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Tolerancia de *Opuntia* spp. del Altiplano Potosino a condiciones de estrés abiótico: respuestas a nivel ecofisiológico, anatómico y molecular

Tesis que presenta:

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Directores de la Tesis:

Dr. Joel David Flores Rivas

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Constancia de aprobación de la tesis

La tesis **"Tolerancia de *Opuntia* spp. del Altiplano Potosino a condiciones de estrés abiótico: respuestas a nivel ecofisiológico, anatómico y molecular"** presentada para obtener el Grado de Doctor en Ciencias Aplicadas en la opción de Ciencias Ambientales fue elaborada por **Pablo Delgado Sánchez** y aprobada el **12 de Octubre de 2011** por los suscritos, designados por el Colegio de Profesores de la División de Ciencias Ambientales del Instituto Potosino de Investigación Científica y Tecnológica, A.C.

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Dr. Joel David Flores Rivas
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Dedicatorias

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Contenido

Anexos

I.- Are fungi important for breaking seed dormancy in desert species? Experimental evidence in *Opuntia streptacantha* (Cactaceae). *Plant Biology* (2011), 13:154-159.

II.- Further evidence from the effect of fungi on breaking *Opuntia* seed dormancy. *Plant Signaling & Behavior* (2010), 5(10):1229-1230

III.- El nopal, una planta adaptada para afrontar el cambio climático del planeta. *Blog Con-ciencia del UNIVERSAL*. 26-Julio-2011.
http://blogs.eluniversal.com.mx/weblogs_detalle14417.html

Resumen

Tolerancia de *Opuntia* spp. del Altiplano Potosino a condiciones de estrés abiótico: respuestas a nivel ecofisiológico, anatómico y molecular

Palabras clave: Efecto de plantas nodriza, establecimiento de plántulas, estacionalidad, estrés hídrico, estrés lumínico, latencia de semillas, plantas CAM.

En zonas áridas y semiáridas los factores ambientales como son sequía, alta radiación solar y temperaturas extremas, limitan tanto el establecimiento de plántulas como el desarrollo de plantas adultas. Las plantas del género *Opuntia* han desarrollado diferentes adaptaciones para poder establecerse en estas condiciones. Estas plantas se caracterizan por tener propagación vegetativa, siendo más exitosa a corto plazo que la propagación sexual, debido a la latencia que presentan sus semillas, sin embargo esta característica les permite permanecer por largos periodos de tiempo en el suelo creando bancos de semillas. En esta investigación, por un lado, se analizó el efecto de los hongos *Penicillium chrysogenum*, *Phoma* sp. y *Trichoderma* spp. en la ruptura de la latencia de semillas de *Opuntia* spp., obteniendo porcentajes de germinación mayores al 90% en semillas almacenadas por 9 años para *O. streptacantha*, y 40% en semillas frescas de *O. leucotricha*. Por otro lado, se sometieron plántulas de *O. leucotricha* y *O. streptacantha* a condiciones contrastantes de riego y radiación solar, en donde se encontró que se establecen mejor bajo la sombra, como ocurre bajo plantas nodriza en condiciones naturales, puesto que presentaron mayor potencial osmótico, acidez y proporción clorofila/carotenoides que las plántulas sin sombra y sin riego. Se encontró también que la sequía y alta radiación solar inducen la expresión de genes como *OpsMT*, *OpsLTP*, *OpsDHN* y *OpsAQP*, así como mayor tasa de transporte de electrones, alta disipación no fotoquímica y agrupamiento de cloroplastos, en comparación con el tratamiento de riego. Además, se evaluaron las respuestas de plantas adultas de *O. streptacantha*, *O. leucotricha* y *O. robusta* durante las estaciones climáticas del año, encontrando mayor daño durante la época de sequía, al presentar menor potencial osmótico, eficiencia cuántica del fotosistema II (Φ_{PSII}) y acidez. Sin embargo; hubo incremento en la disipación no fotoquímica (NPQ), como mecanismo de protección para evitar daños en las plantas. Este estudio es pionero al analizar variables de respuesta a distintos niveles (ecofisiológico, anatómico y molecular), así como al encontrar que la interacción entre hongos y semillas es importante en ambientes desérticos. Esta investigación aporta información muy valiosa sobre los mecanismos de tolerancia que presentan las plantas de *Opuntia* spp. en distintas fases de desarrollo (plántulas y adultas) bajo condiciones de estrés abiótico.

Abstract

Tolerance of *Opuntia* spp. from San Luis Potosi Plateau under abiotic stress conditions: ecophysiological, anatomical and molecular responses

Keywords: CAM plants, nurse-plant effect, light stress, seasonal, seed dormancy, establishment of seedlings, water stress.

Drought, high solar radiation, and extreme temperature are limiting the seedling establishment as well as the adult plants development. *Opuntia* plants have different adaptations to settle in these conditions. These plants have vegetative propagation, which is more successful to short-term than sexual propagation due to their seeds have dormancy, however this characteristic allows them to remain for long time on the soil creating seed banks. In this research, we analyzed the effect of fungi *Penicillium chrysogenum*, *Phoma* sp. and *Trichoderma* spp. in breaking seed dormancy of *Opuntia* spp., having germination over 90% in aged seeds for *O. streptacantha* and 40% in fresh seeds for *O. leucotricha*. On the other hand, *O. leucotricha* and *O. streptacantha* seedlings were subjected to contrasting solar radiation and watering conditions, and it was found that both species established better under shade, similar to natural conditions under nurse plants, showing higher osmotic potential, acidity and chlorophyll/carotenoids ratio than seedlings without both shade and irrigation. Furthermore, it was found that drought and high solar radiation induced the gene expression like *OpsMT*, *OpsLTP*, *OpsDHN*, and *OpsAQP* and increased the electron transport rate, the non-photochemical quenching, and the clumping chloroplasts, in comparison with watering treatment. It was also evaluated the responses of *O. streptacantha*, *O. leucotricha*, and *O. robusta* adult plants during the four climatic seasons, founding higher damage during the dry season, with lower osmotic potential, quantum efficiency of photosystem II (Φ_{PSII}), and acidity content. In addition, it was found increase of non-photochemical quenching as mechanism to tolerate stress and avoid plant damage. This study is groundbreaking in taking into account response variables at different levels (ecophysiological, anatomical and molecular) as well as founding that the interaction between both fungi and seeds is important in desert environments. This research provides valuable information about the tolerance mechanisms of different developmental stages like seedlings and adults from *Opuntia* spp. plants to affront abiotic stress conditions.

CAPÍTULO I

Introducción general

En zonas áridas y semiáridas, las plantas están expuestas a diferentes factores ambientales como la escasa precipitación pluvial, la alta incidencia de luz solar, las altas y bajas temperaturas, el déficit de nutrientes y la salinidad de los suelos, los cuales limitan su establecimiento, crecimiento y supervivencia (Wickens, 1998; Rzedowski, 2006).

Un grupo de plantas que ha logrado establecerse exitosamente en estas regiones son las cactáceas, debido a que han desarrollado adaptaciones que les permiten tolerar las condiciones ambientales que ahí se presentan. Estas plantas son endémicas de América contando con alrededor de 1600 especies (Barthlott y Hunt, 1993; Stevens, 2001). El género *Opuntia* pertenece a esta familia y a la fecha se conocen 258 especies, de las cuales más de 100 se encuentran en México (Anderson, 2001; Cota-Sánchez, 2008). Son plantas fanerógamas, angiospermas, dicotiledóneas, perennes, con hábitos que van desde rastreras hasta arbustivas. Presentan fotosíntesis tipo CAM (metabolismo ácido de las crasuláceas), la cual es considerada como una adaptación a la sequía que permite la supervivencia y dominancia de las plantas en hábitats con periodos inconstantes de suministro de agua (Wickens, 1998; Dodd *et al*., 2002; Pimienta-Barrios *et al*., 2002). Otras adaptaciones que presentan son epidermis revestidas por cutículas gruesas que protegen a las plantas de la evaporación; la acumulación de cristales de oxalato de calcio, lo que refracta y disminuye la absorción de energía luminosa; así como la baja densidad de estomas que evitan la pérdida de agua (Gibson y Nobel, 1986; Malainine *et al*., 2003).

Las plantas de *Opuntia* son importantes por sus diversos usos, como alimenticio y forrajero (Mondragón-Jacobo y Pérez-González, 2001), así como para prevenir la erosión del suelo y combatir la desertificación, por su gran capacidad de adaptación en tierras pobres, inapropiadas para otro tipo de cultivos (Godínez *et al.*, 2003). Sin embargo; se desconocen muchos de sus aspectos ecológicos básicos, por ejemplo, los procesos ecofisiológicos que afectan la germinación de las semillas, el establecimiento de las plántulas y las respuestas de las plantas adultas a las diferentes condiciones ambientales que se presentan durante las estaciones del año. Es decir, conocer el comportamiento que presentan las especies de *Opuntia* en sus distintas fases de desarrollo (semilla, plántula, adulto) ante distintos tipos de estrés ambiental comunes en ambientes áridos y semiáridos, como hídrico y lumínico.

Las plantas de *Opuntia* presentan principalmente propagación vegetativa, debido a la latencia de sus semillas (Mandujano *et al*., 2005; Orozco-Segovia *et al*., 2007). En condiciones naturales, la germinación puede estar influenciada por factores internos y/o externos al embrión (madurez morfo-fisiológica, permeabilidad de la cubierta seminal) o por factores bióticos y/o abióticos que afectan a la semilla en el suelo, como el ataque de depredadores, parásitos, fluctuaciones de temperatura y humedad (Baskin y Baskin 2001). Los bancos de semillas de *Opuntia* permanecen por tiempos prolongados, y después de un período de varios años de post-maduración de las semillas, germinan cuando las condiciones ambientales son favorables (Jurado y Moles, 2003; Jurado y Flores, 2005; Mandujano *et al*., 2005). Se ha tratado de romper la latencia de las semillas de *Opuntia* spp. mediante la escarificación mecánica y/o química, así como la aplicación de reguladores de crecimiento (Mandujano *et al*., 2005, 2007; Orozco-Segovia *et al*., 2007). Sin embargo, los resultados no han sido muy exitosos, obteniendo porcentajes de germinación menores al 50% (Ochoa-Alfaro *et al*., 2008).

Después de la germinación de las semillas, la siguiente fase crítica que presentan las plantas de *Opuntia* es la etapa temprana de desarrollo. Las plántulas son individuos muy vulnerables que están expuestos a los factores abióticos (déficit de agua, altos niveles de radiación solar, temperaturas extremas, etc.) y bióticos (herbivoría, competencia, alelopatía, etc.) que limitan su supervivencia (Flores y Jurado 2003; Flores *et al*., 2004). Por ejemplo, se ha reportado que para *O. rastrera* de 7.2×10^6 semillas/ha, solamente un número muy bajo llega a establecerse (aproximadamente 12 plántulas), y de éstas el número de plantas que llega a la etapa adulta en *Opuntia* spp. es mucho menor (Mandujano *et al*., 1996).

La mayoría de las plántulas de cactáceas (incluyendo *Opuntia* spp.) que llegan a establecerse, lo hacen bajo plantas nodriza (Franco y Nobel, 1989; Flores y Jurado, 2003). La asociación entre plántulas y plantas nodriza, es un fenómeno relevante en ecosistemas áridos y semiáridos, donde el establecimiento de plántulas es mayor debajo de la cubierta de ciertos arbustos, pastos, rosetófilas y plantas suculentas de porte alto que mejoran las condiciones microambientales, en comparación con espacios abiertos (Flores y Jurado, 2003; Flores *et al*., 2004).

Las plantas nodriza amortiguan la temperatura durante los días de calor y protegen de heladas leves; aportan mayor humedad, a través de la canalización del agua y levantamiento hidráulico, aumentan la tasa de infiltración del agua y evitan una mayor evaporación. Además, aportan más nutrientes, protegen del pastoreo o pisoteo, así como de la herbivoría, aportan soporte físico, evitan la compactación y erosión del suelo (Flores y Jurado, 2003; Flores *et al*., 2004). Así, el éxito del establecimiento y supervivencia de las plántulas depende en gran medida de la protección por parte de otras plantas y de su capacidad para adaptarse y/o resistir los factores limitantes.

Por otro lado, tanto plántulas, como juveniles y adultos están expuestos a las condiciones climáticas que se presentan durante las diferentes estaciones del año, y poseen diferentes mecanismos para tolerar la sequía, las altas y/o bajas temperaturas y los altos niveles de luz (Szarek y Ting, 1974; Pimienta-Barrios *et al*., 2000; Ceusters *et al*., 2010). A la fecha, se sabe que las plantas CAM, también responden a la mayoría de los tipos de estrés abiótico por cambios en la expresión de genes de respuesta a estrés (Silva-Ortega *et al*., 2008; Ochoa-Alfaro *et al*., 2011), cambios en su anatomía y morfología (North *et al*., 1995; Chessa *et al*., 2002), acumulación de osmolitos compatibles (Silva-Ortega *et al*., 2008), cambios metabólicos (Ceusters *et al*., 2009), cambios en su crecimiento (tasa relativa de crecimiento) (Singh y Singh, 2003; Cervera *et al*., 2006; Martínez-Berdeja y Valverde 2008; Miquelajauregui y Valverde 2010), cambios en el contenido de clorofilas (Winter y Lesch, 1992; Pimienta-Barrios *et al*., 2005), generación de estrategias fotoprotectivas (agrupamiento de cloroplastos) (Kondo *et al*., 2004), que involucran la combinación de procesos para la utilización de la luz, disipación de energía térmica mediante la inducción de carotenoides (Winter y Lesch, 1992) y cambios en la captura de luz (Goldstein y Nobel 1991; Winter y Lesch, 1992; Nobel *et al*., 1994). Sin embargo, en general existe muy poca información sobre las respuestas de las plantas CAM al ambiente en el cual se establecen, en especial para las especies mexicanas.

En esta investigación los objetivos fueron:

1) Estudiar el rompimiento de la latencia de semillas de *Opuntia* spp., mediante la acción de hongos endógenos de las semillas y de otros hongos benéficos.

2) Determinar cambios anatómicos, ecofisiológicos y moleculares en plántulas de *Opuntia* sometidas a tratamientos de estrés (sequía y luz) en comparación con plántulas cultivadas bajo sombra, simulando en invernadero las condiciones que se presentan en espacios abiertos y bajo plantas nodrizas en zonas áridas y semiáridas.

y 3) Conocer los cambios ecofisiológicos que presentan las plantas adultas silvestres de *O. leucotricha, O. streptacantha* y *O. robusta* en dos orientaciones de los cladodios durante las diferentes estaciones del año.

El presente trabajo de tesis consta de ocho capítulos, el primero es una introducción general en la cual se describen los antecedentes y los objetivos de esta tesis. En el Capítulo 2 se describen los experimentos que se realizaron para evaluar los porcentajes de germinación de semillas de *Opuntia streptacantha* de dos colectas (1998 y 2007), y el efecto de hongos aislados de semillas de *O. streptacantha* y de otros géneros de plantas, en la ruptura de la latencia de semillas. Este capítulo se encuentra publicado en la revista *Plant Biology* (2011) 13:154–159.

En el Capítulo 3 se describe la participación de los hongos aislados previamente, en la ruptura de la latencia de semillas frescas (colecta 2008) de *Opuntia leucotricha*, este capítulo se publicó en la revista *Plant Signaling & Behavior* (2010) 5:1229-1230.

En los Capítulos 4 y 5 se muestran los resultados de experimentos en invernadero simulando el efecto de plantas nodriza sobre los mecanismos de respuesta a estrés hídrico y lumínico, y cómo las plántulas responden mediante cambios anatómicos, ecofisiológicos y moleculares a los factores ambientales a las cuales están expuestas. Los resultados de este primer experimento se analizaron por separado; en el primer análisis (correspondiente al Capítulo 4) se correlacionaron las variables anatómicas con algunas ecofisiológicas y ambientales. De esta parte se tiene un manuscrito enviado a la revista *Environmental and Experimental Botany*. En el Capítulo 5 se correlacionaron las variables moleculares con las ecofisiológicas y el manuscrito será sometido a la revista *Oecologia*.

En el Capítulo 6 se describe un estudio comparativo que se realizó entre plántulas de *O. streptacantha* y *O. leucotricha*. Se muestran las diferentes respuestas ecofisiológicas y moleculares de las plántulas de las dos especies a los diferentes tratamientos de sequía y radiación solar, pero con un tiempo mayor de los tratamientos en comparación con el experimento de los Capítulos 4 y 5.

El Capítulo 7 incluye estudios ecofisiológicos en plantas adultas de *O. streptacantha*, *O. leucotricha* y *O. robusta* nativas del sur del desierto

Chihuahuense. Se describe cómo las plantas responden a las diferentes condiciones ambientales a las que están expuestas durante las distintas estaciones del año. Este capítulo se publicará en el libro “Fisiología Ecológica en México: Estrategias de Respuesta al Ambiente, Diversidad Funcional y Cambio Climático”. Editores: José Luis Andrade (CICY) y Erick de la Barrera (CIECO-UNAM).

Finalmente en el último capítulo se hace una discusión integral de esta investigación y se presentan las conclusiones principales.

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CAPÍTULO II

Are fungi important for breaking seed dormancy in desert species? Experimental evidence in *Opuntia streptacantha* (Cactaceae)

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Running title: Fungi breaking seed dormancy in Opuntia

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Abstract

Opuntia spp. seeds have physiological dormancy; they need a period of after-ripening to break seed dormancy and their embryos have low growth potential. We evaluated the combined effect of seed age and fungi on testa on germination of *Opuntia streptacantha*, an abundant species in the Chihuahuan Desert (Mexico), assuming that old seeds have broken seed dormancy and fungi can reduce the mechanical resistance to germination. In a preliminary experiment, we found no germination from 9-years-old (1998) and freshly-collected (2007) seeds. However, we obtained 67% and 27% germination from 9-years-old and fresh non-sterilized seeds, respectively, and found fungi growing on the testa of all germinated seeds. After this, two fungal strains were isolated and identified by ribosomal internal transcribed spacer (ITS) sequence analysis: *Penicillium chrysogenum* and *Phoma* sp. In a second experiment, we inoculated seeds with strains of *P. chrysogenum* and *Phoma* sp., as well as *Trichoderma koningii* and binucleate *Rhizoctonia* (Gto17S2) to evaluate their ability in breaking seed dormancy. Seeds inoculated with *P. chrysogenum*, *Phoma* sp. and *T. koningii* had higher germination than the controls for both seed ages, but germination was higher in aged seeds. Scanning electron microscope showed that these fungi eroded the funiculus reducing its resistance. Binucleate *Rhizoctonia* produced null germination and controls had almost null germination. Our results strongly indicate that fungi are involved in breaking seed dormancy of *O. streptacantha*, and that the effect of fungi on seeds is species-specific.

