Year-long evaluation of total soluble proteins in the trunk of two pine species from northeastern Mexico

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ABSTRACT
Objective: To quantify the one-year total soluble protein (TSP) concentration in the trunk of two pine species.
Design/Methodology/Approach: The Bradford method (1976) was used to determine the TSP concentration in the two pine species. Statistical tests were subsequently performed with the IBM SPSS 18 Software, using a general linear model (GLM) univariate analysis.
Results: The TSP concentration was different for each month. The highest concentration was recorded in August (6.84 mg gMS^{-1} for *Pinus pinceana* Gordon and 6.82 mg gMS^{-1} for *Pinus cembroides* Zucc), and the lowest was registered in April (5.53 mg gMS^{-1} for *Pinus cembroides* Zucc) and February (6.64 mg gMS^{-1} for *Pinus pincenana* Gordon).
Study Limitations/implications: There is a lack of information regarding these two pine species and scarce studies explain the behavior of the TSP.
Conclusions: The concentration of total soluble proteins varies in each month of the year. Further studies that include more plant organs are required to obtain a broader protein profile for both species.

Keywords: vegetative storage, nutrients, ecophysiology, proteins.

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INTRODUCTION

Pinus pinceana Gordon and *Pinus cembroides* Zucc are two species endemics to Mexico; they are distributed across the states of Coahuila, Nuevo León, Zacatecas, San Luis Potosí, and Querétaro (Villareal *et al.*, 2009). Both species have great economic importance, give the use of their timber as firewood and fuel and of their seeds as food (CONABIO, 2019). The stands of *Pinus pinceana* Gordon are small and its populations are restricted,

additionally, grazing goats and the collection of firewood and seeds have brought this species to the brink of extinction, consequently, it is subject to special protection according to NOM-059-ECOL-2001 and NOM-059-SEMARNAT-2010 (Quiroz *et al*., 2017).

Nitrogen is abundant in plants and may be present as soluble proteins and vegetative storage proteins (El Zein *et al*., 2011), which are crucial to the development of cellular, metabolic, and genetic coding structures. The lack of this element can lead to poor development or growth (Espino *et al*., 2018). The increasing temperatures and droughts caused by climate change and human activities currently threaten the populations of the observed pines in more arid areas (Martiñón *et al*., 2010). Studies about both woody species and the behavior of total soluble proteins are scarce, there are only reports for a few species: *Caladium bicolor* (Ortiz *et al*., 2015), *Quercus petraea* and *Fagus sylvatica* L. (Valenzuela *et al*., 2010), and *Carya illinoinensis* Koch (Núñez *et al*., 2019, 2021). Furthermore, studies about the nitrogenous compound storage in the trunk and roots (El Zein *et al*., 2011) and the mobilization of the said compounds into new growth tissues are very limited and scarce (Villar *et al*., 2015). Despite the economic importance of their seeds, there is no management and conservation program for both pine species (Fuentes *et al*., 2019). Our objective was to evaluate total soluble protein (TSP) concentration in *Pinus pinceana* Gordon and *Pinus cembroides* Zucc, specifically on the trunk, at a 1.3-m height, during an annual cycle.

MATERIALS AND METHODS

This study lasted one year (January-December 2022). It was conducted in the Jagüey de Ferniza ejido, in the south area of the municipality of Saltillo, Coahuila, Mexico (101° 02' 17" N, 25° 14' 47" W). Five individuals of each species were systematically selected according to the diameter of their trunks (≈60 cm). Monthly samples were taken from the trunk cores, 1.3 m above ground level using a Pressler drill (Haglof®, 15mm/0.200" diameter, 3 edges). The methodology reported by Núñez *et al*. (2021) was used for sampling and part of the sample processing. The samples were transported to the Departamento de Recursos Naturales Renovables of the Universidad Autónoma Agraria Antonio Narro, in Saltillo, Coahuila, where they were dehydrated at 50 °C for one week, using a HS45-AIA drying oven (Novatech). The samples where then analyzed using the Bradford method (1976). An analytical balance (Adventurer™ Pro) was used to weigh 10 mg of dry matter, which were placed in 2-mL microtubes. Subsequently, 2 mL of the extraction solution (KH_2PO_4, Na_2HPO_4, and polyvinylpyrrolidone) were added to each of the microtubes. The samples were stirred in a vortex mixer for 10 min and centrifuged for 15 min at 10,000 rpm. Afterwards, 500 μL of the resulting solution were poured into plastic cuvettes, to which 500 μL of the reagent were added (Quick Start™ Bradford Protein Assay). The cuvettes with the solution were stirred and left to settle for five minutes, before a sensitive UV spectrophotometer (JENWAY 6320D) was used to analyze the solution at a 595-nm absorbance.

