solution) and 5 ml of Carrez II (15% $K_4[Fe(CN)_6]$ solution) solutions is performed. Finally, a volumetric flask is filled up by distilled water, its content is properly mixed, and filtrated using a filtration funnel. The obtained filtrate is then transferred to a polarisation tube (2 dm) and measured using a polarimeter. The extent of polarisation is related to the concentration of the optically active molecules in solution by the **Eq. 1** $\left[\alpha = [\alpha]_\lambda^t \cdot \ell \cdot c\right]$. Where α is the measured angle of rotation, $[\alpha]_\lambda^t$ is the optical activity (which is a constant for each type of molecule), ℓ is the path length and c is the concentration. The overal angle of rotation depend on the temperature and awvelength of light used and olso these parameters are usually standarddised (e.g. 20 °C and 589.3 nm (the D-line for sodium). The obtained value is firstly corrected for a laboratory temperature (t) drift using **Eq.2** $\alpha_{crrected} = \alpha_{meaured}\left(\ddot{S}\right) - 0.0144(t - 20)\ \left[\ddot{S}\right]$ followed by multiplying by a factor of **0.3462**. The amount of starch (X) in the sample is calculated using **Eq.3** $\left[X = \frac{10^4 . \alpha}{[\alpha]_\lambda^t . \ell . m}\right]$.

Where α is calculated value of optical rotation, $[\alpha]_\lambda^t$ is the optical activity (specific rotation) depending on the discharge lamp and wavelength of light used and variety

Table1. The studied species list their ages and origin

Family	Species	Origin	Age of plants
Pittosporaceae	*Pittosporum tobira* L	Japan	6
Palmaceae	*Trachycarpos fortunei* (HOOK) H. WENDL	China	6
Lauraceae	*Laurus nobilis* L.	Mediterranean	6

Table 2 Average temperature in °C and Sum of rainfalls in mm (Nitra, 2011–2012–2013)

Average temperature in °C				Sum of rainfalls in mm			
Month	**2011**	**2012**	**2013**	**Month**	**2011**	**2012**	**2013**
January	-0.90	1.36	-0.8	**January**	25	61.1	71.2
February	-0.60	-2.49	1.5	**February**	6	23.5	75.6
March	5.90	7.41	3.1	**March**	27	2.8	113.9
April	12.70	11.23	12.1	**April**	13	36.1	20.4
May	15.80	17.29	15.6	**May**	48	19.6	77.8
June	19.80	20.86	19.3	**June**	91	70.1	46.7
July	19.70	22.77	22.8	**July**	122	61.4	2.1
August	20.90	21.47	21.9	**August**	152	7.3	73.9
September	17.70	17.02	14.7	**September**	92	32.7	60.0
October	9.90	10.46	12.1	**October**	37	76.1	30.5
November	3.00	7.45	6.8	**November**	1	34.6	71.3
December	2.20	-0.91	2.3	**December**	42	44.4	11.0
Year Average Temperature	10.51	11.16	11.0	**Year sum of Rainfalls**	656	469.7	654.4

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of starch, ℓ is the path lenght (2 dm), and *m* is the sample weight (5 g). For a mercury discharge lamp and a wavelength (λ) of 546.1 nm, the $[\alpha]^t_\lambda$ values are 214.7, 216.3, 213.3, 213.1, 218.5, 217.0 and 215.5 for wheat, rye, barly, oat, rice, maize and unknown cereal starch, respectivly. (Not: the correction for moisture is not account in the equation). Samples randomly picked out from all parts of the plants. The starch and total sugar were measured. An experiment was laid out as Randomized Complete Design (*RCD*) in three replications, the data were analyzed with the general linear model procedures in Statistical Analysis System (SAS), and Duncan test at level 0.05 was used for the means separation.