Keywords

Binucleate *Rhizoctonia*; Cactaceae; Fungi; *Opuntia streptacantha; Penicillium chrysogenum*; *Phoma* sp.; Physiological dormancy; Prickly pear; Seed germination; *Trichoderma koningii.*

Introduction

Seed dormancy is a very common adaptive plant strategy in unpredictable and harsh environments, such as arid and semiarid landscapes (Jurado and Moles, 2003; Jurado and Flores, 2005), and it is common in the Cactaceae (Rojas-Aréchiga and Vázquez-Yanes, 2000; Flores *et al.*, 2005; 2006; 2008), especially the genus *Opuntia* (Mandujano *et al.*, 2005; 2007; Orozco-Segovia *et al.*, 2007, Ochoa-Alfaro *et al.*, 2008). The first *Opuntia* species found to have dormant seeds was *O. auranthiaca* (Archibald, 1939), and currently seed dormancy has been found for more than 20 species (Ochoa-Alfaro *et al.*, 2008). In most cases, different responses have been achieved with the use of several treatments (Mandujano *et al.*, 2005; 2007; Orozco-Segovia *et al.*, 2007; Ochoa-Alfaro *et al.*, 2008), but although seed dormancy in *Opuntia* spp. can be broken by scarification, seeds have physiological dormancy also, *i.e*. they have low growth potential of the embryo and thus need a period of after-ripening to break seed dormancy (Mandujano *et al.*, 2005; 2007; Orozco-Segovia *et al.*, 2007). If the coat of seeds is scarified, mechanical restriction is decreased, and the embryo is able to germinate. However, when physiological dormancy is broken in intact seeds, the embryo gains sufficient growth potential to overcome the restraint of the seed coat (Orozco-Segovia *et al.*, 2007).

Seeds in the soil interact with micro-organisms that could help them to break seed dormancy. Fungi attack the testa, eroding or cracking the hard/stony endocarp, and thus potentially can reduce the mechanical resistance to germination in seeds with physiological dormancy (Morpeth and Hall, 2000). In arid environments, some fungi have a role in seedling establishment, growth, water relations and nutrition of cacti (González-Monterrubio *et al.*, 2005), but the effects of fungi on breaking seed dormancy in cacti have received very little attention. The previous studies are by Arredondo *et al.* (2007), who found that *Rhizopus* sp. moderately breaks seed dormancy of *Thelocactus hexahedrophorus*, another cactus species from the Chihuahuan Desert, and by Olvera-Carrillo *et al.* (2009), who found that 7-month-old exhumed seeds from *Opuntia tomentosa* showed fungal hyphae penetrating the funicular envelope through the openings, favoring the germination of a weak embryo (an embryo with low growth potential).

In this study, assuming that old seeds have broken seed dormancy and fungi can reduce their mechanical resistance to germination, we present experiments in which we evaluated: (i) the combined effect of seed age (seeds collected in 1998 and in 2007) and the natural fungi in seed testa, and (ii) the combined effect of seed age and the fungal inoculation of each of four fungi species (two of them isolated from *Opuntia streptacantha* seed testa) in breaking the seed dormancy of *O. streptacantha* Lem (Prickly Pear), a perennial arborescent cactus of economical interest (it has edible fruits and young pads) distributed on the semiarid lands of central Mexico. In addition, we used PCR and sequencing techniques in order to identify the fungi species found in *O. streptacantha* seed testa. We also tested the effect of *Trichoderma koningii* on breaking seed dormancy of *O. streptacantha*, because some researchers have suggested that *Trichoderma* spp. could promote seed germination (Kleifeld and Chet, 1992; Ousley *et al.*, 1994). In addition, we tested binucleate *Rhizoctonia* (Gto17S2), since it has been suggested that *Rhizoctonia* spp. promotes seed germination of some species, such as orchids (Vujanovic *et al.*, 2000). This binucleate *Rhizoctonia* isolate (Gto17S2), which was previously isolated by our group, is known to protect pepper plants against root pathogens.

Materials and methods

Experiment 1. Seed germination of aged and fresh seeds

The life span of an *Opuntia streptacantha* seed in the soil is not known, but *Opuntia* seeds are morphologically and physiologically heterogeneous, and so is their response to soil factors, which suggests that their seeds may remain in the seed bank for a year or more or until conditions are adequate for germination (Olvera-Carrillo *et al.*, 2009). Thus, in order to know if old seeds have broken seed physiological dormancy we used two seed collections. Seeds were collected from mature fruits when available over two years (1998 and 2007) and stored in paper bags at room temperature.

There were two treatments, washed seeds and non-washed seeds. Washed (sterilized) seeds were immersed in 150 ml of 70% ethanol for 5 min, then in 150 ml of 20% sodium hypochlorite (6% free chlorine) for 2 min and finally washed four times using sterile distilled water (under aseptic conditions inside a laminar flow cabinet). Seeds were incubated in water-agar plates for 40 days in an automatic germination chamber with a 16 h light and 8 h dark photoperiod at 25 °C ± 2 °C. There were five replicates per treatment with 20 seeds per replicate.

Isolation and characterization of fungi from the testa

We found fungi growing on the testa of unsterilized germinated seeds. Two fungal strains, fungus A (HP1P) and fungus B (HP1C), were isolated from seeds of both seed ages grown in the Petri dishes on water-agar medium. Morphology of HP1P and HP1C colonies was determined by visual observation on potato-dextrose-agar (PDA) at 28 °C for 3-4 days. Additionally, samples of mycelia of HP1P and HP1C grown on PDA were observed on a Motic microscope Model BA300 and images were captured using a Moticam 2000 and the software Mitic Images Plus 2.0 ML.

Ribosomal internal transcribed spacer (ITS) sequence analysis

HP1P and HP1C isolates were grown on PDA media overlaid with cellophane for 5-7 days at 28 °C. DNA from each strain was then extracted according to Raeder and Broda (1985). PCR analysis of genomic DNA was carried out using the oligonucleotide primers ITS-4 5'-TCCTCCGCTTATTGATATGC-3' (sense) and ITS-5 5' GGAAGTAAAAGTCGTAACAAGG-3' (anti sense) (White *et al.*, 1990) which span the ITS1, ITS2 and 5.8S ribosomal sequences using the following reaction mixture: 50 ng fungal genomic DNA, 10 pmol/µL ITS-4 and ITS-5 primers, 2 mM $MgCl_2$, 200 µM each dNTP, 20 mM Tris–HCl, pH 8.4, 50 mM KCl and 1.5 U Taq DNA polymerase (Invitrogen). PCR conditions were carried out at 95 ºC for 5 min followed by 35 cycles of 30 s at 94 ºC, 45 s at 60 ºC and 1.5 min at 72 ºC and a final extension of 8 min at 72 ºC in a MyCycler model thermocycler (Biorad). Amplified fragments were cloned in pCR4-TOPO vector (Invitrogen) following the manufacturer instructions. Sequencing of cloned fragments was carried out on an ABI PRISM 377 automatic sequencer (Applied Biosystem) and *in silico* analysis was carried out using NCBI BLAST utilities. Sequences were deposited at the GenBank under the accession numbers EU869181 (HP1P) and EU869182 (HP1C). The sequences and the phylogenetic tree were aligned and created by the Clustal W method using the MegAlign Program (DNASTAR, Inc. Madison, WI).

Experiment 2. Effect of fungi on seed germination

HP1P, HP1C, *Trichoderma koningii* and binucleate *Rhizoctonia* (Gto17S2) fungi were grown on PDA plates at 28 °C for 3 days. The spores (HP1P and *T. koningii*) and mycelium (HP1C and binucleate *Rhizoctonia* (Gto17S2)) were collected applying sterile distilled water and counted in a Neubauer chamber to carry out the inoculations on the two *O. streptacantha* seed collections (1998 and 2007). Sterilized seeds were grown on water-agar plates and inoculated with 2 µL of a solution of spores or mycelium (6 x 10^7/mL) of each fungus. There were five replicates per treatment with 20 seeds per replicate. At the end of the experiment, the external morphology of the control and inoculated seeds was observed with a high resolution scanning electron microscope (ESEM/QUANTA 200 FEI, Low Vacuum/Water). Photomicrographs were taken with ESEM with a pressure chamber of 100.00 Pa, and voltage of 30.0 Kv.

Statistical analysis

A two-way ANOVA was conducted on the germination percentage of each experiment, as suggested by Baskin and Baskin (2001). We used sterilization (two levels) and seed age (two levels) as factors in Experiment 1, and seed age (two levels) and fungus species (three levels, because seeds inoculated with binucleate *Rhizoctonia* (Gto17S2) did not germinate) in Experiment 2. Before the analysis was done, the percentage data were normalized using a square root arc-sine transformation (Sokal and Rohlf, 1994). For each species, differences among treatments were explored using orthogonal contrasts.

Results

Experiment 1. Seed germination of aged and fresh seeds

There was a significant effect of sterilization (F = 67.88, P < 0.0001), seed age (F = 7.88, P = 0.01), and the interaction of both factors (F = 7.88, P = 0.01) on *Opuntia streptacantha* seed germination. Non-sterilized 1998 and 2007 seeds germinated to 67% ± 6.5 (mean ± SE) and 27% ± 6, respectively, while none of the sterilized 1998 and 2007 seeds germinated. Ungerminated seeds tested viable after dissection.

Identification of fungi isolated from the seeds of Opuntia streptacantha

The fungus A (HP1P) showed a growth of hyaline mycelium and dark green spores which are characteristic of the genus *Penicillium*. Fungus B (HP1C) had a white mycelium and dark brown structures similar to “spores”, but these characteristics were insufficient for identification (Fig. 1). Subsequently, with the aim of identifying the fungi isolated from the seeds of *O. streptacantha,* a portion of HP1P and HP1C mycelium from non-sterilized seeds was isolated. The DNA spanning the ITS-1, ITS-2 and the 5.8S ribosomal sequences was amplified, cloned and sequenced to aid in identification. Amplified fragments of 598 and 589 bp were obtained for isolates HP1P and HP1C respectively, using the universal fungal primers (ITS1/ITS4) (deposited in GenBank under accession numbers EU869181 and EU869182). The sequences of isolated A (HP1P) and B (HP1C) showed high identity with *Penicillium chrysogenum* (98%) and *Phoma medicaginis* (97%), respectively. Phylogenetic analysis confirmed the association of isolated A (HP1P) with *P. chrysogenum*, and the association of isolated B (HP1C) with *P. medicaginis* (Fig. 2).

Experiment 2. Effect of fungi on seed germination

There was a significant effect of fungus species (F = 95.99, P < 0.0001) and seed age (F = 42.16, P < 0.0001), but not of the interaction of both factors (F = 1.31, P > 0.5) on *O. streptacantha* seed germination. Seeds inoculated with *Phoma* sp., *T. koningii*, and *P. chrysogenum* had higher germination than the controls for both seed age, although *P. chrysogenum* had lower germination than the others. This three species promoted higher seed germination than both seeds inoculated with binucleate *Rhizoctonia* (Gto17S2) and control treatment (Table 1). Ungerminated seeds tested viable after dissection. Photomicrographs taken with ESEM showed that fungi eroded the endocarp reducing its resistance (Fig. 3).

Discussion

In arid and semiarid environments, seed germination and seedling establishment are infrequent due to extreme temperatures and low water availability (Flores and Jurado, 2003; Flores *et al.*, 2004). Thus, a common plant strategy in these environments is having seed dormancy, which has been found in several plant families (Baskin and Baskin, 2001; Jurado and Moles, 2003; Jurado and Flores, 2005) including the Cactaceae (Rojas-Aréchiga and Vázques-Yanes, 2000; Flores *et al.*, 2005; 2006; 2008).

Opuntia species, including *O. streptacantha* (Ochoa-Alfaro *et al.*, 2008) have hard to break dormancy in their seeds. Several dormancy-breaking treatments, including mechanical scarification, cold stratification, soaking in hot water, after-ripening, scarification with sulfuric acid, leaching, passage through the digestive tract of animals, osmolytes (proline), polycationic compounds (polyamines), and some combinations of these treatments have been used to enhance germination of *Opuntia* seeds. However, these treatments do not always promote germination and, in some cases, treated seeds have germinated to lower percentages than those in the control (Rojas-Aréchiga and Vázquez-Yanes, 2000; Mandujano *et al.*, 2005; 2007; Orozco-Segovia *et al.*, 2007; Ochoa-Alfaro *et al.*, 2008). Gibberellic acid (GA_3) alone seems to be only moderately effective in promoting germination of seeds of *Opuntia* spp. (Wang *et al.*, 1996). However, it induces a high percentage of germination in combination with other treatments such as rinsing/washing, mechanical or chemical scarification (Orozco-Segovia *et al.*, 2007).

In our experiments, we found that three fungi species erode the endocarp and break seed dormancy of *O*. *streptacantha*. Since seeds of *Opuntia* are known to have physiological dormancy, *i.e.* embryo has low growth potential (Orozco-Segovia *et al.*, 2007), it is very reasonable that fungal attack of the tests would reduce the mechanical resistance of the testa, thus making it easier for the embryo to germinate. Controls in both experiments were different, the reason the germination of control seeds in the second experiment for fresh seeds is only 1% whereas control fresh seeds in the first experiment reached 27% is that seeds from the second experiment were sterilized, which inhibited fungal growth. The reason the 1998 seeds germinated to high percentages than the 2007 seeds is that after-ripening had occurred, *i.e.* growth potential of the embryo had increased (Orozco-Segovia *et al.*, 2007). Thus, even a small amount of weakening of the testa of 1998 seeds by fungi allowed them to germinate. The small amount of weakening of the testa of 2007 seeds by fungi may not have been enough release of mechanical resistance to allow these more physiological dormancy seeds (compared to 1998 seeds) to germinate. The measurement of puncture force to determine the effect of the fungus weakening the seed coat is worthy of consideration in future studies.

The effect of fungi on seed germination has been studied for tropical (Porras-Alfaro and Bayman, 2007; Stewart and Kane, 2007) or temperate species (Bidartondo and Bruns, 2005; Massicotte *et al.*, 2007), in which fungi are very common due to high moisture availability. However, the effect on desert species has not been sufficiently studied, perhaps due to a lack of tradition for fungi studies in deserts (Porras-Alfaro *et al.*, 2008). Arredondo *et al.* (2007) have found such interaction in a related Cactaceae species. Olvera-Carrillo *et al.* (2009) found that

7-month-old exhumed seeds from *Opuntia tomentosa* showed fungal hyphae penetrating the funicular envelope through the openings, favoring the germination of a weak embryo, although they did not identified fungi species and their effects on *Opuntia* seed germination. Our main results are the identification of fungi species living on testa and their differential effect on seeds. Here, local fungi strains are identified and tested after cultivation.

Our study revealed that seeds inoculated with *Phoma* sp. and *T. koningii* had higher germination percentages than those inoculated with *P. chrysogenum*, but these three fungal species promoted higher seed germination than both seeds inoculated with binucleate *Rhizoctonia* (Gto17S2) and control treatment. Binucleate *Rhizoctonia* is commonly found in nature, where it may exist as a saprophyte in the soil and plant debris or as beneficial endophyte or harmful parasite in plants (Sneh *et al.*, 1991). Binucleate *Rhizoctonia* (Gto17S2) however did not promote breaking seed dormancy in *O. streptacantha*, suggesting that the effect of fungi on seeds is species-specific.

The effect of *T. koningii* on seed germination was similar to *Phoma* sp. *Trichoderma* spp. are fungi that protect plants against pathogenic fungi, since they produce enzymes that degrade the cell wall of phytopathogenic fungi (Sharon *et al.*, 2001). *Trichoderma* spp. also helps protecting seeds (Tsahouridou and Thanassoulopoulos, 2002), and some researchers have suggested that they could promote seed germination (Kleifeld and Chet, 1992; Ousley *et al.*, 1994). In addition, it has been found that *Penicillium* spp. promotes seed germination, as well as seedling growth and even seedling protection in *Picea glehnii* (Yamaji *et al.*, 2001) and *Xyris complanata* (Tamura *et al.*, 2008). Now, we have evidence that *T.*

koningii and *P. chrysogenum* break mechanical (not physiological) seed dormancy, and promote seed germination *in O. streptacantha*.

In conclusion, *Phoma* sp. and *T. koningii*, and in less proportion *P. chrysogenum*, help to break seed dormancy of *O. streptacantha*, maybe by the action of enzymes that degrade the testa. Fungi attack the testa and thus potentially can reduce the mechanical resistance to germination in seeds with physiological dormancy, *i.e.*, embryos have low growth potential.We suggested that fungi play an important role in breaking seed dormancy of desert species, opening new insights regarding the effect of fungi in breaking seed dormancy of arid and semiarid plants.

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Table 1. Effect of fungus species on germination percentage of *Opuntia streptacantha* seeds.