Statistical analysis

The Kolmogorov-Smirnov test was performed to verify that the data met the normality and homoscedasticity assumptions. Once the assumptions were confirmed, the TSP concentration of each species was compared using a GLM univariate analysis, considering month and height as the main factors in the model. The statistical analyses were performed with the help of the IBM SPSS 18 software (significance level: $\alpha \leq 0.05$).

RESULTS AND DISCUSSION

The TSP concentration was statistically different for each month evaluated (F=13.335, g.l.=11,365, P=0.000). Figure 1 shows that August recorded the highest TSP concentration for both species (6.84 mg gMS^{-1} in *Pinus pinceana* Gordon and 6.82 mg gMS^{-1} in *Pinus cembroides* Zucc), while the lowest TSP concentrations occurred in April for *Pinus cembroides* Zucc (5.53 mg gMS^{-1}) and February for *Pinus pinceana* Gordon (6.64 mg gMS^{-1}).

Plants can mobilize nutrients that will subsequently contribute to their optimal development in each season of the year (Perdomo *et al.*, 2010). Therefore, *Pinus pinceana* Gordon and *Pinus cembroides* Zucc showed variations in TSP concentration.

TSP concentrations recorded the highest results for both species in August (6.84 mg gMS^{-1} in *Pinus pinceana* and 6.82 mg gMS^{-1} and *Pinus cembroides*). These results differ from the *Quercus robur* study conducted by Valenzuela *et al.* (2011), who reported the highest concentration in October (0.15±0.03 g·100g^{-1}) and a significant decrease of TSP in June. In this study, a decreasing TSP concentration could only be observed in Pinus cembroides in June (6.23 Mg gMS^{-1}), while the lowest record of these compounds in *Pinus pinceana* occurred in February (6.64 Mg gMS^{-1}).

The results also differ from the observations made by Muñoz *et al.* (1993), who reported an increase in N in the tissues of *Prunus persica* during autumn (August to November). However, this study failed to detect a fixed pattern in TSP concentrations. Núñez *et al.* (2021)

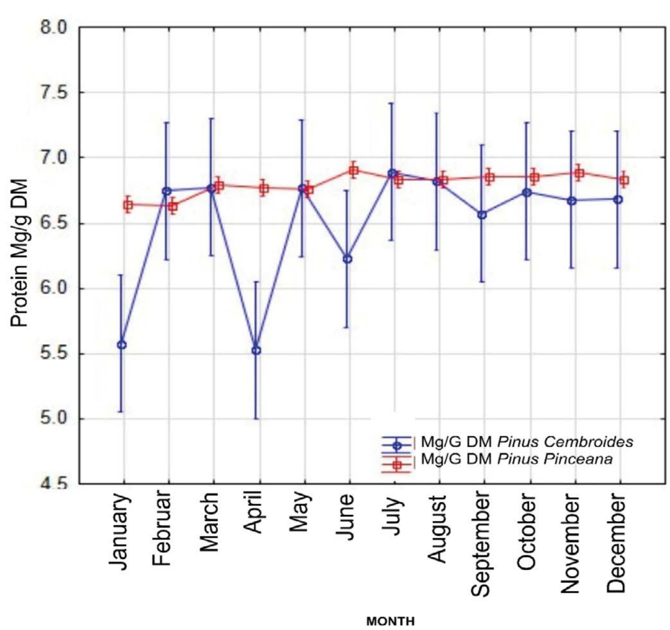

Figure 1. Monthly variation in TSP concentration in the trunk of two *Pinus* species over the course of one year.

Table 1. Average TSP concentration (mg protein/g DM) in two *Pinus* species.

Month	protein Mg /g DM *Cembroides*	Protein mg /g DM *Pinceana*
August	6.82 a	6.84 a
July	6.89 a	6.83 a
January	5.58 ab	6.64 bc
March	6.77 ab	6.79 a
May	6.77 ab	6.76 abc
December	6.69 abc	6.83 a
February	6.74 abc	6.64 b
June	6.23 abc	6.90 a
November	6.68 abc	6.89 a
October	6.74 abc	6.86 a
September	6.57 abc	6.85 a
April	5.53 c	6.77 abc

Milligrams of protein over grams of dry matter (protein mg /g DM).
$_z$Different lines between columns indicate significant differences (Tukey, $p<0.05$).

found a proportional relation between protein nitrogen concentration and temperature increase in *Carya illinoinensis* —*i.e.*, the percentage of protein nitrogen increases along with temperature. For their part, Taulavuori *et al.* (2014) reported that protein nitrogen content increased with high temperatures.

