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RELACIÓN ENTRE EL CONTENIDO DE CLOROFILA Y EL COLOR DE LAS ACÍCULAS DE *Abies religiosa* KUNTH, SCHLTDL. *et* CHAM

RELATIONSHIP BETWEEN CHLOROPHYLL CONTENT AND NEEDLE COLOR OF *Abies religiosa* KUNTH, SCHLTDL. *et* CHAM

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SUMMARY

Evaluation of the physiological performance of plants as an environmental response is crucial to understand the ecological succession in plant communities and to implement strategies that increase plant survival and growth for restoration efforts and commercial plantations; however, evaluating plant physiology often requires complex and expensive technical equipment not normally accessible to all researchers and non-specialized people. In the present study, we explored the relationship of the visual condition of the needles with an indicator of their photosynthetic capacity related to chlorophyll content in *Abies religiosa.* A visual stress index was developed to evaluate the response to environmental stress in seedlings of *A. religiosa* during a reforestation effort, and the correlation between leaf chlorophyll content and different categories of the stress index. The replicability of the index was also tested to determine its applicability by non-specialized people. A high correlation was detected between the index and chlorophyll concentration (r^2 = 0.76; P < 0.0001). Additionally, there was a significant agreement on the index among several observers (W = 0.95; P < 0.0001). Overall, the stress index is related the physiological condition of the plants and can be adopted by people, after training, to make evaluations of the physiological status of *A. religiosa* seedlings.

Index words: *Abies religiosa,* chlorophyll, ecophysiology, reforestation, stress index.

RESUMEN

Evaluar el desempeño fisiológico de las plantas como respuesta al ambiente es fundamental para entender los procesos de sucesión ecológica en las comunidades de plantas y para implementar estrategias que incrementen su supervivencia y crecimiento, tanto en esfuerzos de restauración como en plantaciones comerciales; sin embargo, evaluar la fisiología de las plantas muchas veces requiere equipo técnico complejo y costoso, que normalmente no es accesible a todo los investigadores o al público no especializado. En el presente estudio se exploró la relación entre la condición visual de las acículas y un indicador de la capacidad fotosintética relacionado con el contenido de clorofila en *Abies religiosa.* Se desarrolló un índice visual para evaluar la respuesta al estrés ambiental en plántulas de *A. religiosa* en una reforestación y se exploró la correlación entre el contenido de clorofila de las acículas y distintas categorías del índice de estrés; así mismo, se probó la replicabilidad del índice para determinar su aplicabilidad por parte de personas no especializadas. Se detectó una alta correlación entre el índice y la concentración de clorofila (r^2 = 0.76; P < 0.0001). Adicionalmente, existió una concordancia significativa sobre el índice entre varios observadores (W = 0.95; P < 0.0001). En general, el índice de estrés se relaciona con la condición fisiológica de las plantas y puede ser adoptado por personas, previa capacitación, para hacer evaluaciones sobre el estado fisiológico de plántulas de *A. religiosa.*

Palabras clave: *Abies religiosa,* clorofila, ecofisiología, índice de estrés, reforestación.

INTRODUCTION

The physiology of plants varies during ontogeny or as a response to fluctuation in the resource levels (Lambers *et al*., 2008). The quantification of the physiological plant response is vital to increase plant survival during the first stages of establishment. Particularly of interest are chlorophyll pigments which capture light related to carbon fixation during photosynthesis (Shinkarev, 2004). A variation in pigment content is expressed as a greenish tone change in the leaf, which relates to chlorophyll content and with overall plant growth. Not all plant pigments capture photons exclusively for photosynthesis; several prevent damage to the photosynthetic apparatus during environmental stress (Lambers *et al*., 2008). The plant stress response can quickly and precisely be quantified by chlorophyll fluorescence variation. This is a standard ecophysiological method that measures plant endurance to environmental stress, specifically for the photosynthetic apparatus (Binder and Fielder, 1996; Oxborough, 2004).

On the other hand, the measurement of the plant physiological response tends to require expensive biochemical tests and equipment, thus limiting its applicability. For example, the fluorometer, which estimates chlorophyll content is a complicated and expensive piece of equipment. However, some plant physiological processes, like wilting loss point, might be visually assessed (Tyree *et al*., 2003). Visual evaluation of the plant physiological status has enormous applicability potential (Rodríguez-Laguna *et al*., 2015; Viveros-Viveros *et al*., 2006); therefore, designing

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low-budget strategies to estimate plant response would massify their application, especially in situations where no sophisticated equipment is available; however, a precise relationship between the plant physiological status and the visual assessment should be established.