Seeds (inoculated*)	9 years old seeds (S.E.)	Fresh seeds (S.E.)
Control	8% (± 3.39)	1% (± 1.00)
*Fungus A (HP1P) (*Penicillium chrysogenum*)	68% (± 5.14)	39% (± 6.40)
*Fungus B (HP1C) (*Phoma* sp.)	87% (± 2.54)	68% (± 4.06)
**Trichoderma koningii*	91% (± 3.67)	58% (± 8.15)

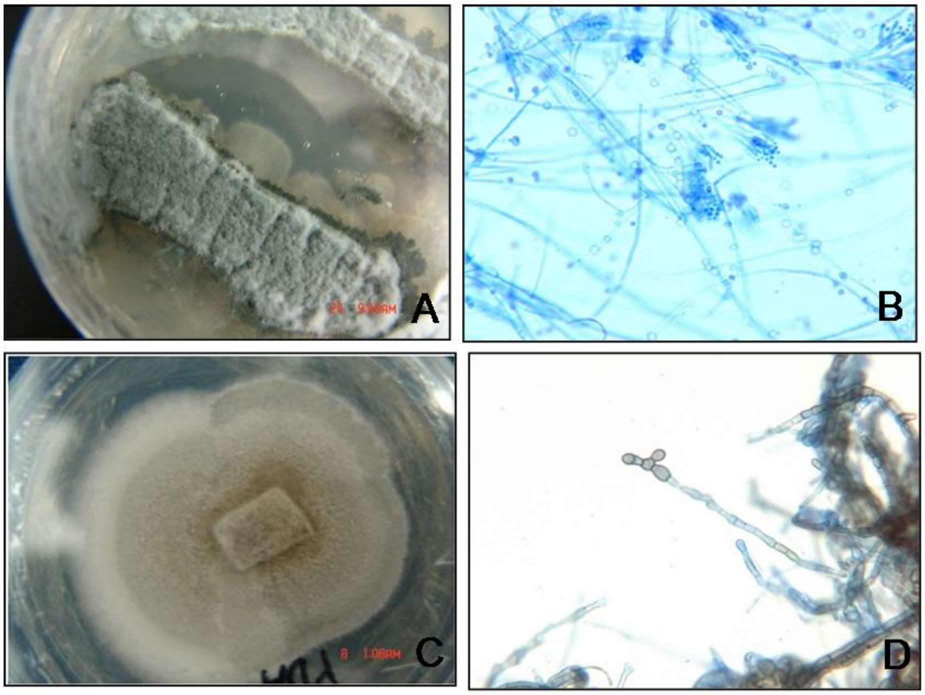

Figure 1. Identification of fungi isolated from *Opuntia streptacantha*. A) Growth of mycelium of fungus A (HP1P) identified as *Penicillium chrysogenum*; B) Microscopy of fungus A (HP1P); C) Growth of mycelium of fungus B (HP1C) identified as a *Phoma* sp.; D) Microscopy of fungus B (HP1C).

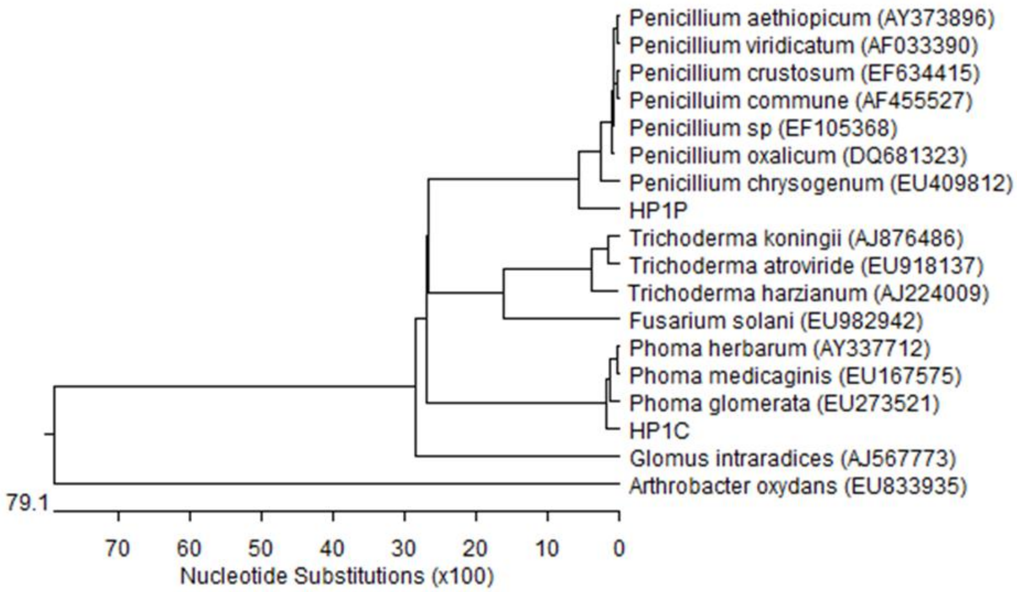

Figure 2. Phylogenetic tree based on ITS analysis showing fungi isolated from *Opuntia streptacantha* (HP1P (EU869182) and HP1C (EU869182)) and relatives, created by the Clustal W method using the MegAlign Program (DNASTAR, Inc. Madison, WI).

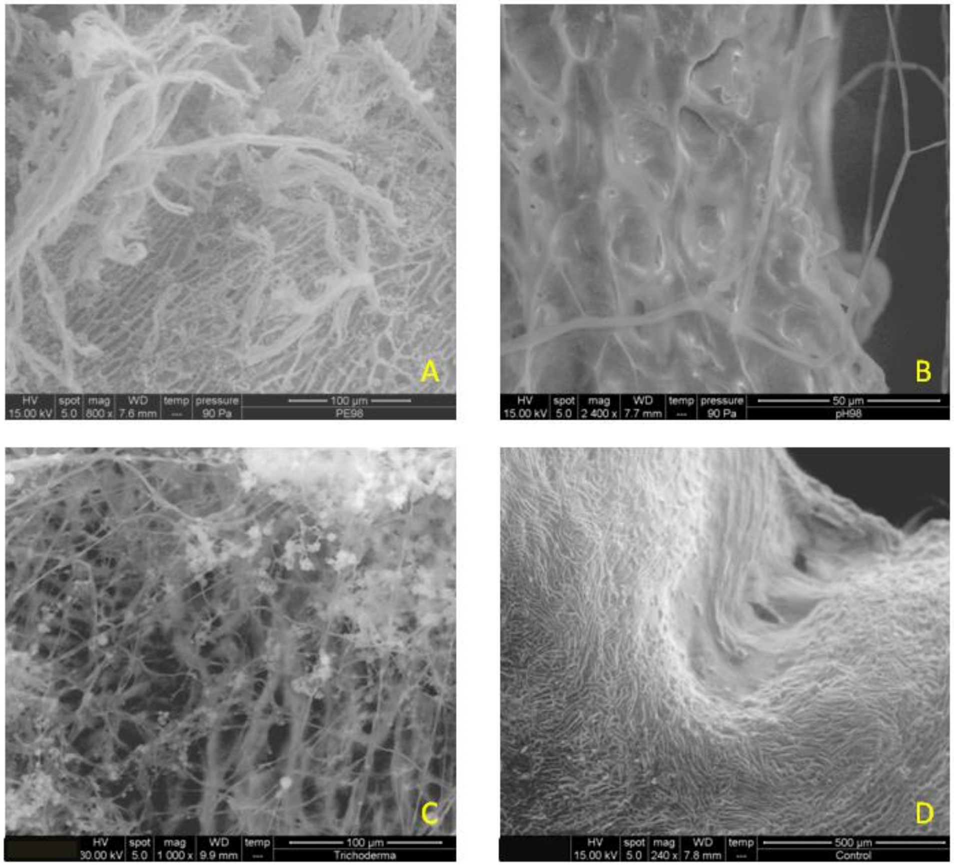

Figure 3. Photomicrographs taken with an environmental scanning electron microscope of fungal hyphae eroding the endocarp of seeds: A) *Penicillium chrysogenum*, B) *Phoma* sp., C) *Trichoderma koningii*, and D) control (sterile seed).

CAPÍTULO III

Further evidence from the effect of fungi on breaking *Opuntia* seed dormancy

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Addendum to: Delgado-Sánchez P, Ortega-Amaro MA, Jiménez-Bremont JF, Flores J. Are fungi important for breaking seed dormancy in desert species? Experimental evidence in *Opuntia streptacantha* (Cactaceae). *Plant Biology* (2011), 13:154-159

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Abstract

Recently, we found that fungi are involved in breaking seed dormancy of *Opuntia streptacantha*, and that the effect of fungi on seeds is species-specific. However, the effect of fungi on seed germination from other *Opuntia* spp has not been evaluated. Thus, we evaluated the effect of four fungal species (*Penicillium chrysogenum*, *Phoma* sp., *Trichoderma harzianum*, *Trichoderma koningii*) on the germination of *Opuntia leucotricha*, an abundant species in the Chihuahuan Desert, Mexico. We found that seeds inoculated with the four fungal species had higher germination than control seeds. *Trichoderma* spp. were the most effective. Our results strongly indicate that fungi are involved in breaking seed dormancy of *O. leucotricha*. Thus, we suggest that these fungi could promote seed germination from other *Opuntia* species.

Keywords: Cactaceae; *Opuntia leucotricha*; *Penicillium chrysogenum*; *Phoma* sp.; Physiological dormancy; Prickly pear; Seed germination; *Trichoderma* spp.

Introduction

Seeds in the soil interact with micro-organisms that could help them break seed dormancy. Fungi attack the testa, eroding or cracking the hard/stony endocarp, and could reduce the mechanical resistance to germination in seeds with physiological dormancy.[1] In arid environments, the effects of fungi on breaking seed dormancy in cacti have received very little attention. Recently, our work group found that *Phoma* sp. and *Trichoderma koningii*, and in less proportion *Penicillium chrysogenum*, help break seed dormancy of *Opuntia streptacantha*, maybe by the action of enzymes that degrade the testa.[2] However, the effect of fungi on seed germination from other *Opuntia* species has not been evaluated.

In this study, we test the effects of four fungal species (two isolated from *O. streptacantha* testa) in breaking seed dormancy of *Opuntia leucotricha*; a perennial arborescent cactus of economic interest distributed on the semiarid lands of central Mexico.

Since seeds of *Opuntia* spp. have physiological dormancy, they need a period of after-ripening to break dormancy, and the embryos have low growth potential; we used 2-year-old seeds, assuming that old seeds have broken physiological seed dormancy and that fungi can reduce mechanical resistance to germination.[2] *O. leucotricha* seeds were collected from mature fruits, in 2008, and stored in paper bags at room temperature during two years.

Penicillium chrysogenum, *Phoma* sp., *Trichoderma harzianum*, and *T. koningii* were grown on PDA plates at 28°C for 3 days. The spores (*P. chrysogenum*, *T. harzianum*, and *T. koningii*) and mycelia (*Phoma* sp.) were collected in sterile distilled water and counted in a Neubauer chamber for later inoculation of *O. leucotricha* seeds. Sterilized seeds were grown on water-agar plates and inoculated with 2 µl of spore solution or mycelium (6 X 10^7 ml^{-1}) from each fungus. Seeds were incubated in water-agar plates for 35 days in an automatic germination chamber with a 16-h light and 8-h dark photoperiod at 25ºC ± 2ºC. There were five replicates per treatment and 20 seeds per replicate.

After one-way ANOVA, we found a significant effect of fungal species (F = 52.198, $P < 0.0001$) on *O. leucotricha* seed germination. Seeds inoculated with the four fungal species had higher germination than control, although *Trichoderma* spp. promoted higher seed germination than the other fungi examined (Table 1).

Opuntia species have hard to break dormancy in their seeds.[2-8] Seed dormancy is a common plant strategy in arid and semiarid environments, which has been found in several plant families,[9-11] including Cactaceae.[12-16]

In our experiment, we found that four fungal species erode the endocarp and break seed dormancy of *O. leucotricha*. Since seeds of *Opuntia* are known to have physiological dormancy, *i.e.* the embryo has low growth potential[6], it is possible that fungal attack of the tests would reduce the mechanical resistance of the testa, thus promoting embryo growth.

Arredondo *et al.*[16] found that *Rhizopus* sp. moderately breaks seed dormancy of *Thelocactus hexahedrophorus*, another cactus species from the Chihuahuan Desert. Olvera-Carrillo *et al.*[8] found that 7-month-old exhumed seeds from *O. tomentosa* showed fungal hyphae penetrating the funicular envelope through the openings, favoring germination but with a weak embryo (an embryo with low growth potential).

Conclusions

We found that *O. leucotricha* seeds inoculated with the four fungal species had higher germination than control seeds, similar to findings for *O. streptacantha.*[2] These results show that fungi play an important role in breaking seed dormancy of *Opuntia* species; they contribute to understanding germination biology of cactus species, opening new insights regarding the effect of fungi on breaking seed dormancy of arid and semiarid plants.

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Table 1. Effect of fungal species on germination of *Opuntia leucotricha* seeds.

Treatment	Germination percentage (±S.E.)
Control	0% [c]
Penicillium chrysogenum	15% (± 3.35)[b]
Phoma sp.	10% (± 2.23)[b]
Trichoderma harzyanum	40% (± 8.94)[a]
Trichoderma koningii	37% (± 8.27)[a]

CAPÍTULO IV

Mechanisms behind the nurse effect: evaluating ecophysiological and anatomical responses in *Opuntia streptacantha* seedlings

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Abstract

Opuntia streptacantha seedlings establish mostly under the canopy of nurse plants which provide a less stressful micro-environment, although mechanisms underlying this process are unknown. There are clearly complex ecophysiological and anatomical adaptations as a result of interactions between drought and light, which could have direct consequences in the establishment of seedlings. In this study, the impact of the various combinations of light and watering treatments (*i.e.,* shade – watering, shade and non-watering, light – watering, and light and non-watering) on seedlings was examined. Ecophysiological [titratable acidity and osmotic potential (Ψ_s)], anatomical (chloroplast density, chloroplast frequency, and cell area), and environmental [photosynthetic photon flux density (PPFD), air temperature, and soil water availability] variables were evaluated. Three harvests were carried out fortnightly, at days 15, 30, and 45. The two ecophysiological variables were found to be the most important responses for seedling establishment. The main anatomical and environmental variables were chloroplast density and water availability, respectively. *Opuntia streptacantha* seedlings establish better in the shade – watering treatment, due to higher Ψ_s and acidity, unaffected chloroplasts, and lower PPFD. The chloroplasts of cells under light and non-watering treatment were clumped closer to the center of the cytosol than to those under shade and non-watering. Thus, these results suggest that *Opuntia* seedlings grow better under the shade of nurse plants, although they can tolerate high-light and drought in open spaces by increasing and moving chloroplasts and also avoiding drastic decreases in their osmotic potential. Our results are especially important in predicting the impact of climate change on natural desert regeneration, reforestation-afforestation practices, and rural land uses.

***Keywords*:** CAM plants; chloroplast density; light stress; prickly pear; titratable acidity; water stress.

***Abbreviations*:** CAM, crassulacean acid metabolism; EPI, environmental productivity index; LNW, light – non-watering treatment; LW, light – watering treatment; PPFD, photosynthetic photon flux density; PSII, photosystem II; SNW, shade – non-watering treatment; SW, shade – watering treatment; Ψ_s, osmotic potential.

Introduction

Plant recruitment in arid environments often occurs only in years with above average rainfall or in safe sites under the canopy of nurse plants that provide shelter from high temperatures and low moisture. This phenomenon is thought to be more frequent in harsh than in mild environments and appears to occur frequently in arid and semi-arid biomes (Flores and Jurado, 2003). For example, in the southern Chihuahuan Desert of Mexico, *Opuntia streptacantha* establish mostly under the canopy of nurse plants (Yeaton and Romero-Manzanares, 1986; Vargas-Mendoza and González-Espinoza, 1992). However, although it is known that *Opuntia* species are succulent plants with high water-use efficiency, at least in the adult stage (Nobel, 1988; North and Nobel, 1992; Pimienta-Barrios et al., 2002; Pimienta-Barrios et al., 2003; Zañudo-Hernández et al., 2010), physiological and anatomical mechanisms underlying the nurse effect on *Opuntia* seedlings are unknown in arid environments. For tropical ecosystems, Winter et al. (2011) found strong C_3-photosynthesis component and facultative crassulacean acid metabolism (CAM-photosynthesis) features in young *O. elatior*.

Cactus plants generally exhibit CAM-photosynthesis, in which gas (CO_2) exchange occurs at night when the water vapor pressure difference between the air and the transpiring surfaces is lowest, resulting in high water-use efficiency in adult plants (Nobel, 1988; Winter et al., 2005) but not necessarily during seedling stages and when water from rainfall is plentiful (Nobel, 2010; Winter et al., 2011). However, nocturnal acid accumulation decreases for light - exposed cactus seedlings but increases for shaded cactus seedlings. Thus, the presence of CAM in shaded cactus seedlings increases water-use efficiency and reduces the risk of photoinhibition (Gallardo-Vasquez and De la Barrera, 2007; Hernández-González and Briones-Villarreal, 2007). The CAM pathway is very common in arid and semiarid environments, where plants are frequently exposed to low soil moisture and high solar radiation (Cushman, 2001; Nobel, 2010). It is possible that *Opuntia* seedlings established in open, highly illuminated spaces, show lower acid accumulation than those established in shade. Indeed, the Cactaceae family includes the highest number of species protected by nurse plants (Flores and Jurado, 2003).

Light is the ultimate source of energy for photosynthesis. Plants under the canopy of nurse plants are exposed to diminished light, which could limit or improve their photosynthesis and growth. For example, Franco and Nobel (1989) found a higher environmental productivity index (EPI), a measure of the quantitative effects of water, temperature, and photosynthetic photon flux density (PPFD) on net CO_2 uptake, for *Ferocactus acanthodes* seedlings at exposed sites compared to those located under nurse plants. However, other authors have found higher growth for shaded than for exposed columnar cactus seedlings (Gallardo-Vasquez and De la Barrera, 2007; Hernández-González and Briones-Villarreal, 2007). Thus, photosynthesis and growth under nurse plants are diminished for *F. acanthodes* but improved for the columnar cactus species.

As reported by other workers, high solar radiation could cause persistent damage to the photosynthetic complexes of chloroplasts (Valladares and Pearcy, 1997). To help overcome this inherent photoinactivation of photosystem II (PSII), plants have different photoprotective mechanisms. Each species may have a different level of photoprotection, allowing it to survive and even to flourish in its natural habitat (Park et al., 1996; Li et al., 2010), such as high-light-induced chloroplasts movement, for example, which prevents PSII from light stress (Haupt and Scheuerlein, 1990; Park et al., 1996; Sharon and Beer, 2008).