The variation in TSP concentration could be the result of differences between species and their development stages; one species could be more sensitive to biotic or abiotic variables (Tromp and Ovaa, 1973). It is worth highlighting that the concentration of protein compounds tends to increase or decrease in a matter of hours or days, due to the plasticity of plants, various species modify their behavior to survive in heterogeneous environmental conditions (Villar *et al.*, 2013). The plasticity of plants is defined as the ability of a particular genotype or population to express distinct phenotypes in the face of any biotic or abiotic variation, through changes in their morphology and physiology (Hernández *et al.*, 2015; Villamizar *et al.*, 2012).

The results of the present study show that TSP concentration in plants can fluctuate and that plants can retranslocate these compounds to meet their needs (Aerts and Chapin, 1999). The fluctuation of TSP may be impacted by different causes, such as environmental variables or the abiotic stress that the plant suffers (Upendra and Dagla, 2016). Tuberosa (2012) and Brunner *et al.* (2015) reported that, when plants experience high levels of drought stress, they activate protein-based mechanisms to protect themselves from cellular damage.

CONCLUSIONS

The concentration of total soluble proteins in the two observed species varies in each month of the year. An analysis of the complete proteic profile of both species is still required to characterize the mobilization dynamics of nitrogenous compounds, taking the effect of environmental variables into consideration.

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REFERENCES

Aerts, R., & Chapin, F. S. (1999). The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. *Advances in Ecological Research, 30*(C), 1–67. https://doi.org/10.1016/S0065-2504(08)60016-1

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. In *ANALYTICAL BIOCHEMISTRY* (Vol. 72).

Brunner, I., Herzog, C., Dawes, M. A., Arend, M., & Sperisen, C. (2015). How tree roots respond to drought. *Frontiers in Plant Science, 6*(JULY). https://doi.org/10.3389/fpls.2015.00547

CONABIO. (2019). *Pinus pinceana* Gordon.

El Zein, R., Bréda, N., Gérant, D., Zeller, B., & Maillard, P. (2011). Nitrogen sources for current-year shoot growth in 50-year-old sessile oak trees: An *in situ* 15N labeling approach. *Tree Physiology, 31*(12), 1390–1400. https://doi.org/10.1093/treephys/tpr118

Espino Castillo, D. A., Valenzuela-Nuñez, L. M., Legaria-Solano, J. P., Briceño-Contreras, E. A., Esparza-Rivera, J. R., Rodríguez-Bautista, G., & García-de-la-Peña, C. (2018). Evidencia de una proteína de reserva vegetativa de 20 kDa en raíz de nogal (*Carya illinoensis* Koch) durante la etapa de letargo. *Ecosistemas y Recursos Agropecuarios, 5*(14), 309–317. https://doi.org/10.19136/era.a5n14.1616

Fuentes Amaro, S. L., Legaria Solano, J. P., & Herrera Ramírez, C. (2019). Estructura genética de poblaciones de *Pinus cembroides* de la región central de México. *Fitotec. Mex.*, 1, 57–65.

Hernández Verdugo, S., González-Sánchez, R. A., Porras, F., Parra-Terraza, S., Valdez-Ortiz, A., Pacheco-Olvera, A., & López-España, R. G. (2015). Plasticidad fenotípica de poblaciones de chile silvestre (*Capsicum annuum* var. *glabriusculum*) en respuesta a disponibilidad de luz. *Botanical Sciences, 93*(2), 275–290. https://doi.org/10.17129/botsci.237

Martiñón Martínez, R. J., Jesús Vargas-Hernández, J., López-Upton, J., Gómez-Guerrero, A., & Vaquera-Huerta, H. (2010). Respuesta de *Pinus pinceana* gordon a estrés por sequía y altas temperaturas. In Artículo Científico *Rev. Fitotec. Mex* (Vol. 33, Issue 3).