Results and discussion

The data in (Table 3) showed that the highest sugar content was found in *Pittosporum tobira* which were planted in pots (12.92 % in dry weight) and the lowest was in *Trachycarpos fortunei* which were planted in the ground (0.40 % in dry weight). The highest starch content was found in *Laurus nobilis* which were planted in pots (5.12 % in dry weight) and the lowest starch content was in *Trachycarpos fortunei* (0.8 % in dry weight) there are no significant difference between *Laurus nobilis* and *Pittosporum tobira* in total sugar content see Table 4. Planting in pots led to increase each of starch and sugar content in plants and value was (6.70% in dry weight total sugar and (4.18% in dry weight) starch content (Figure 1 and 3). *Laurus nobilis* showed significant deference in starch and sugar content comparing with other species see Figure 2 and 4.

Planting in pots led to increase each of starch and sugar content in

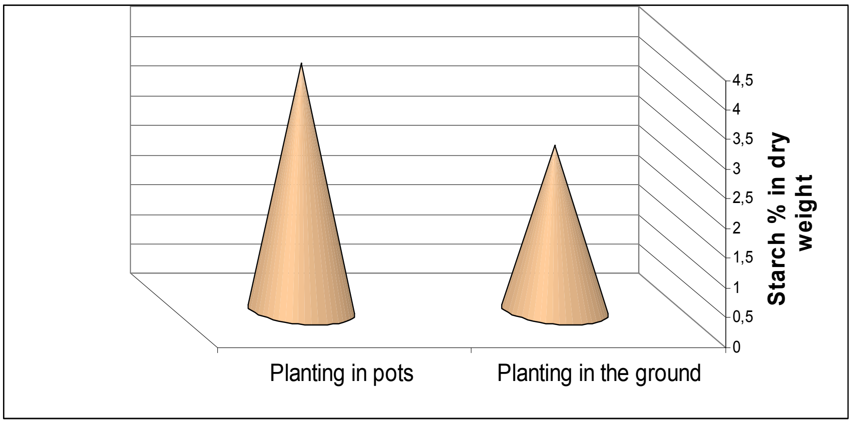

Figure 1 Starch content in the leaves affected by planting type

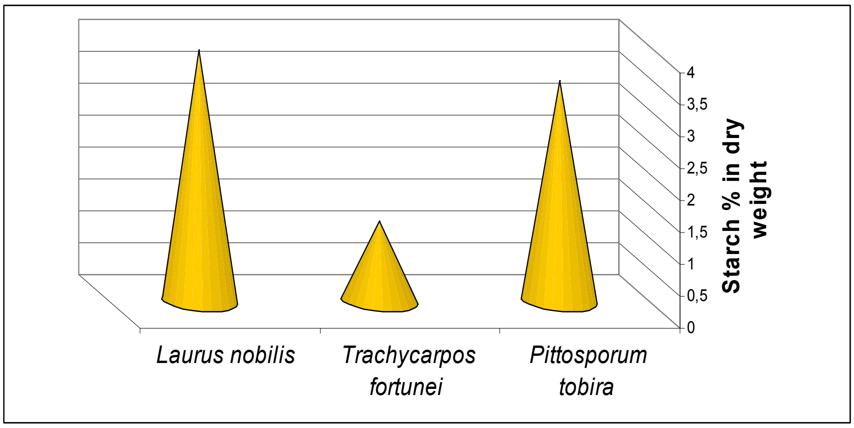

Figure 2 Starch content in the leaves affected by species

Table 3 Planting type affected on total sugar in different ever green species

Total sugar % in dry weight		**Factor species**			**Mean planting**
		Pittosporum tobira L	*Trachycarpos fortunei* (HOOK) H. WENDL	*Laurus nobilis* L	
Factor planting types	**planting in the ground**	2.26de	0.40e	9.83b	4.16b
	planting in pots	12.92a	2.99d	7.86c	6.70a
Mean species		7.59a	1.69c	8.85a	5.43

*Means not followed by the same letters are significant at 5% level of probability

Table 4 Planting type affected on starch in different ever green species

Starch % in dry weight		**Factor species**			**Mean planting**
		Pittosporum tobira L	*Trachycarpos fortunei* (HOOK) H. WENDL	*Laurus nobilis* L	
Factor planting types	**planting in the ground**	2.41cd	0.8e	2.76c	2.79b
	planting in pots	4.5ab	1.65de	5.12a	4.18a
Mean species		3.46bc	1.25d	3.94a	3.49