Chloroplasts are the location for chlorophyll and hence for photosynthesis (Nobel, 2010). They are usually positioned with their broad surfaces parallel to the cell wall, but they can reorient along cell walls perpendicular to the leaf surface, parallel to the direction of incident light to avoid absorption (Evert and Eichhorn, 2006; Li et al., 2009). Diurnal movement of chloroplasts in the CAM plant *Zygocactus truncates* (epiphytic cactus) has also been reported under the combination of light and water stress (Kondo et al., 2004), but not in *Opuntia* species or in seedlings which require the protection of nurse plants.

Aridity is expected to increase in arid and semi-arid ecosystems (IPCC, 2007). Nobel (2010) suggested that the future of succulents like cacti is extremely bright under new climatic conditions (global warming) expected worldwide in the 21st century. Thus, understanding how water availability and light intensity influence the ecophysiological and anatomical performance of *Opuntia* seedlings is crucial in predicting the impact of climate change on natural desert regeneration.

In this study, the effect of the combination of light and watering treatments (shade and watering, shade and non-watering, light and watering, and light and non-watering) has been examined on *O. streptacantha* seedlings. *Opuntia streptacantha* Lem. (Prickly Pear) is a perennial arborescent cactus of economic interest which has edible fruit and young pads. It is distributed throughout the semiarid lands of central Mexico (Littlejohn Jr. and Williams III, 1983; Winter and

Lesch, 1992). We assessed three types of response variables: ecophysiological [titratable acidity and osmotic potential (Ψ_s)], anatomical (chloroplast frequency, chloroplast density, and cell area), and environmental (PPFD, air temperature, and soil water condition).

Materials and methods

Study design

Opuntia streptacantha seeds were collected at Mexquitic de Carmona municipality of San Luis Potosi, Mexico (22º16’ N, 101˚07’ W at 2,020 m asl), in the Southern Chihuahuan Desert, in 2007. Seeds were sown in plastic trays with Sunshine Mix # 3 substrate (Sun Gro Horticulture Canada Ltd), which were placed in a greenhouse and watered every other day. Four months later, the seedlings were transplanted into pots with soil from the *O. streptacantha* habitat and maintained at field capacity for 20 days. Then, four treatments were randomly assigned to 100 seedlings corresponding to a total of 25 replicates after treatment. Treatments included: shade (25 % light) with watering (SW) at field capacity every other day, shade without watering (SNW), light (100 % light) with watering (LW), and light without watering (LNW). The waterless treatments mimic dry periods with no rain. Treatments with water were intended to model years in which rains were frequent throughout the entire wet season. In addition, watering provided a good control of added water quantity to experimental unities. Treatments with high solar radiation simulated solar radiation in open spaces without vegetation, whereas shaded treatments represented the low solar radiation (≈ 50 % of light reduction) found under shrubs in the area. All of these treatments allowed us to measure the ecophysiological and anatomical responses of plants to contrasting environmental conditions.

In order to simulate low solar radiation, plants were covered with a plastic film mesh which created shade; for high radiation treatments, plants were not covered. Seedlings in the low moisture treatments were not watered, while those in the high moisture treatments were watered every other day to field capacity during the entire course of the experiment.

Three harvests were made fortnightly, after 15, 30, and 45 days. PPFD and temperature were measured using a portable fluorometer (pulse-amplitude modulated photosynthesis yield analyzer, Mini-PAM; Walz GmbH, Effeltrich, Germany). The following parameters were evaluated: osmotic potential (Ψ_s), titratable acidity content, chloroplast density, chloroplast frequency, and cell area. In addition, chloroplast arrangement was described.

Osmotic potential (Ψ_s)

Water status measurements were completed at predawn. The experimental unit consisted of one seedling *per* pot to obtain sufficient sap extract. In order to evaluate osmotic potentials (Ψ_s), three harvests (three seedlings *per* harvest) were carried out. *Ex situ* determinations were made of the Ψ_s of the sap obtained by mechanical compression (Pearcy et al., 1991). Ψ_s was recorded in C-52 sampling chambers connected to a Wescor HR-33T dew-point microvoltmeter (Wescor Inc., Logan, Utah, U.S.A.). Sampling chambers were calibrated prior to use with standard NaCl solutions (Rundel and Jarrel, 1991).

Titratable acidity content

The malic acid content of seedlings was determined at 6:00 a.m., when its concentration is highest (Nobel, 2010). Transverse samples of plant tissue were obtained from seedling shoots using a steel borer of cross section 0.01 to 0.02 m^2. The plant material was sectioned and preserved in ethanol (80 %) in 1.5 mL Eppendorf tubes. Titration was carried to neutrality with a 0.01 N NaOH solution to determine the malic acid concentration (mmol equiv. H^+ m^{-2}) *per* tissue according to Pearcy et al. (1991).

Light microscopy

Small samples of *O. streptacantha* seedlings, including epidermis, hypodermis, and chlorenchyma were collected and immediately fixed with cold 4 % glutaraldehyde in a phosphate buffer at pH 7.4, and dehydrated with graded ethanol series. Samples were hand-sectioned with a low profile microtome blade and used for light microscopy (Leica DM2000; Leica Microsystems Inc., Buffalo Grove, Il, USA) examination. Cell micrographs were obtained (LAS Imaging Software; Leica Microsystems Inc., Buffalo Grove, Il, USA) and digitized to determine the number of chloroplasts *per* cell and the cell cross-sectional area for every cell for five cells *per* sample. Chloroplasts were counted in one plane for each cell to estimate the number of chloroplasts on the top surface of each cell. Chloroplast density was calculated as the number of chloroplasts *per* 100 μm^2 of cell area, and the chloroplast frequency as the number of occasions that chloroplasts are observed *per* grid unit within the cell, divided by the total grid number.

Statistical analysis

A canonical discriminant analysis was selected to understand the complex relationship between three groups of variables [ecophysiological (Ψ_s and titratable acidity), anatomical (chloroplast frequency, chloroplast density, and cell area), and environmental (PPFD, air temperature, and soil water condition)] and the relative contribution of the original variables (within each group) to explain the effect of our experimental design.

Data were performed using a canonical discriminant analysis that allowed the identification of differences among the four combined treatments (SW, SNW, LW and LNW) mentioned previously, using data obtained from variables from individuals belonging to each group (Ψ_s, titratable acidity, chloroplast frequency, chloroplast density, and cell area) and by utilizing linear functions from the quantified variables. Then the groups of individuals were separated to maximize the variance among treatments and to minimize the variance within them. The standardized canonical coefficients show the contribution of each of the joint variables which were analyzed to each of the canonical functions, and individually indicate the relative importance of each variable (McGarigal et al., 2000; Pires Da Silva et al., 2001; Yáñez-Espinosa et al., 2006). In addition, a classificatory discriminant analysis was used to estimate the number and percent of entities classified correctly or incorrectly into each group of combined treatments. Both analyses were carried out using PROC CANDISC and PROC DISCRIM procedures.

The relationship among the three sets of variables (ecophysiological, anatomical, and environmental) was determined by performing a generalized canonical correlation analysis (Ortega-Alcalá et al., 1992). The goal of this analysis is to determine the relationships among more than two sets of variables, in order to measure the magnitude of the relationships and to delineate simple canonical correlation (McGarigal et al., 2000).

From the generalized canonical correlation results, a simple canonical correlation analysis was applied to the original variables related to their canonical variates (ecophysiological, anatomical, and environmental), arranged as two sets of plant and environmental variables. The goal of canonical correlation is to analyze the relationships between two sets of variables, and to elucidate the relationship between the two sets of variables of high dimension by generating two sets of low dimension. One set of variables consists of response variables and the other set consists of explanatory variables. A redundancy analysis was also performed to calculate the variance in a set of original variables explained by a canonical variate of another set (McGarigal et al., 2000). All statistical analyses were performed with SAS software (SAS Institute Inc.).

Results

Descriptive results are demonstrated below and after of this we showed the canonical discriminant analysis, which identify differences among the four combined treatments (SW, SNW, LW, and LNW) and all of the response variables that were analyzed.

Ecophysiological variables

Osmotic potential (Ψ_s)

In order to know the water status of *O. streptacantha* seedlings, the osmotic potential (Ψ_s) was evaluated. The highest Ψ_s (-0.29 ± 0.007 MPa) was found in seedlings in the shade – watering (SW) group for harvests after 15, 30, and 45 days, while the lowest Ψ_s (to -1.46 ± 0.01 MPa) was found in the light and non-watering (LNW) group. Seedlings in the shade and non-watering (SNW) group exhibited medium osmotic potential (between -0.77 and -1.0 MPa). In both non-watering treatments, there is a gradual decrease in osmotic potential which is more drastic for seedlings which were exposed to high sunlight (-1.46 ± 0.01MPa; Fig. 1a).

Titratable acidity content

In order to evaluate CAM activity, the malic acid content was measured. Seedlings in the water treatment groups had higher titratable acidity content, which was highest for plants exposed to direct sunlight, reaching a maximum of 120.53 ± 10.5 mmol equiv. H^+ m^{-2} after 45 days of treatment (Fig. 1b). In a contrary way, the acidity content for the non-watering treatment groups decreased significantly, particularly for the longer period of 45 days of water stress which had a titratable acid content of 32.66 ± 4.62 and 27.86 ± 4.00 mmol equiv. H^+ m^{-2} under high light and shade conditions, respectively (Fig. 1b).

Anatomical variables

Chloroplast density

As shown in Fig. 2a, the cells of seedlings from the non-watering treatments (LNW and SNW) have more chloroplasts than those from the watering groups (LW and SW). From the harvest at day 30, the highest number of chloroplasts was found in LNW (8.39 ± 1.10) and SNW (5.82 ± 0.48 chloroplasts *per* 100 μm^{-2}). However, at the highest water stress period of 45 days of non-watering, a decrease in the chloroplast number was observed (~5 chloroplasts *per* 100 μm^{-2}). Seedlings from the SW treatment have the lowest chloroplast density (1.80 ± 0.01 chloroplasts *per* 100 μm^{-2}).

Chloroplast frequency

The chloroplast frequency was higher in the LNW and SNW treatment groups (46.14 ± 8.21 and 39.97 ± 6.93 %, respectively) at day 15 than in the LW and SW groups. At days 30 and 45, the chloroplast frequency was higher (48.63 ± 10.00; 33.03 ± 0.76 %) in cells from the LNW group than from the other treatment groups, although the values were lower at day 30 than at the last harvest (Fig. 2b).

Cell size

Cell area of the seedlings was higher for the SW and SNW groups (~2000 μm^2) than for the LW group (1731.66 ± 542.77 μm^2) and LNW group (1467.22 ± 193.62 μm^2) at day 15, but it was highest for the SW treatment at day 45 (3319.44 ± 32.03 μm^2; Fig. 2c).

Chloroplasts arrangement

We found different chloroplast arrangements among the treatments in chlorenchyma cells. For cells from the watering treatment groups in both light and shade conditions, chloroplasts are always dispersed in the cytosol of the cells in the three harvests (Fig. 3a-f). The chloroplasts of cells from the LNW treatment group show a gradual aggregation toward the center of the cytosol for three harvests, but this effect is most pronounced for the last harvest (Fig. 4b, d, f). A similar response of chloroplast in cells from the SNW group is observed, but chloroplasts are less aggregated than cells of LNW treatment group (Fig. 4a, c, e). In this sense, a similar behavior of chloroplast arrangement is observed when the seedlings were exposed to non-watering treatments, particularly at 45 days of the SNW treatment in comparison to LNW treatment at 30 days.

Environmental variables

Photosynthetic photon flux density (PPFD)

Photosynthetic photon flux density (PPFD) received by *O. streptacantha* seedlings was ~1031 µmol m^{-2} s^{-1} for the light treatments and ~250 µmol m^{-2} s^{-1} for the shade treatments. Similar photo flux densities were observed in the three harvests (Fig. 5a).

Temperature

Temperatures for the four treatments were of ~39 °C in the light treatments for the three harvests, and ~33 °C for the shade treatments (Fig. 5b).

Canonical discriminant analysis

To understand which variables contributed to the responses of seedlings under the four combined treatments, we employed a canonical discriminant analysis and determined that three discriminant functions accounted for 99 % of the data set total variation, contributing significantly to the separation among treatments (Wilks' λ: $P < 0.0001$, $n = 36$). The first function (eigenvalue of 171.52; $P < 0.0001$)

explained 94.5 % of the total variation, the second (eigenvalue of 6.26; $P < 0.0001$) explained 3.5 % and the third (eigenvalue of 2.11; $P < 0.0003$) explained the remaining variation.

Variables that contributed significantly to the three canonical functions were present in the standardized canonical coefficients (Table 1), and those indicating the relative importance of each variable were Ψ_s in the first function, titratable acidity in the second function, and chloroplast density in the third function. The mean class variables for each treatment are given in Fig. 6.

The classificatory discriminant analysis showed that centroids of each group were significantly different ($P < 0.0001$). All of the observations were correctly classified for all treatments, except those for the LW-30 days and LNW-15 days treatments, of which 70 % of the observations were correctly classified. The remaining 30 % of the observations were classified as SW-30 days and SNW-45 days.

Canonical correlation analysis

The generalized canonical correlation revealed that there was only one canonical variate. Vector defining canonical variates to produce the vector of the first canonical variates are shown in Table 2.

The first eigenvalue ($\lambda_1 = 2.00$) accounted for most of the variance of the first canonical variate, indicating that the first canonical variate is generated by only one common factor. The eigenvalue indicated that the three sets of variables are highly correlated and all sets had similar weight in the determination of the first canonical variate. The second ($\lambda_2 = 0.68$) and third eigenvalues ($\lambda_3 = 0.29$) accounted for less of the variance. The first eigenvector elements ($e_1 = 0.63_{\text{ecophysiological}}$; $0.52_{\text{anatomical}}$; $0.56_{\text{environmental}}$) showed that the anatomical variables set had the lowest value among the ecophysiological and environmental sets. The results from the correlation matrix among canonical variates indicated that highest correlation between ecophysiological and environmental variates (0.63), followed by ecophysiological and anatomical (0.55), and the lowest between anatomical and environmental (0.32).

Table 3 shows correlations between the original variables and the canonical variates within sets. These correlation coefficients corroborated that Ψ_s and titratable acidity were highly correlated with ecophysiological (their first canonical) variate, which means that these variables were important in the resolution of the canonical variate. The highly associated variable with anatomical variables was chloroplast density. With respect to the environmental variables, water treatment in the harvests and PPFD were highly correlated. These were the variables selected for the canonical correlation analysis.

The first canonical correlation is 0.74 (74 % of variance, $P < 0.002$), however, the remaining correlation was not significant. Data for the first pair of canonical variates (cross-loadings) appear in Table 4, showing the correlations between the variables and the canonical variates, standardized canonical variate coefficients, with opposite-set variance accounted for by the canonical variate (% of variance), redundancies, and canonical correlations. Percentage of variance (97

%), redundancy (0.53), and canonical correlation (0.74) indicate that the first pair of canonical variates was highly correlated. The cross-loadings showed that Ψ_s was negatively correlated with PPFD, water treatment, titratable acidity, and chloroplasts density. In addition, titratable acidity was correlated with chloroplast density, and both variables were correlated with PPFD and water treatment (Table 4).

From the canonical correlation analysis, we found that SW-15, 30, and 45 days treatments were grouped with the highest values of osmotic potential and lower density of chloroplasts, while LNW-15, 30, and 45 days treatments showed the lowest values of osmotic potential and higher density of chloroplasts (Fig. 7).

Discussion

Opuntia plants tolerate drought stress by several anatomical, physiological, and biochemical mechanisms, all of which contribute to improved water-use efficiency (Nobel, 1988; North and Nobel, 1992; Pimienta-Barrios et al., 2002, 2003; Zañudo-Hernández et al., 2010). However, until this study there was no information related to *Opuntia* seedlings mechanisms in arid environments, particularly during early stages of growth.

In order to examine the effects of these factors, we simulated shade under nurse plants as well as light in open spaces, both under conditions of drought and rainfall, in *O. streptacantha* seedlings. In this study, ecophysiological variables are the most important for *O. streptacantha* seedling establishment, followed by anatomical and environmental variables. In terms of seedling biology, ecophysiological variables such as Ψ_s and titratable acidity are very important for CAM plants, because acidity is regulated mainly by the water status of the plant (Hanscom and Ting, 1978; Cushman, 2001; Cushman and Borland, 2002; Winter et al., 2005). In this case, we found that Ψ_s of *O. streptacantha* seedlings is negatively correlated with titratable acidity. Interestingly, the Ψs was not particularly low (-1.5 MPa) for seedlings in the most stressful treatment (light and non-watering) at 45 days. This suggest that higher mucilage content in the *Opuntia* cells help to maintain Ψ_s, thus favoring water retention (Nobel et al., 1992). Our results are similar to findings for adult *Opuntia* plants, in which acidity content and Ψ_s decrease in cladodes under drought conditions (Acevedo et al., 1983; Goldstein et al., 1991; Snyman, 2004; 2006). The importance of water status for the establishment of *O. streptacantha* seedlings is also in agreement with studies on other cactus seedlings (Franco and Nobel, 1988, Gallardo-Vasquez and De la Barrera, 2007).

The causes of nurse-seedling relationships can vary between species and environments, and it is possible that multiple causes produce this association, such as seed trapping, nutrient, moisture, protection from browsing or trampling and support availability. Shade and higher soil humidity under nurse plants are two important environmental factors facilitating seedling establishment (Flores and Jurado, 2003). The results clearly show that seedlings under shade can survive for a longer time in the dry season because the water status and the CAM activity did not decrease as rapidly as in high light conditions; this protection under nurse plants slows the effects of stress. Thus, *Opuntia* seedlings appear to have adapted to the protection of the shade of nurse plants, similar to findings for other cacti (Gallardo-Vasquez and De la Barrera, 2007; Hernández-González and Briones-Villarreal, 2007).