Muñoz, N., Guerri, J., Legaz, F., & Primo-Millo, E. (1993). Seasonal uptake of SN-nitrate and distribution of absorbed nitrogen in peach trees. *Plant and Soil*, 150, 263–269.

Núñez Colima, J. A., García de la Peña, C., Valenzuela Núñez, L. M., González Torres, A., Rivera Esparza, R., & Martínes Rodríguez, R. (2021). Factores ambientales y su relación con el nitrógeno contenido en proteínas solubles totales (PST) en *Carya illinoinensis*. *Acta Universitaria*, 1–10.

Núñez Colima, J. A., Moreno Reséndez, A., Valenzuela Núñez, L. M., Rodríguez Martínez, R., González Torres, A., García De La Peña, C., Esparza Rivera, J. R., & Molina Ochoa, J. (2019). Influencia de variables climáticas en el contenido de N en *Carya illinoensis* Koch. *Nova Scientia*, *11*(22), 207–223. https://doi.org/10.21640/ns.v11i22.1823

Ortiz Sánchez, A. I., Álvarez Reyna, V. de P., González Cervantes, G., Manuel Valenzuela Núñez, L. M., Potisek Talavera, M. del C., & Chávez Simental, J. A. (2015). Concentration of Starch and soluble proteins in tubers of *Caladium bicolor* under different phenological stages. *Revista Mexicana de Ciencias Agrícolas*, 6, 483–494.

Perdomo, C., Barbazán, M., & Durán Manzoni, J. M. (2010). Nitrógeno.

Quiroz-Vázquez, R. I., López-Upton, J., Cetina-Alcalá, V. M., & Ángeles-Pérez, G. (2017). Capacidad reproductiva de *Pinus pinceana* gordon en el límite sur de su distribución natural. *Agrociencia*, 51, 91–104.

Taulavuori, K., Taulavuori, E., & Sheppard, L. J. (2014). Truths or myths, fact or fiction, setting the record straight concerning nitrogen effects on levels of frost hardiness. *Environmental and Experimental Botany*, 106, 132–137. https://doi.org/10.1016/j.envexpbot.2013.12.022

Tromp, J., & Ovaa, J. C. (1973). Mobilization of protein nitrogen: in apple Spring Mobilization of Protein Nitrogen in Apple Bark. In *Physiol. Plant* (Vol. 29).

Tuberosa, R. (2012). Phenotyping for drought tolerance of crops in the genomics era. *Frontiers in Physiology*, 3 SEP. https://doi.org/10.3389/fphys.2012.00347

Upendra, J. M., & Dagla, H. R. (2016). Growth and biochemical analysis of evergreen haloxeric tree species *Salvadora oleoides* and *Salvadora persica* under NaCl stress. *Acta Physiologiae Plantarum, 38*(5). https://doi.org/10.1007/s11738-016-2121-y

Valenzuela Nuñez, L. M., Gérant, D., Maillard, P., & Bréda, N. (2010). Seasonal dynamics of total soluble proteins in adult trees of *Quercus petraea* (Matts.) Liebl. and *Fagus sylvatica* L. *Revista Mexicana Ciencias*, 1, 76–83.

Valenzuela Núñez, L. M., Maillard, P., & Nathalie, B. (2011). Evidence for a 26kDa vegetative storage protein in the stem sapwood of mature pedunculate oak. https://www.researchgate.net/publication/230675753

Villamizar Cújar, J. M., Rodríguez López, N. F., & Tezara Fernández, W. (2012). Phenotypic Plasticity in Plants of *Lippia dulcis* (Verbenaceae) Subjected to Water Deficit. In *Acta biol. Colomb* (Vol. 17, Issue 2).

Villareal Quintanilla, J. Á., Mares Arreola, O., Cornejo Oviedo, E., & Capó Arteaga, M. A. (2009). Estudio florístico de los piñonares de *Pinus Pinceana* Gordon. *Acta Botánica Mexicana*, 87–124.

Villar Salvador, P., Peñuelas, J. L., & Jacobs, D. F. (2013). Nitrogen nutrition and drought hardening exert opposite effects on the stress tolerance of *Pinus pinea* L. seedlings. *Tree Physiology, 33*(2), 221–232. https://doi.org/10.1093/treephys/tps133

Villar Salvador, P., Uscola, M., & Jacobs, D. F. (2015). The role of stored carbohydrates and nitrogen in the growth and stress tolerance of planted forest trees. *New Forests, 46*(5–6), 813–839. https://doi.org/10.1007/s11056-015-9499-z