The distribution of conifers in southern latitudes of the northern hemisphere is predicted to be negatively impacted by diverse scenarios of climate change (Mátyás, 2010; Rehfeldt *et al*., 2014; Sáenz-Romero *et al*., 2019). In México, *Abies religiosa* Kunth, Schltdl. *et* Cham. is experiencing a reduction in abundance by mortality events possibly related to climate change (Brower *et al*., 2017; Sáenz-Romero *et al*., 2012). Reforestation and restoration efforts are continually implemented to revert the negative impacts on stand size of *A. religiosa* populations (Blanco-García *et al*., 2011; Carbajal-Navarro *et al.,* 2019; Castellanos-Acuña *et al*., 2014; Ortiz-Bibian *et al*., 2017; SEMARNAT and CONANP, 2018). Reforestation endeavors do not incorporate plant physiological responses, which would provide information on optimum microenvironmental conditions for seedling establishment and the most stressful seasons of the year limiting plant growth. Therefore, in the present study, a method was designed to visually evaluate seedlings condition in the field as a response to the dry season and to explore how it relates with the photosynthetic apparatus physiology. Notably, it is expected that a change in the color of leaves during drought would indicate a reduction in chlorophyll content.

MATERIALS AND METHODS

In June 2015, a site was planted with seedlings of *A. religiosa* Kunth, Schltdl. *et* Cham. This site is located at 19º 34' 21'' N and 100º 14' 0'' W, at 3460 masl, within the Monarch Butterfly Biosphere Reserve at ejido La Mesa, municipality of San José del Rincón, State of Mexico. A total of 360 two-and-a-half-year-old seedlings were transplanted with a mean height of 12.84 (± 0.29) cm and mean basal diameter of 3.94 (± 0.05) mm at a distance of 1.5 × 1.5 m between seedlings. A year and a half later, at the beginning of the dry season, the condition of the plants was evaluated monthly through a visual stress index.

The index was designed to quantify changes in the color of the needles visually. An index value was assigned to each plant based on the proportion of color types at the whole plant canopy. The index ranges from level 1 to 5 (Figure 1; Table 1): level 1 represents the healthiest condition with all the leaves in the plant dark green, complete and shiny; at level 2, plants begin to exhibit slightly circular discolorations (of yellowish tones) in 80 % of the leaves, but the rest of the leaf has a dark green color, as in level 1; at level 3, leaves change to a greenish-yellow tone, while at level 4, between 40 and 50 % of the leaves are yellow and between 50 and 60 % are brown; and at the level 5, 98 % of the foliage is reddish-brown and apparently dry. Although five categories of the needle color were defined, intermediate conditions between the levels can be quantified.

Concurrently with the determination of the stress index, the chlorophyll concentration was measured in the leaves using a portable chlorophyll content meter (CCM-300, Opti-Sciences, Hudson, New Hampshire, USA). The device is a modulated fluorometer that quantifies the ratio between chlorophyll fluorescence at 735 and 700 nm and measures the chlorophyll concentration of the needle-like leaves in mg m^{-2}. Chlorophyll concentration (on an interval between 0 and 1500 mg m^{-2}) was measured in five needles in the field, representative of each of the visual stress index levels of each plant. For levels 4 and 5 of the index, the chlorophyll concentration represents the average of two needles with yellow coloration, two with brown coloration and one with intermediate coloration (yellow-brown). The measurement was done between 10 and 13 h because at those times environment temperature varies the least (mean 16.4 °C, maximum 26.35 °C, minimum 10.6 ºC). The quantification was done over a total of 276 seedlings.

The stress index was designed to be applied by any non-specialized observer after brief training. Therefore, the replicability of the index among four independent observers (undergraduate Biology students from Universidad Michoacana de San Nicolás de Hidalgo) was tested. By using pictures representative of each of the stress index levels, we explained criteria for each level to the four students who then used those photos in the field to compare them to seedlings representatives of each index. Subsequently, each one independently assigned a stress level to each plant in the plot.

Statistical analysis

Average chlorophyll concentration per seedling was calculated, and the relationship between the stress index and the chlorophyll concentration was explored with a linear regression analysis. At the same time, to confirm the applicability of the stress index by non-specialized people and identify if different observers reach the same conclusion, Kendall's concordance test was applied to the stress index data (Berlanga *et al*., 1997; Legendre, 2005; Madrigal-Fritsch *et al*., 1999). This test is non-parametric and calculates the agreement among three or more evaluators of categorical variables.

Figure 1. Representation of the five levels of the stress index for *Abies religiosa* seedlings. Numbers in the figure indicate the different levels of the stress index; level 1: healthiest condition; level 2: plants begin to exhibit slightly circular discolorations (of yellowish tones) in 80 % of the leaves; level 3: the plant leaves change to a greenish yellow tone; level 4: between 40 and 50 % of the leaves are yellow, and between 50 and 60 % are brown; level 5: 98 % of the foliage is reddish brown (see materials and methods for further description).