The chloroplasts in cells of many plants can be displaced and rearranged in accordance with the incidence of PPFD, with chloroplasts exhibiting maximum surface area at low PPFD and aggregated under high PPFDs (Powles, 1984; Nauš et al., 2008). Light-induced chloroplast movement is an adaptive response (Kagawa et al., 2001), because the aggregation of chloroplasts tends to avoid photoinhibition and/or to better distribute or utilize the penetrating light in the plant leaf tissue (Königer et al., 2008; Yamada et al., 2009). We suggest that *Opuntia* seedlings established in open, highly illuminated spaces show chloroplasts movement in order to tolerate light and/or water stress. In this study, we found that chloroplasts are highly clumped in the center of the cell under high solar radiation and drought, which is in agreement with findings that chloroplast aggregation occurs under combined light and water stress (Kondo et al., 2004; Königer et al., 2010). This behavior is also observed under shade and drought treatment, although to a lesser degree, chloroplasts also aggregate. Indeed, chloroplast arrangement is similar for LNW treatment at day 30 and SNW treatment at day 45. Seedlings from these treatments have similar Ψ_s.

In field conditions, *O. streptacantha* seedlings are primarily found in shade under nurse plants (Janzen, 1986, Yeaton and Romero-Manzanares, 1986; Vargas-Mendoza and González-Espinoza, 1992). Hence, we suggest that the chloroplasts of seedlings under nurse plants move to maximize photosynthesis, because light coming through the canopy provided by nurse plants is weak, diffuse, and transient owing to the movement of the sun, as proposed by Park et al. (1996) for shade plants. Interestingly, seedlings under no watering treatments, both in light and shade, show higher density of chloroplast, contrary to that described for other plants in which the number of chloroplasts decreases under water stress or when exposed to intense light (Maxwell et al., 1999; Rizhsky et al., 2003; Nagy-Déri et al., 2011). This could be a mechanism used by *O. streptacantha* seedlings to maintain photosynthetic activity under stress conditions.

In drought condition with lower Ψ_s, chloroplast clumping was also correlated with high chloroplast density and titratable acidity. Chloroplasts move towards a position of higher CO_2 concentration for efficient photosynthesis (Sato and Kadota, 2007), because higher concentration of CO_2 in CAM plants is found in the vacuole

(Borland et al., 2009), and perhaps chloroplasts, being closer to the vacuole, have easier access to stored malic acid.

In conclusion, there are clearly complex interactions between drought and light that promote different ecophysiological and anatomical adaptations, which have direct consequences in the seedling establishment under nurse plants or in open spaces. Ecophysiological variables are the most important for *O. streptacantha* seedlings, followed by anatomical and environmental variables. Ecophysiological variables are more sensitive to environmental conditions, and these would be mainly affecting anatomical variables, but at the same time, anatomical variables are affected in less degree by environmental conditions that ecophysiological variables. We found that *O. streptacantha* seedlings establish well in exposed sites with watering, but these conditions are not so common in arid and semi-arid environments. Thus, these plants can tolerate high-light and drought in open spaces by increasing and moving chloroplasts and also avoiding drastic decrease in their osmotic potential, although they could suffer damage in longer water stress periods. Higher soil humidity under nurse plants could be longer during the year than in open sites, for this reason, *O. streptacantha* seedlings establish better in conditions simulating microhabitat under the shade of nurse plants with high soil humidity in the rainfall period, having higher Ψ_s and titratable acidity, as well as dispersed (unaffected) chloroplasts. All of these responses help *O. streptacantha* seedlings to tolerate drought and high-light in arid environments. This tolerance could have important implications for predicting the impact of climate change on natural desert regeneration, as well as for planning reforestation-afforestation practices, and rural land uses.

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Table 1. Total sample canonical coefficients.

	Function coefficients		
Variables	Function 1	Function 2	Function 3
Osmotic potential (Ψ_s)	10.78*	-2.63	1.16
Titratable acidity	0.13	3.39*	-0.68
Chloroplast frequency	0.05	-0.32	-0.70
Chloroplast density	0.11	0.39	1.80*
Cell area	0.26	0.53	1.40

* Characteristics with a high contribution to centroid separation among treatments

Table 2. Results from the first canonical variates vector.

Sets	Original variables	Canonical variates
Ecophysiology	Osmotic potential (Ψ_s)	-1.55
	Titratable acidity	0.95
Anatomy	Chloroplast frequency	-0.73
	Chloroplast density	1.32
	Cell area	0.20
Environment	PPFD	0.57
	Air temperature	0.01
	Watering treatment	0.78

Table 3. Correlation between original and canonical variables within groups.

Sets	Original variables	Canonical variates
Ecophysiology	Osmotic potential (Ψ_s)	-0.79
	Titratable acidity	-0.26
Anatomy	Chloroplast frequency	0.04
	Chloroplast density	0.80
	Cell area	-0.14
Environment	PPFD	0.65
	Air temperature	0.33
	Watering treatment	0.78

Table 4. Canonical cross-loadings of the first pair of canonical variables.

		First Pair of Canonical Variates	
		Correlation	Coefficient
Mixed*	Osmotic potential (Ψ_s)	-0.46	-1.59
	Titratable acidity	0.18	1.42
	Chloroplast density	0.25	-0.02
Environmental	PPFD	0.59	0.77
	Watering treatment	0.46	0.59

* Anatomical and ecophysiological variables

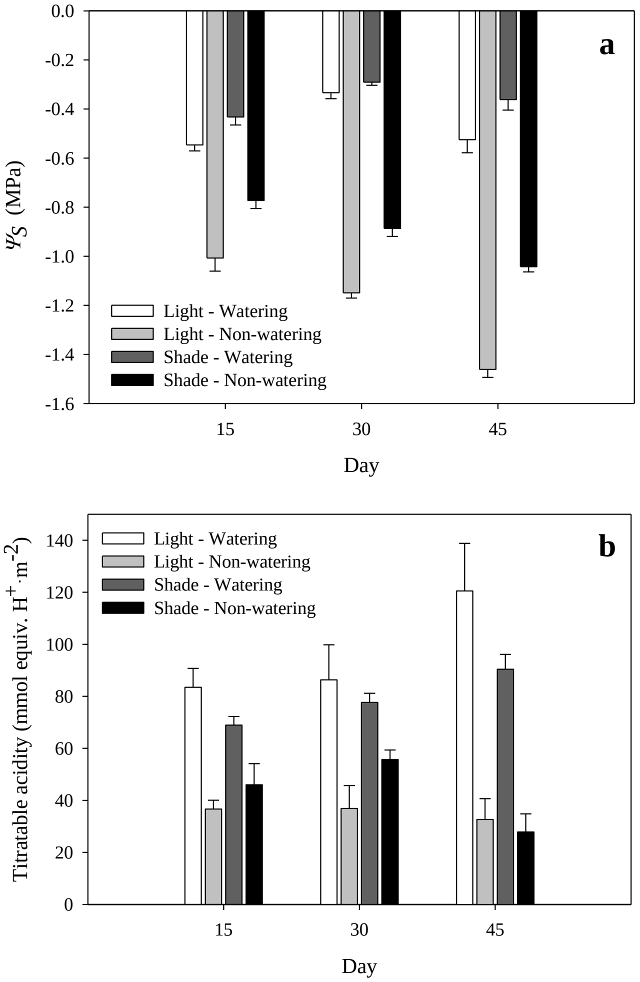

Figure 1. Responses of *Opuntia streptacantha* seedlings under combined water and light treatments at 15, 30 and 45 days. a) osmotic potential (MPa) and, b) titratable acidity (mmol equiv. $H^+ \cdot m^{-2}$). Bars represent the means ± SE (n = 3).

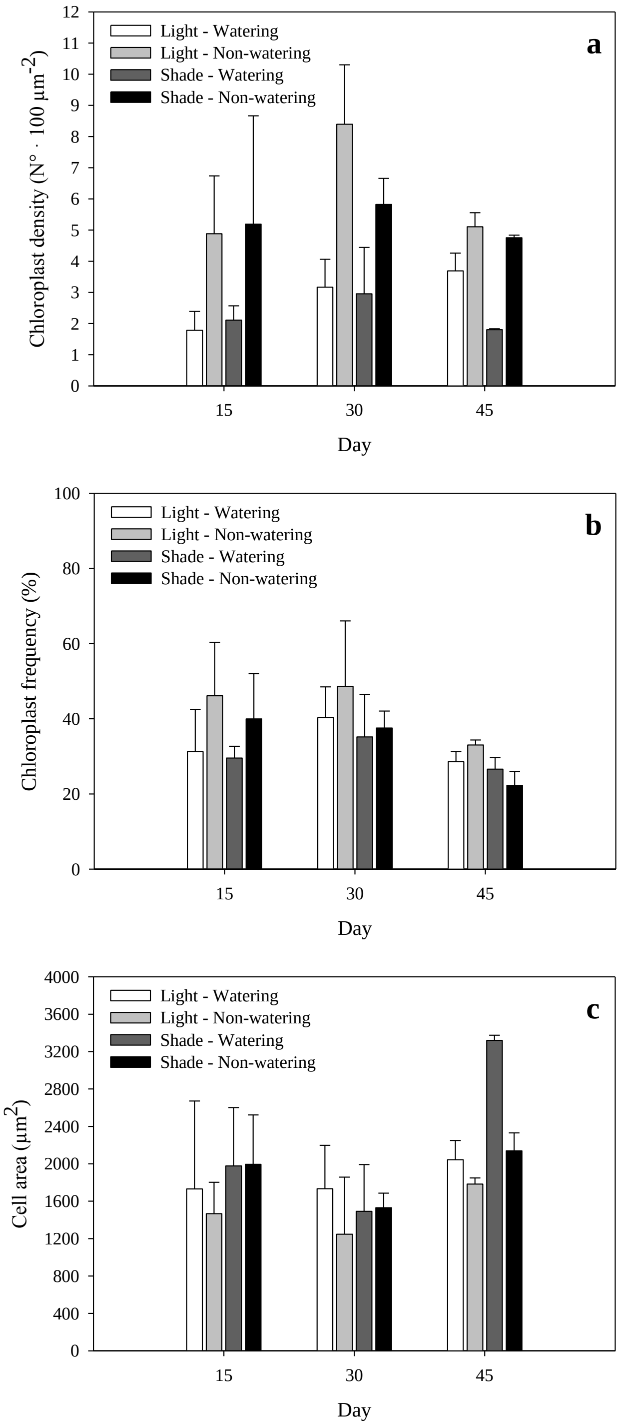

Figure 2. Responses of *Opuntia streptacantha* seedlings under combined water and light treatments at 15, 30 and 45 days. a) chloroplast density (N° · 100 µm^{-2}); b) chloroplast frequency (%) and, c) cell area (µm^{2}). Bars represent the means ± SE (n = 3).

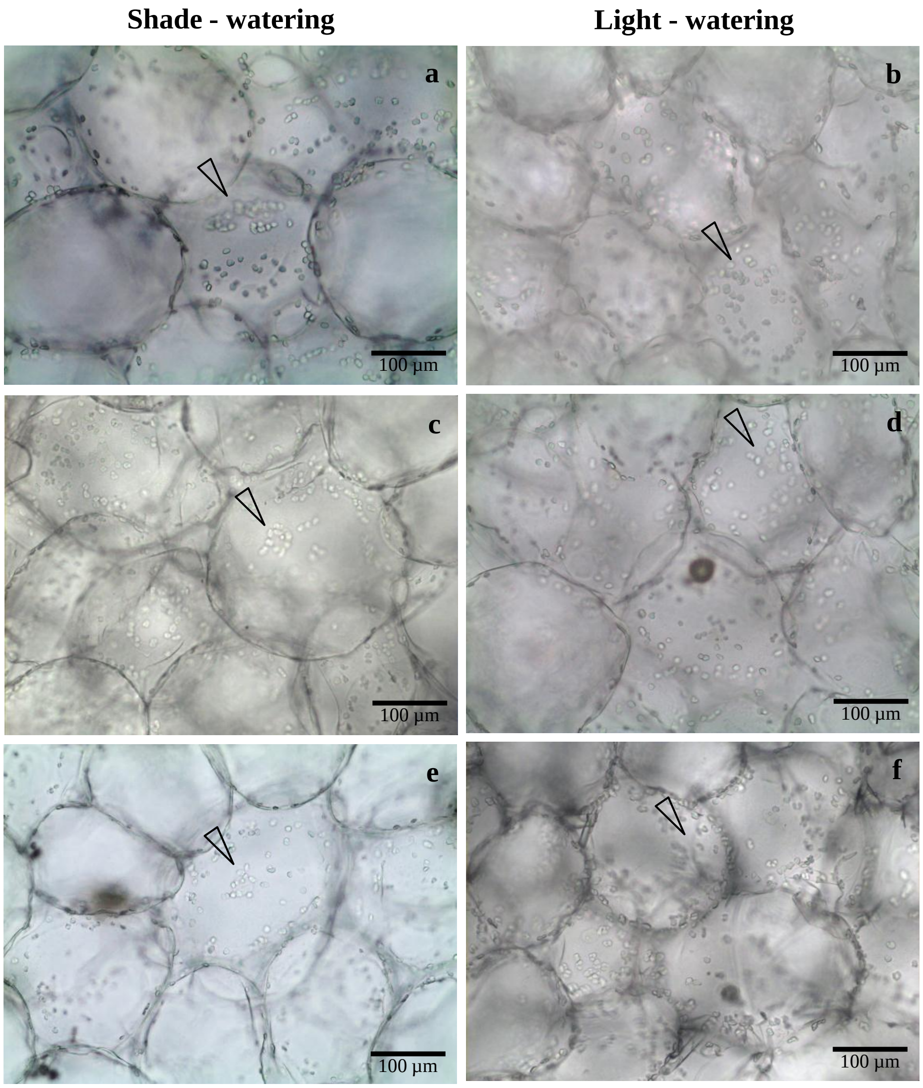

Figure 3. Chloroplasts arrangement along cell walls of *Opuntia streptacantha* seedlings under shade – watering (SW) and light – watering (LW) treatments. SW (a, c, e) and LW (b, d, f) at 15, 30 and 45 days, respectively. An arrow indicates the chloroplasts arrangement.

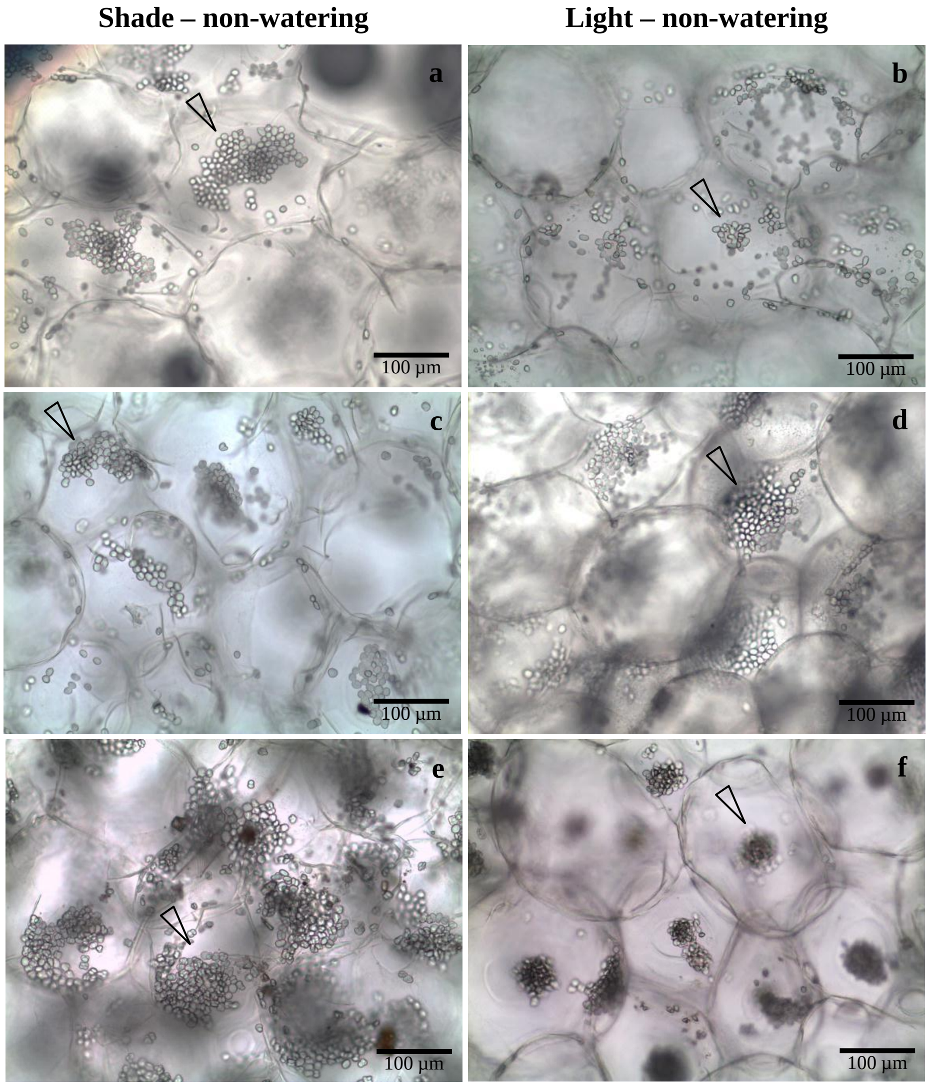

Figure 4. Chloroplasts arrangement of *Opuntia streptacantha* seedlings under shade – non-watering (SNW) and light – non-watering (LNW) treatments. a) SNW at 15 days, chloroplasts clumped in one area of the cytosol (arrow); b) LNW at 15 days, chloroplasts clumped in the peripheral cytosol (arrow); c) SNW at 30 days, chloroplasts clumped in one area of the cytosol (arrow); d) LNW at 30 days, chloroplast clumped located close together at adjoining cells (arrow); e) SNW at 45 days, chloroplasts clumped in one area of the cytosol (arrow) and f) LNW at 45 days, chloroplasts densely clumped in the center of the cytosol (arrow).