*Means not followed by the same letters are significant at 5% level of probability

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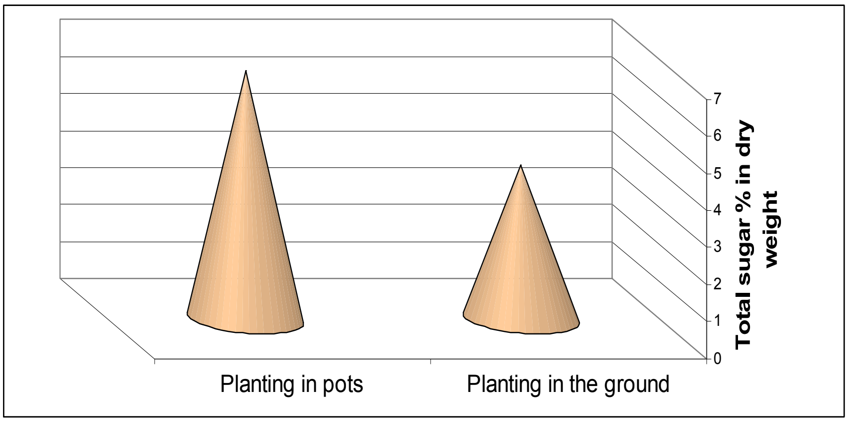

Figure 3 Total sugar content in the leaves affected by planting type

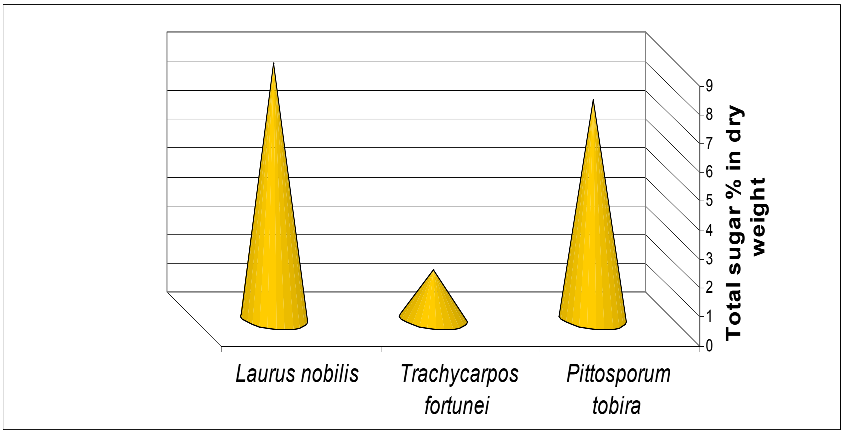

Figure 4 Total sugar content in the leaves affected by species

plants. Low temperature is one of the important a biotic stress that affected survival, growth, reproduction and geographic distribution of plants. Temperature factor influence all plant growth processes such as photosynthesis, respiration, transpiration, breaking of seed dormancy, seed germination, protein synthesis, and translocation. This is agree with (Pressman et al, 1989) Long term low temperature treatment led to a sharp decrease in the sugar content in *Asparagus*. Probably low temperatures can affect plants in several ways, temperatures near the minimum for plant growth will reduce the plant's rate of metabolism and growth. If the temperature, and therefore the metabolism, remain low for an extended period. A strikingly linear relationship between starch breakdown and temperature is observed in *Populus canadensis* (Sauter, 1988). The highest value of starch and total sugar found in *Laurus nobilis*, while the lowest value of starch and total sugar found in *Trachycarpos fortunei*. Plants are composed of a variety of compounds, besides water, certain plant organs have high concentrations of carbohydrates, proteins and lipids that can vary in different organs in the plant and between species. Paganová (2003) obtained that there has been found new information about distribution of *Sorbus domestica* in growth abilities.

Conclusion

Through this study we found that starch and total sugar affected by planting type, planting in greenhouse led to increase, starch and total sugar significantly comparing with planting out side during the winter time, Laurus nobilis showed the highest value of starch and total sugar content. But the lowest value of was in Trachycarpos fortunei comparing with other species.

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