RESULTS AND DISCUSSION

A highly significant correlation between the stress index and the leaf chlorophyll concentration was found ($r^2 = 0.76$; $P < 0.0001$; Figure 2; Table 1). Overall, the average chlorophyll concentration was 1101.58 mg m^{-2} (SE ± 9.01). Particularly, the chlorophyll concentration at the needle begun to reduce at level 2 of the stress index (1109.9 ± 15.6 mg m^{-2}), and the concentration dropped around 80 % from level 2 to level 5. Overall, plants visually scored as being stressed had lower chlorophyll concentration in their leaves. Stress generally caused a decrease in photosynthetic pigments content and induced impairment of the photosynthetic pigments biosynthesis or pigment degradation (Ashraf and Harris, 2013; Hörtensteiner, 2006). With the onset of the dry season, the soil water content decreased; consequently, *A. religiosa* seedlings experienced stress which was expressed as a change in the needle color.

Overall, variation in needle color during dry season relates with nutrient and chlorophyll content, influencing the leaf capacity to capture photons; thus, potentially affecting CO_2 fixation (Lambers *et al*., 2008; van den Berg and Perkings, 2004). This behavior relates to re-translocation during leaf senescence, which is an essential mechanism for nutrient conservation (Aerts, 1996); this strategy favors the sprouting capacity and leaf production at the next favorable season (Rentería *et al*., 2005). In temperate conifers, the

Table 1. Description of each of the five levels of the stress index and their average concentration of chlorophyll.

Levels of the visual index	Description	Concentration of chlorophyll (mg m^{-2}) (SE)
1	All the leaves of the plant are dark green, complete and shiny	1239.61 (13.9)
2	Plants begin to exhibit slightly circular discolorations (of yellowish tones) in 80 % of the leaves	1123.68 (13.3)
3	Leaves of plants change to a greenish yellow tone	951.73 (17.8)
4	Between 40 and 50 % of the leaves are yellow, and between 50 and 60 % are brown	779.2 (28.5)
5	98 % of the foliage is reddish brown, making leaves seem dry	423.12 (17.3)

SE: standard error

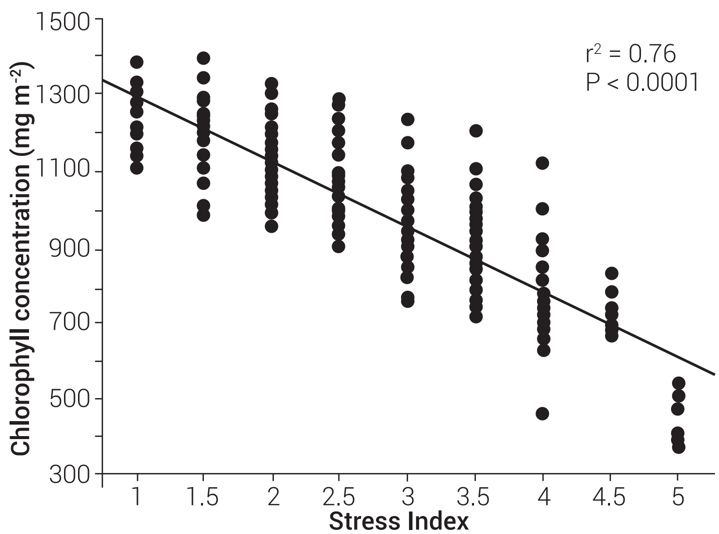

Figure 2. Relationship between needle chlorophyll concentration (mg m^{-2}) and the stress index in *Abies religiosa* seedlings planted on the field at 3460 m of altitude, Monarch Butterfly Biosphere Reserve, ejido La Mesa, State of Mexico.

rate of nutrient re-translocation directly relates to plant growth rate (Sadanandan and Fife, 1991). However, current knowledge has not determined the magnitude carbon fixation is affected at the different stages of stress in *A. religiosa*, or whether the reduction in chlorophyll content is a consequence of a re-translocation process for nutrient conservation.

The visual index designed is a straightforward and affordable method to assess plant response to the environment. This proposal adds to other efforts where the visual condition relates to the physiology of the plant (*i.e.,* in response to water stress) (Tyree *et al*., 2003). For example, in studies of assisted migration, the most suited genotypes for upward movement along an altitudinal gradient were selected based on visual evaluation of the percentage of plant necrosis caused by frost damage (Martínez *et al*., 2005; Viveros-Viveros *et al*., 2006).

Finally, there was statistically significant agreement among observers, according to Kendall's coefficient of concordance ($W = 0.95$; $P \leq 0.0001$); results show that independent observers reached the same conclusion about the condition of the plant. It can thus be concluded that the stress index can be replicated and adopted by any individual trained to make the field evaluation of *A. religiosa* plants performance. Overall, the stress index can be a reliable alternative for the assessment of the plant response to different types of stress when the equipment required for this kind of evaluation is not available.

In the present study, the definition of different scoring levels based on needle color of *A. religiosa* seedlings allowed tracking of different stages of stress with the onset of the dry season. Design of a visual assessment scale of the plant response is thus possible, and it could easily be in reforestation efforts, as an inexpensive and expedite way to evaluate the seedling physiological status.

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