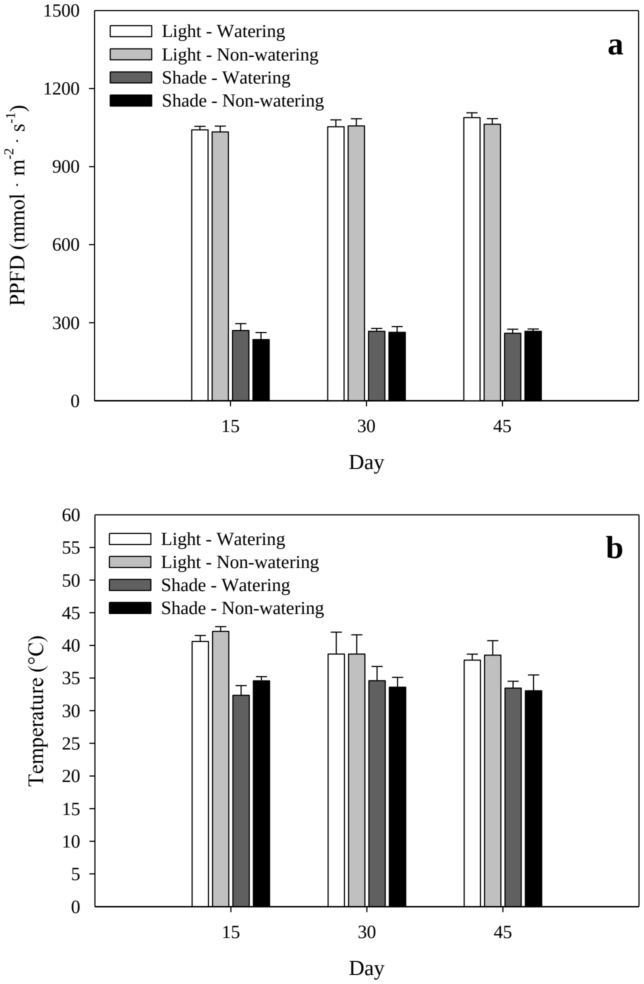

Figure 5. Environmental conditions that receive *Opuntia streptacantha* seedlings under combined water and light treatments at 15, 30 and 45 days. a) photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) and, b) temperature (°C). Bars represent the means ± SE (n = 3).

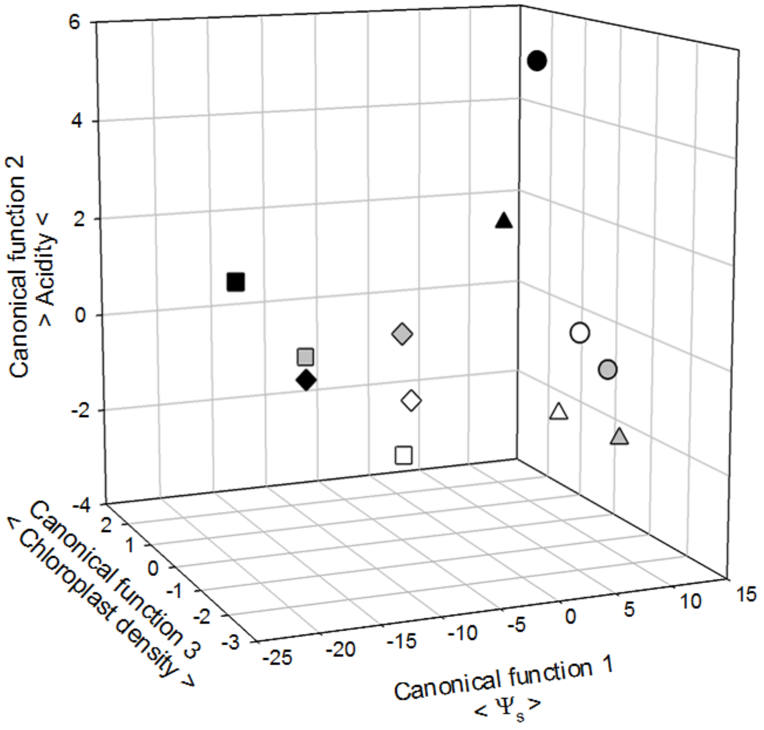

Figure 6. Ordination of treatments using three canonical discriminant functions by mean class variables. ○) Light – Watering (LW) at 15 d; ◍) LW at 30 d; ●) LW at 45 d; □) Light – Non-watering (LNW) at 15 d; ▣) LNW at 30 d; ■) LNW at 45 d; Δ) Shade – Watering (SW) at 15 d; ◬) SW at 30 d; ▲) SW at 45 d; ◊) Shade – Non-watering (SNW) at 15 d; ◈) SNW at 30 d; and ♦) SNW at 45 days of treatment.

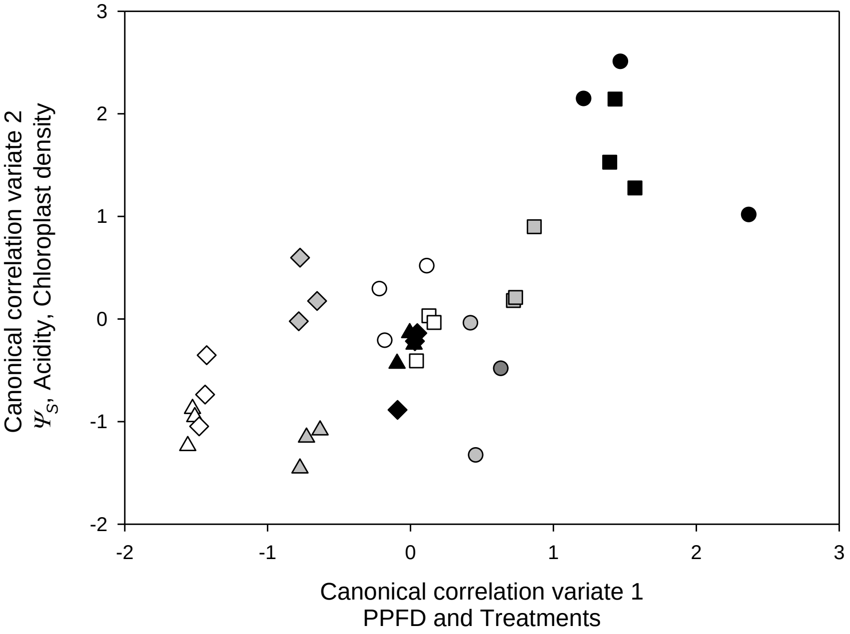

Figure 7. Scatter plot showing the relationship between the first pair of canonical variates canonical correlation. ○) Light – Watering (LW) at 15 d; ◍) LW at 30 d; ●) LW at 45 d; □) Light – Non-watering (LNW) at 15 d; ▣) LNW at 30 d; ■) LNW at 45 d; Δ) Shade – Watering (SW) at 15 d; ◬) SW at 30 d; ▲) SW at 45 d; ◊) Shade – Non-watering (SNW) at 15 d; ◈) SNW at 30 d; and ♦) SNW at 45 days of treatment.

CAPÍTULO V

How nurse plants help to seedlings to tolerate water and light stress in semiarid environments? Ecophysiological and molecular changes in *Opuntia streptacantha* seedlings

Abstract

Arid and semiarid environments are characterized by limited and variable rainfall during the year. Nevertheless, in these environments nurse plants play an important role in the establishment of *Opuntia streptacantha* seedlings, avoiding the desiccation and facilitating their establishment during the dry season. In this study, we simulated the nurse plants effect in greenhouse and evaluated three types of response variables ecophysiological [osmotic potential (Ψ_s), relative growth rate (RGR) and their components, photosynthetic pigments, carotenoids, and titratable acidity], molecular [metallothionein (*OpsMT*), lipid transfer protein (*OpsLTP*), dehydrin (*OpsDHN*) and aquaporin (*OpsAQP*)], and environmental [photosynthetic photon flux density (PPFD), air temperature, and soil water availability] in *O. streptacantha* seedlings under four treatments (shade – watering, shade – non-watering, light – watering, and light – non-watering) by 45 days. Our results emphasizes that firstly the molecular responses (*OpsMT* and *OpsLTP* up-expression) helped to tolerate the water deficit condition and high light radiation. In addition, the water availability is an important factor, because the seedlings in drought treatments are affected in their *Ψs*, acidity content, total chlorophylls, and chlorophylls/carotenoids ratio, affecting the seedling development. We conclude that *O. streptacantha* seedlings under nurse plants can tolerate stress conditions for a longer time than in open spaces, responding mainly with changes in the molecular architecture and after with ecophysiological changes. This is the first time that both gene expressions and ecophysiological responses are studied together to determine mechanisms behind the nurse effect on seedling performance, which is also crucial to solving natural desert regeneration problems.

Keywords: CAM plants; abiotic stress; ecophysiology; metallothionein protein; lipid transfer protein.

Introduction

Cactus seedlings often establish under nurse plants which modify environmental conditions by increasing moisture and decreasing solar radiation, which may cause beneficial and detrimental effects, respectively, on seedling growth (Franco and Nobel 1989; Flores and Jurado 2003; Flores et al. 2004). Thus, environmental conditions differ between open and under nurse plants microsites, and act in concert to determine plant growth and survival. However, the mechanisms behind the nurse effect on growth response are not enough known. For example, there are studies about the relative growth rate (RGR) and root:shoot ratio (R/S) on cactus seedlings in microhabitats under nurse plants, with lowest RGR (Martínez-Berdeja and Valverde 2008) and highest R/S (Miquelajauregui and Valverde 2010) at lower soil moisture. Nevertheless, there are no studies about the morphological and physiological components of the RGR. The changes in seedling biomass through time –*i.e.* the relative growth rate (RGR) – can be broken down into the morphological components [the specific leaf area (SLA), which indicates the plant investment in leaves or other photosynthetic structures of the plants, and the leaf weight ratio (LWR), which measures the allocation of biomass to leaves *vs*. other plant parts], and a physiological component, the net assimilation rate (NAR) or plant mass increment per leaf area or photosynthetic area (Poorter 1999; Shipley 2002).

In arid environments, drought and high solar radiation can modulate seedling performance or even survivorship (Flores et al. 2004; Martínez-Berdeja and Valverde 2008; Miquelajauregui and Valverde 2010). Drought is buffered under the shade of nurse plants, but the associated decrease in solar radiation (and the concomitant reduction in photosynthetic rate) represents an additional challenge for growth. Shading by the nurse plant decreases the photosynthetic photon flux density (PPFD) available for the associated seedling, which would limit its CO_2 uptake if any of the photosynthetic surfaces are below the PPFD saturation level, as is the case for several desert CAM plants (Woodhouse et al. 1980; Nobel 1986; Franco and Nobel 1989).

In another hand, nocturnal acid accumulation (CAM activity) decrease for exposed than for shaded cactus seedlings. Thus, the presence of CAM in shaded cactus seedlings increases water-use efficiency (WUE) and reduces the risk of photoinhibition (Gallardo-Vasquez and De la Barrera 2007; Hernández-González and Briones-Villarreal 2007). It could be that also the concentrations of photosynthetic pigments decrease under full sunlight in cactus seedling, as found for adult cacti (Raveh et al. 1998), as well as for other adult CAM plants (Adams III et al. 1987). It could also be that carotenoids (pigments involved in photoprotection and photorepair) increase under full sunlight, avoiding changes in the photosystems (Osmond et al. 1994, 1999; Förster et al. 2005, 2009). In addition, carotenoids (xanthophyll pigments like violaxanthin, antheraxanthin, and zeaxanthin) transfer energy to the photosynthetic reaction centers as accessory pigments to chlorophylls, and they also play a central role in the transformations of the photosynthetic apparatus, especially in thermal energy dissipation and detoxification of reactive oxygen species caused by the high radiation (Osmond et al. 1999; Förster et al. 2005; Takahashi and Badger 2011).

A few studies have explored the genetic basis of interactions among nurse and protégé plants (Michalet et al. 2011), because the complex plant response to abiotic stress also involves many genes and biochemical-molecular mechanisms (Wang et al. 2003; Shao et al. 2006, 2007). The ongoing elucidation of the molecular control mechanisms of abiotic stress tolerance is based on the expression of specific stress-related genes. These genes include three major categories: (i) those that are involved in signaling cascades and in transcriptional control, such as MyC, MAP kinases and SOS kinase, phospholipases, and transcriptional factors such as HSF, and the CBF/DREB and ABF/ABAE families; (ii) those that function directly in the protection of membranes and proteins, such as heat shock proteins (Hsps) and chaperones, late embryogenesis abundant (LEA) proteins, osmoprotectants, and free-radical scavengers; (iii) those that are involved in water and ion uptake and transport such as aquaporins and ion transporters (Wang et al. 2003). Thus, identify genes related with mechanism behind the nurse effect is very relevant.

In this study the impact of combined drought and high-light was examined on *O. streptacantha* seedlings. *Opuntia streptacantha* Lem (Prickly Pear) is a perennial arborescent cactus of economical interest (it has edible fruits and young pads) distributed on the semiarid lands of central Mexico (Littlejohn Jr. and Williams III 1983; Winter and Lesch 1992). We assessed three types of response variables: ecophysiological (Ψ_s, RGR and their components, photosynthetic pigments, carotenoids, and titratable acidity), molecular [metallothionein (*OpsMT*), lipid transfer protein (*OpsLTP*), dehydrin (*OpsDHN*) and aquaporin (*OpsAQP*)], and environmental (PPFD, air temperature, and soil water availability in the harvests).

Research into the plant responses to drought is becoming increasingly important, as climate change scenarios suggest an increase in aridity in many areas of the world (IPCC 2007). Thus, understanding how water and light availabilities influence the molecular and ecophysiological responses of *Opuntia* seedlings is crucial in understanding the mechanisms behind the nurse effect, as well as in predicting the impact of climate change on natural desert regeneration.

Materials and methods

Study design

For the experiments, *Opuntia streptacantha* seeds were collected in Mexquitic de Carmona, San Luis Potosi, Mexico (22º16' N, 101˚07' W at 2,020 m asl), at Southern Chihuahuan Desert, in 2007. Seeds were germinated and the seedlings were watered constantly. Seedlings of four month-old were placed in four treatments with 25 seedlings by treatment: shade (25% light) and watering (SW), shade and non-watering (SNW), light (100% light) and watering (LW) and in light and non-watering (LNW). The non-watering treatments represented dry times without rain and treatments with watering were performed for simulated the wet season. Treatments with high solar radiation simulated the solar radiation of open spaces without nurse plants and shaded treatments represented the low solar radiation (≈ 50 % of light reduction with a plastic film mesh to create shade) found under nurse plants. All these treatments permitted to get the ecophysiological and molecular expression of the plants under contrasting environmental conditions.

Three harvests were done fortnightly. The following parameters were evaluated: ecophysiological (Ψ_s, RGR, NAR, SLA, LWR, LAR, chlorophyll *a* and *b*, total chlorophyll, carotenoids, chlorophylls/carotenoids ratio, and titratable acidity), and molecular (*OpsMT*, *OpsLTP*, *OpsDHN*, and *OpsAQP* genes). Photosynthetic photon flux density (PPFD) was measured using a portable fluorometer (pulse-amplitude modulated photosynthesis yield analyzer, Mini-PAM; Walz GmbH, Effeltrich, Germany).

Osmotic potential (Ψs)

Water status measurements were realized at predawn. The experimental unit was one seedling *per* pot to obtain enough sap extract. In order to evaluate Ψs, three harvests (three pots *per* harvest) were done. *Ex situ* determinations were made of the Ψs of the sap obtained by mechanical compression (Pearcy et al. 1991). Ψs was registered in C-52 sampling chambers connected to a Wescor HR-33T dew-point microvoltmeter (Wescor Inc., Logan, Utah, U.S.A.).

Photosynthetic area

To determine the photosynthetic area of *Opuntia streptacantha* stems, three seedlings were collected at 15, 30 and 45 days in the four treatments-watering combination of light (LW, LNW, SW and SNW). Seedlings were scanned with an HP 2400 ScanJet scanner. The images were analyzed with the software ImageJ 1.40g (Wayne Rasband National Institutes of Health, USA).

Seedling growth

To determine *O. streptacantha* seedling growth in the four treatments, we measured the relative growth rate (RGR) and its components, as well as the root:shoot ratio ($\text{Weight}_{root}/\text{Weight}_{shoot}$). Three seedlings were yielded at 15, 30, and 45 days in the four combined treatments. The calculations were realized using the formula Relative growth rate (RGR) = Net assimilation rate (NAR, g cm^{-2} day^{-1})

* Specific leaf area (SLA, cm^2 g^{-1}) * leaf weight ratio (LWR, g g^{-l}), following Shipley (2000). NAR is a physiological component because it is a measure of whole-plant daily net photosynthetic rate weighted by the rate of change in plant carbon content (Poorter and van der Werf 1998; McKenna and Shipley 1999). SLA is a morphological component because it is determined by leaf dry matter concentration and leaf thickness (Witkowski and Lamont 1991; Shipley 1995, 2002). LWR measures the allocation of biomass to leaves or photosynthetic organs *vs.* other plant parts (Shipley 1995, 2002). Leaf area ratio (LAR, cm^2 g^{-1}) is a morphological component, and is the result of SLA * LWR (Shipley 2002). In addition, due R/S is a ratio, it does not have units; in this case, a value near unity is an indication that the plant allocates similar amounts of biomass to roots and to aerial parts (Miquelajauregui and Valverde 2010).

Pigments content

To determine the concentrations of chlorophylls *a*, *b*, and total, as well as carotenoids, and total chlorophylls/carotenoids ratio, samples were collected from three individuals of each treatment at 7:00 am, at days 15, 30 and 45. The pigments were extracted according to the procedure of Hendry and Price (1993). Samples of seedlings (50 to 100 mg fresh weight) were taken and transported in cold 2 mL Eppendorf tubes, filling them in the light, the tissue was macerated with 2 mL of acetone to 80% in extracts at cold and the absorbance was determined at 645 and 663 nm for chlorophylls *a* and *b*, and at 470 nm for total carotenoids, using a spectrophotometer (Thermo Scientific ®, Model Spectronic Genesys 10 Bio).

Titratable acidity content

The malic acid content of seedlings was determined at 6:00 a.m., when its concentration is highest (Nobel, 2010). Transverse samples of plant tissue were obtained from seedling shoots using a steel borer of cross section 0.01 to 0.02 m^2. The plant material was sectioned and preserved in ethanol (80 %) in 1.5 mL Eppendorf tubes. Titration was carried to neutrality with a 0.01 N NaOH solution to determine the malic acid concentration (mmol equiv. H^+ m^{-2}) *per* tissue according to Pearcy et al. (1991).

RT-PCR Analyses of genes expressed

To study gene expression of abiotic stress response in *O. streptacantha* seedlings subjected to four treatments combined, first used the SuperScript II Kit ™ First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) following the protocol recommendations included in the kit. It was added 1 mL of cDNA in each PCR reaction of 25 mL, using specific primers to amplify the actin gene as an internal control of loading. Specific primers were designed for four genes of *O. streptacantha* by PrimerSelect program of Lasergene (DNASTAR), these primers were genes for metallothionein (*OpsMT*) Forward 5’-CCGTTACATCTCAGAAAATGTC-3’ and Reverse 5’-TCATTTGCAGTTACATGGGTTG-3’, lipid transfer protein (*OpsLTP*) Forward 5’-CAGAATGGCTAGCTCAGCGGTT-3’ and Reverse 5’-CATCTAGTTCACCTTGGAGCAGTC, dehydrin (*OpsDHN*) Forward 5’-

GATCGCGGACTGTTCGACTT-3’ and Reverse 5’-GTCGGAACGATGGAGCTTGT-3’, and aquaporin (*OpsAQP*) Forward 5’-AGAGAGGTCATCATGTCCAAG-3’ and Reverse 5’-ATGTATGCGCTGGTGCTAGTG-3’ which were previously identified in a cDNA *O. streptacantha* library obtained in our work group. All amplified fragments corresponded to the expected size based on the primers design. The expression levels of genes in each sample of *O. streptacantha* were calculated based on the intensity of the bands by the analysis software Quantity One 1-D 4.5 (BIO-RAD, Hercules, CA, USA), carrying out a normalization process through the expression of an actin gene . The program for the amplification conditions was: an initial cycle of 95 ° C for 5 min, followed by 95 ° C for 30 s (denaturation), 60 ° C for 45 s (alignment), 72 ° C for 1.5 min (extension), between 25 or 30 cycles, depending on the transcript analysis. Amplified products were separated by electrophoresis on agarose gels with 1% 1X TAE stained with EtBr and photodocumented.

Statistical analysis

Data were performed using a canonical discriminant analysis that allowed to identify differences among four combined treatments (SW, SNW, LW and LNW), using the measurements of variables from individuals belonging to each group; ecophisiologycal (Ψ_s, RGR, NAR, SLA, LWR, LAR, R:S ratio, chlorophylls *a* and *b* and total, carotenoids, chlorophylls/carotenoids, and titratable acidity), molecular (*OpsMT*, *OpsLTP*, *OpsDHN*, *OpsAQP*) and environmental (PPFD and soil water condition) and estimating linear functions from the quantified variables, and then separating the groups of individuals maximizing the variance among treatments and minimizing the variance within them.

Some variables showed collinearity (chlorophylls *a* and *b*, carotenoids, NAR, SLA, LWR, and LAR); therefore, they were not included in the canonical analysis. The standardized canonical coefficients show the contribution of the joint variables analyzed for each canonical function, and individually indicate the relative importance of each variable (McGarigal et al. 2000; Pires Da Silva et al. 2001; Yáñez-Espinosa et al. 2006). In addition, a classificatory discriminant analysis was applied to provide the number and percent of entities classified correctly or incorrectly into each group of combined treatments. Both analyses were carried out using PROC CANDISC and PROC DISCRIM procedures.

Subsequently, we performed a generalized canonical correlation. The goal of this correlation is to analyze the relationships among more than two sets of variables, in order to measure the intensity of the relationships and to delineate strategies of simple canonical correlation (McGarigal et al. 2000). In the method of maximum variance, the first vector of canonical variates presents the maximum variance of the first eigenvector, corresponding to maximization of the first eigenvalue of the vector of the canonical variates' correlation matrix (Yáñez-Espinosa et al. 2006). The relationship among the three sets of variables, ecophysiological (Ψ_s, RGR, total chlorophylls, chlorophylls/carotenoids ratio, and titratable acidity), molecular (*OpsMT*, *OpsLTP*, *OpsDHN* and *OpsAQP*) and environmental (PPFD, and soil water condition), was determined by the generalized canonical correlation analysis.

From the generalized canonical correlation results, a simple canonical correlation analysis was applied to the original variables more related to their canonical variates (ecophysiological, molecular, environmental), arranged in two sets, one for plant and the other for environmental variables. The goal of canonical correlation is to analyze the relationships between two sets of variables, and to elucidate the relationship between the two sets of variables of high dimension by generating two sets of low dimension. One set of variables may be response variables and the other set explanatory variables, like in a multiple regression, except that there are several variables in both sides of the equation. Sets of variables on each side are combined to produce a predicted value that has the highest correlation with the predicted value on the other side (McGarigal et al. 2000).

A redundancy analysis was also performed to calculate the variance in a set of original variables explained by a canonical variate of another set (McGarigal et al. 2000).

Results

Figures 1-4 show the results for each response variables.

Canonical discriminant analysis

The canonical discriminant analysis identified differences among the four combined treatments (SW, SNW, LW, and LNW) and all response variables that were analyzed. It showed that two discriminant functions explained 94 % of the total variation, contributing significantly to the separation among treatments (Wilks' λ: $P < 0.0001$, $n = 36$). The first function (eigenvalue of 1919.35; $P < 0.0001$) explained 88 % of the total variation and the second (eigenvalue of 148.94; $P < 0.0001$) explained 6 %.

Variables that contributed significantly to the first canonical functions are present in the standardized canonical coefficients (Fig. 5, Table 1), and those indicating the relative importance from each variable were *OpsLTP*, *OpsMT*, total chlorophylls/carotenoids, Ψ_s, total chlorophylls, and titratable acidity.

The classificatory discriminant analysis showed that centroids of each group were significantly different ($P < 0.0001$). All of the observations were correctly classified for all treatments.

Canonical correlation analysis

The first canonical correlation was 0.93 (85 % of variance, $P < 0.0001$), however the remaining correlations were no significant. Data on the first pair of canonical variables (cross-loadings) appear in Table 2 showing the correlations between the variables and the canonical variables, standardized canonical variable coefficients, with opposite-set variance accounted for by the canonical variable (% of variance), redundancies and canonical correlations. Percentage of variance (56 %), redundancy (0.30), and canonical correlation (0.93) indicate that the first pair of canonical variables was highly related. The cross-loadings showed that environmental conditions were positively associated with *Opuntia streptacantha* variables (Table 2).

With the canonical correlation analysis, we found treatment of SW at 15, 30, and 45 days had the highest osmotic potential, chlorophylls content, acidity, and chlorophylls/carotenoids ratio, and the lowest *OpsLTP* and *OpsMT* expression, while contrary results were found for both drought treatments (Fig. 6).

Discussion

Shade and higher soil humidity under nurse plants are two important environmental factors helping seedling establishment in arid environments (Callaway 1995; Flores and Jurado 2003). However, the mechanisms behind the nurse effect on growth response are not enough known. The present study reveals that molecular variables (the induction in the expression of *OpsLTP* and *OpsMT*) were the most important responses for *O. streptacantha* seedlings under combined treatments, especially under water and light stress. Plant lipid transfer proteins (LTPs) are ubiquitous lipid-binding proteins that are characterized by their ability to act as intracellular lipid transporters between the organelles in *in vitro* plant cells (Jung et al. 2003, 2005). These LTPs are involved in several abiotic stress responses in crop plants (Plant et al. 1991; Hwang et al. 2005; Oshino et al. 2007; Choi et al. 2008) and in plants of cold environments as *Bromus inermis* (Wu et al. 2004). For native desert plants there are few research, *i.e.* the non-succulent plants *Prosopis juliflora* and *Tamarix hispida* registered up-regulation of *LTPs* genes under conditions of drought and oxidative stress (George et al. 2007, 2010; Wang et al. 2009).

MTs are small cysteine rich proteins that range in size from 4 to 8 kDa and bind several metals like copper, cadmium, zinc, nickel, etc. (Hamer 1986). Recently, several results have indicated MTs play important roles in abiotic stress tolerance as a ROS scavenger (Akashi et al. 2004; Wong et al. 2004; Yang et al. 2009), and act as possible cellular redox sensors (Fabisiak et al. 2002). Severe drought stress impairs the function of cellular ions, transporters and membrane-associated enzymes, leading to generation of ROS subsequently (Mahajan and Tuteja 2005). We suggest that *OpsMT* is involved in the elimination of ROS caused by water and high light stresses in which *O. streptacantha* seedlings were subjected.

Inside ecophysiological variables, Ψ_s was an important response variable, which confirms that water availability is the factor controlling the activity and responses of the plant (Cushman 2001; Cushman and Borland 2002; Winter et al. 2005; Winter et al. 2011). *Opuntia* plants tolerate drought, displaying water use efficiency in adult plants (Nobel 1988; North and Nobel 1992; Pimienta-Barrios et al. 2002, 2003; Zañudo-Hernández et al. 2010), but there is no information about *Opuntia* seedlings from arid environments under drought, just for *Opuntia elatior* from tropical regions (Winter et al. 2011). We found that *O. streptacantha* seedlings had lower Ψ_s under drought and high solar radiation than under watering and shade. Thus, *Opuntia* seedlings appear to be adapted to establish under the shade of nurse plants.

Owing the plants need CO_2 up-take for the photosynthesis process, the acidity content is very important for CAM plants, however acidity is regulated mainly by the water status of the plant (Hanscom and Ting, 1978; Cushman, 2001; Cushman and Borland, 2002; Winter et al., 2005). Our results clearly show that seedlings under shade can survive for a longer time in the dry season because the water status and the CAM activity did not decrease as rapidly as in high light conditions; this protection under nurse plants slows the effects of stress.

Under conditions of excess light, the production of ROS cause damage at PSI and PSII in chloroplasts, and decrease the chlorophylls content whilst increase the carotenoids content (Havaux and Niyogi 1999). We found that total chlorophylls and chlorophylls/carotenoids ratio are important for ecophysiological variables, as well as water availability is important for environmental variables. We also found higher total chlorophylls and chlorophylls/carotenoids ratio under shade and watering than under high solar radiation and drought, which is in agreement with findings that in plants exposed to high PPDF both chlorophylls content and chlorophylls/carotenoids ratio decrease considerably (Adams III et al. 1987; Barker and Adams 1997; Raveh et al*.* 1998; Pimienta-Barrios et al. 2005). Our results suggest that both synthesis and carotenoid accumulation are very important as a mechanism for attenuating stress caused by high irradiance and drought; similar to findings by Baker and Adams (1997) for *Opuntia macrorhiza.* Therefore, *O. streptacantha* seedlings induced and accumulated carotenoids for avoid damage to chlorophylls and PSI and PSII, through thermal energy dissipation and detoxification of ROS generated by the high radiation and drought (Osmond et al. 1999; Takahashi and Badger 2011).

Relative growth rate (RGR) frequently decreases under low water availability in a large number of species (Khurana and Singh 2004; Galmés et al. 2005; Bunker and Carson 2005), including globose cactus seedlings (Martínez-Berdeja and Valverde 2008). However, RGR of *O. streptacantha* seedlings was not important inside the ecophysiological variables, probably because our study species is an obligatory CAM-photosynthesis (Nobel and Hartsock 1987) and globose cactus seedlings could be CAM facultatives (Altesor et al. 1992; De La Barrera and Smith 2009). Similar findings were found under drought for *Clusia hilariana* seedlings, another obligatory CAM-photosynthesis plant (Cavalcante et al. 2010). In addition, root to shoot ratio (R/S) frequently increases in seedlings subjected to sporadic watering compared to those with frequent watering (Miquelajauregui and Valverde 2010). However, this growth variable neither was important inside the ecophysiological variables, although it was low values, which is in agreement with Osmond et al. (2008), who found that *Opuntia* plants have an exceptional water-conserving potential, because of its extraordinarily low root to shoot ratio dominated by above-ground cladode biomass that is fully invested in photosynthetic surface.

In conclusion, there are clearly complex interactions between drought and light that involve different molecular and ecophysiological traits, however in *O. streptacantha* seedlings molecular variables are more sensitive to environmental conditions, and these would be mainly affecting ecophysiological variables which have direct consequences in the seedlings establishment under nurse plants or in open spaces. Up-regulated molecular variables like *OpsMTs* and *OpsLTP* are the most important stress tolerance responses in *O. streptacantha* seedlings, followed by ecophysiological variables as Ψ_s, total chlorophylls, chlorophylls/carotenoids, and finally environmental like the effect of water availability and light intensity. We found that *O. streptacantha* seedlings establish better with high soil humidity and shade, conditions simulating microhabitat under shade of nurse plants in the rainfall period, having higher Ψ_s and total chlorophylls. In the other hand, seedlings under drought and high solar radiation had up-regulation of both *OpsLTP* and *OpsMT*, helping to detoxification and to protecting against oxidative stress. Thus, *Opuntia* seedlings appear to be adapted to establish under the shade of nurse plants; however, they have tolerance mechanisms to survive in exposed areas.

This is the first time that both gene expressions and ecophysiological responses are studied together to determine mechanisms behind the nurse effect on seedling performance, which is also crucial to solving natural desert regeneration problems

Acknowledgments

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Table 1. Total sample canonical coefficients.

	Function coefficients	
Variables	Function 1	Function 2
Lipid transfer protein (*OpsLTP*)	-0.96*	0.23
Metallothionein (*OpsMT*)	-0.92*	0.24
Chlorophylls/Carotenoids	0.91*	0.35
Relative Growth Rate (RGR)	0.04	0.54
Osmotic potential (Ψ_s)	0.73*	0.52
Total chlorophylls	0.86*	0.38
Root/Shoot (R/S)	0.17	0.16
Titratable acidity	0.80*	0.10

* Characteristics with a high contribution to centroid separation among treatments

Table 2. Canonical cross-loadings of the first pair of canonical variables.

		First Pair of Canonical Variates	
		Correlation	Coefficient
*Opuntia streptacantha**	Lipid transfer protein (*OpsLTP*)	-0.3566	1.8449
	Metallothionein (*OpsMT*)	-0.4195	-1.9970
	Chlorophylls/Carotenoids	0.0752	1.2720
	Osmotic potential (Ψ_s)	-0.3021	-1.8660
	Total chlorophylls	0.0861	-0.3821
	Titratable acidity	0.1286	0.9722
Environmental	PPFD	0.4329	0.0314
	Watering treatment	0.2971	0.4883

* Molecular and ecophysiological variables

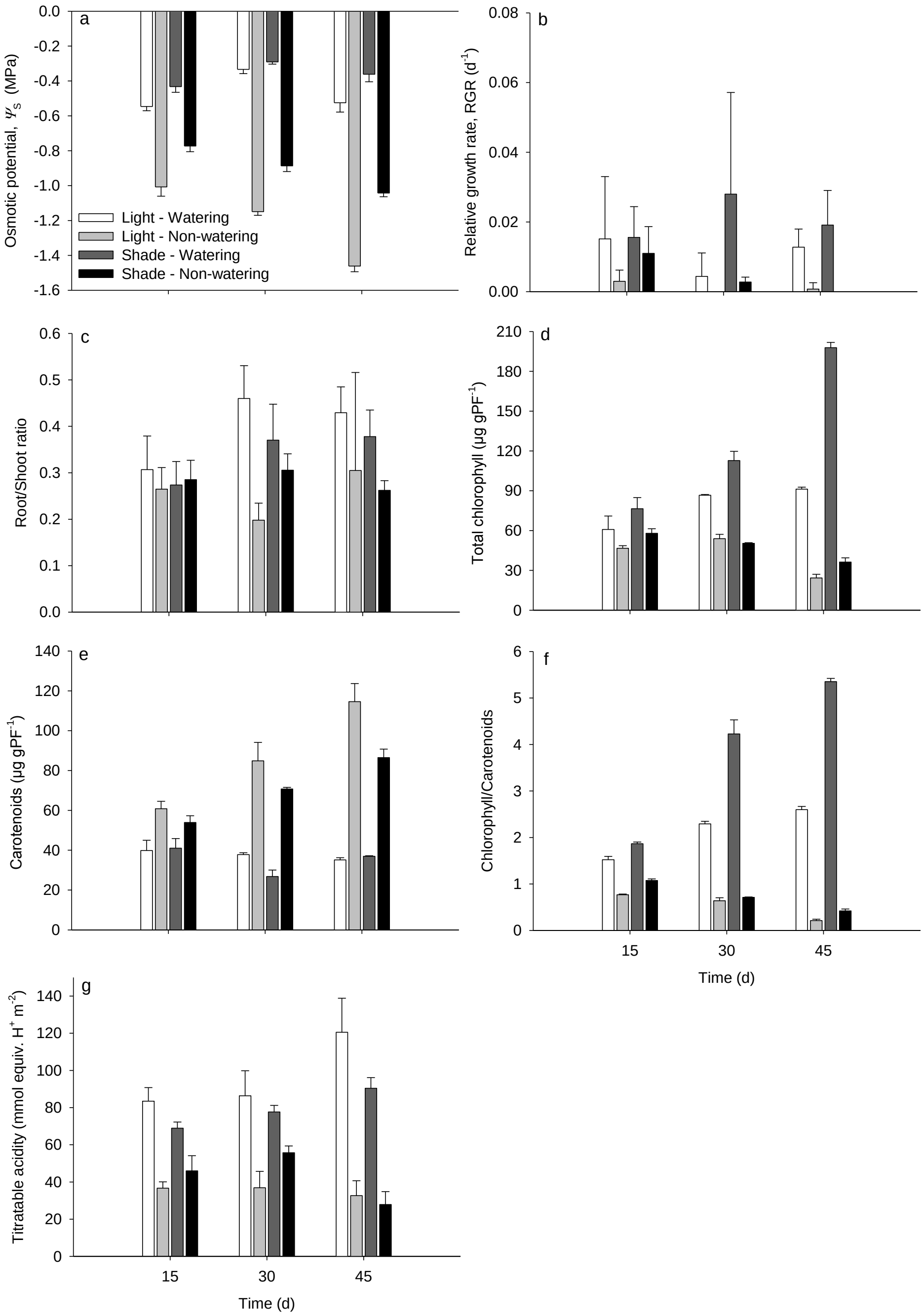

Figure 1. Responses of *Opuntia streptacantha* seedlings under combined water and light treatments at 15, 30 and 45 days. a) Osmotic potential (MPa), b) relative growth rate (d^{-1}), c) Root:Shoot radio (R/S), d) total chlorophylls content (µg gFW^{-1}), e) total carotenoids content (µg gFW^{-1}), f) chlorophylls/carotenoids ratio, and g) titratable acidity (mmol equiv. H^+ m^{-2}). Bars represent the means ± SE (n = 3).

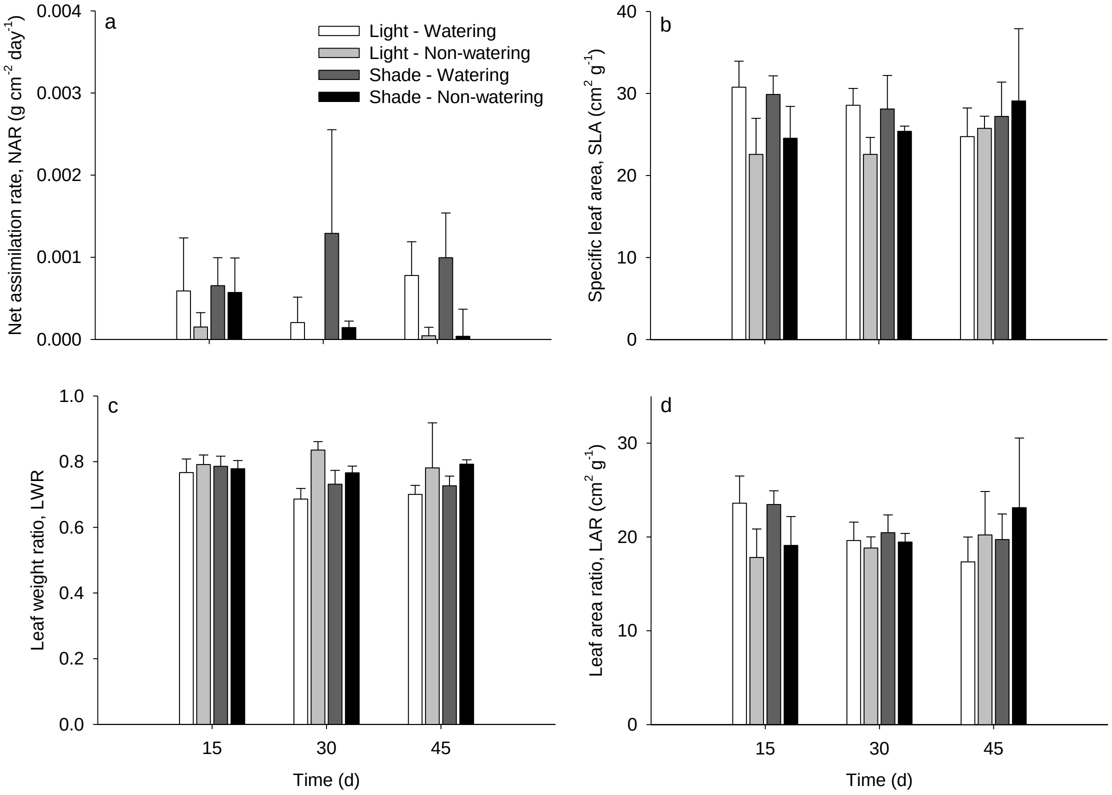

Figure 2. Relative growth rate components of *Opuntia streptacantha* seedlings under combined water and light treatments at 15, 30 and 45 days. a) net assimilation rate (NAR, g cm^{-2} day^{-1}), b) specific leaf area (SLA, cm^{2} g^{-1}), c) leaf weight ratio (LWR, g g^{-l}), and d) leaf area ratio (LAR, cm^{2} g^{-1}). Bars represent the means ± SE (n = 3).

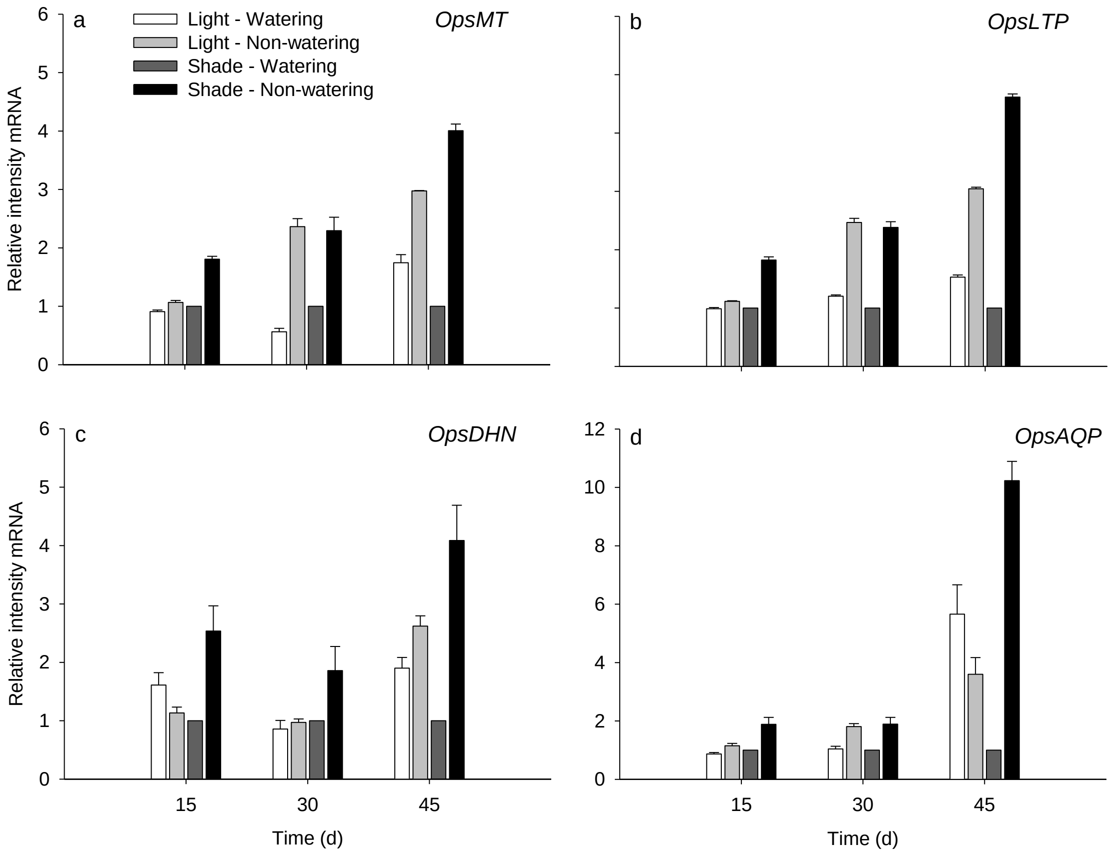

Figure 3. Expression of *Opuntia streptacantha* genes under four combined treatments of light and watering at 15, 30, and 45 days. a) Metallothionein (*OpsMT*), b) lipid transfer protein (*OpsLTP*), c) dehydrin (*OpsDHN*), and d) aquaporin (*OpsAQP*) genes. Total RNA was isolated from seedlings. The relative amount of each transcript was normalized to that of the actin product. Bars represent the means ± SE (n = 3).

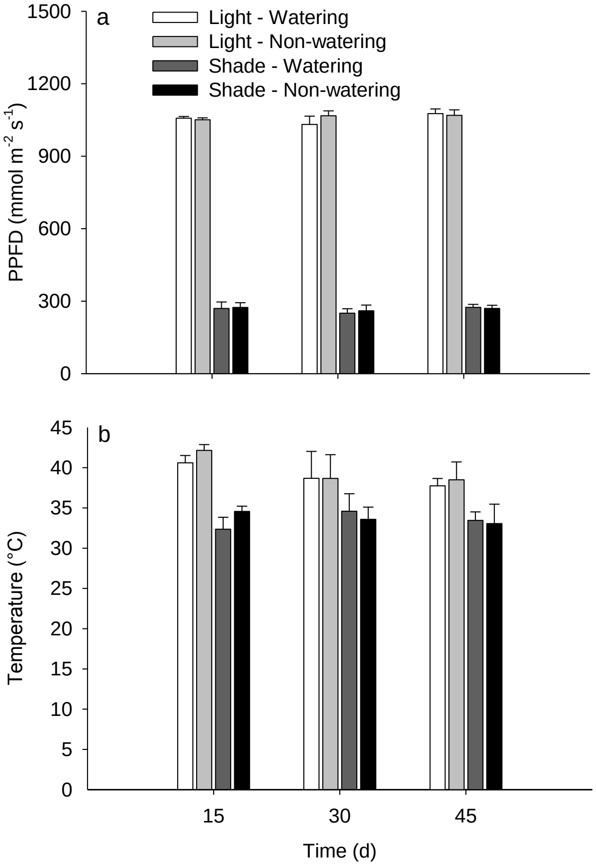

Figure 4. Environmental conditions that receive *Opuntia streptacantha* seedlings under combined water and light treatments at 15, 30 and 45 days. a) Photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) and, b) temperature (°C). Bars represent the means ± SE (n = 3).

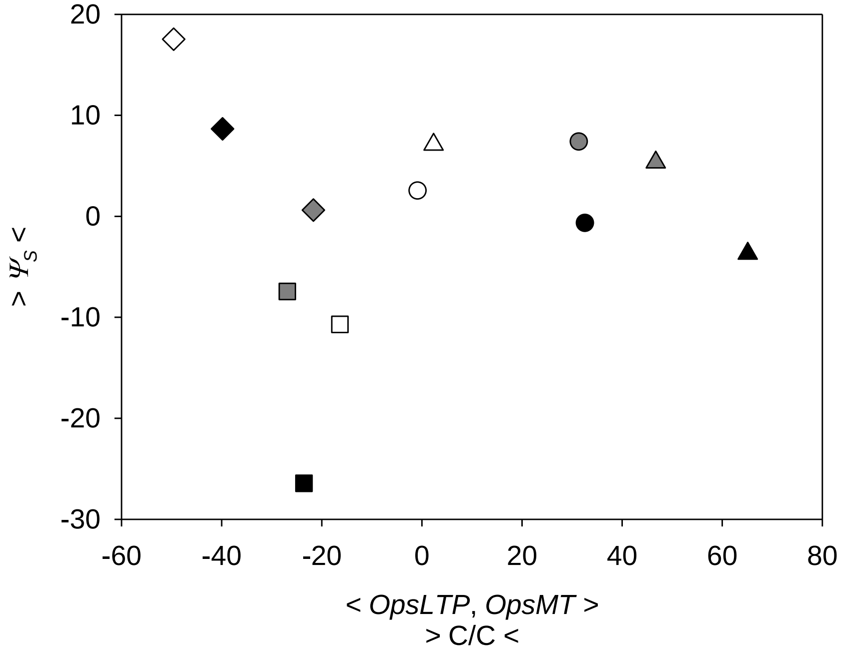

Figure 5. Ordination of treatments using canonical discriminant functions by mean class variables. ○) Light – Watering at 15 d; ◍) Light – Watering at 30 d; ●) Light – Watering at 45 d; □) Light – Non-watering at 15 d; ▣) Light – Non-watering at 30 d; ■) Light – Non-watering at 45 d; △) Shade – Watering at 15 d; ◬) Shade – Watering at 30 d; ▲) Shade – Watering at 45 d; ◊) Shade – Non-watering at 15 d; ◈) Shade – Non-watering at 30 d; and ♦) Shade – Non-watering at 45 days of treatment. Osmotic potential (Ψ_s), lipid transfer protein (*OpsLTP*), metallothionein (*OpsMT*), and chlorophylls/carotenoids ratio (C/C).

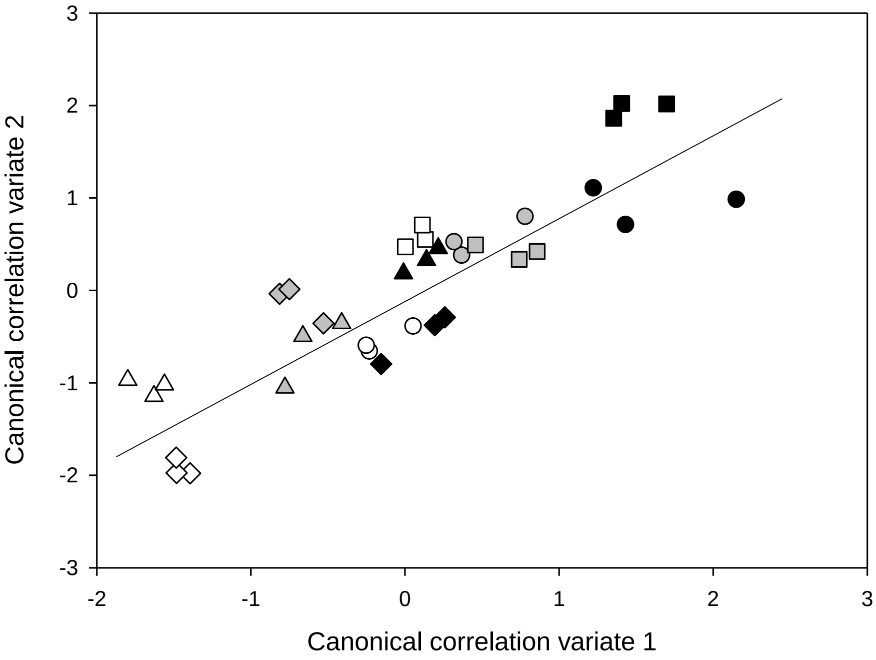

Figure 6. Scatter plot showing the relationship between the first pair of canonical variables canonical correlation. ○) Light – Watering at 15 d; ◍) Light – Watering at 30 d; ●) Light – Watering at 45 d; □) Light – Non-watering at 15 d; ▣) Light – Non-watering at 30 d; ■) Light – Non-watering at 45 d; Δ) Shade – Watering at 15 d; ◬) Shade – Watering at 30 d; ▲) Shade – Watering at 45 d; ◊) Shade – Non-watering at 15 d; ◈) Shade – Non-watering at 30 d; and ♦) Shade – Non-watering at 45 days of treatment.

CAPÍTULO VI

Ecophysiological and molecular responses in *Opuntia streptacantha* and *Opuntia leucotricha* seedlings as mechanisms behind the nurse effect

Abstract

In arid and semi-arid environments, there are clearly complex ecophysiological and molecular mechanisms as a result of interactions between drought and light, which could have direct consequences in the seedling phase. Seedlings of *Opuntia* spp. establish mostly under the canopy of nurse plants which provide a better micro-environment, although mechanisms underlying this process are unknown. In this research, we evaluated the responses of *Opuntia streptacantha* and *Opuntia leucotricha* seedlings, two species from the Southern Chihuahuan Desert, under four combined treatments (Light – watering; Light – non-watering; Shade – watering and Shade – non-watering) at 30 and 60 days. Ecophysiologycal (chlorophyll fluorescence, ETR, NPQ, Ψ_s, titratable acidity, chlorophylls *a*, *b*, and total, as well as carotenoids, R/S ratio, RGR, NAR, SLA, LWR, and LAR), molecular (*OpsMT*, *OpsLTP*, *OpsDHN* and *OpsAQP*) and environmental (PPFD, air temperature, and soil water condition) variables were evaluated. The molecular variables were found to be the most important responses for seedling establishment. Variables that contributed significantly were *OpsMT*, *OpsLTP*, *OpsDHN*, *OpsAQP* in the first function, Ψ_s and chlorophylls/carotenoids in the second function, and ETR and titratable acidity in the third function. With the canonical correlation analysis, we found that plants in the shade – watering treatment at 30 and 60 days had the highest osmotic potential and chlorophylls/carotenoids ratio, as well as acidity, and the lowest expression of *OpsMT*, *OpsLTP*, *OpsDHN*, *OpsAQP*, and ETR, while contrary results were found for both light and shade - drought treatments. We found that *O. streptacantha* and *O. leucotricha* seedlings establish better with high soil humidity and shade, conditions simulating microhabitat under shade of nurse plants in the rainfall period, having higher Ψ_s and total chlorophylls. In the other hand, seedlings under drought and high solar radiation had up-regulation of *OpsLTP*, *OpsMT*, *OpsDHN* and *OpsAQP* helping to detoxification and to protecting against oxidative stress. Thus, *Opuntia* spp. seedlings appear to be adapted to establish under the shade of nurse plants; however, they have tolerance mechanisms to survive in exposed areas. This is the first time that both gene expressions and ecophysiological responses are studied together to determine mechanisms behind the nurse effect on seedling performance.

Keywords: CAM plants; abiotic stress; ecophysiology; lipid transfer protein; metallothionein; dehydrin; aquaporin; chlorophyll fluorescence.

Introduction

Opuntia mature plants have different adaptations to tolerate extreme environmental conditions such as high water use efficiency (WUE), and CAM-photosynthesis (CAM), a water-conserving mode of photosynthetic CO_2 assimilation which is characterized by both the uptake of CO_2 and the acid accumulation at night when the driving forces for transpirational water loss are low (Winter et al., 2005; Nobel, 2010). In addition, also for *Opuntia* adult plants the nocturnal CO_2 uptake and acid accumulation are influenced by the daytime PAR, because it has been found nocturnal CO_2 uptake and acid accumulation at the higher PAR levels (Nobel and Hartsock, 1983), which may represent a form of photoinhibition, where the light-harvesting reactions and hence CO_2 assimilation ability of the chloroplasts are inhibited (Osmond et al., 1982; Adams III et al., 1987; Osmond, 1994). Also, for *Opuntia* adult plants a decrease in Chlorophyll per unit cladode area at the higher total daily PAR (Nobel and Hartsock, 1983) suggests that photooxidation has occurred.

For seedlings, nocturnal acid accumulation decreases for light - exposed cactus but increases for shaded cactus. Therefore, the presence of CAM in shaded cactus seedlings increases water-use efficiency and reduces the risk of photoinhibition (Gallardo-Vasquez and De la Barrera, 2007; Hernández-González and Briones-Villarreal, 2007). It is possible that *Opuntia* seedlings established in open, highly illuminated spaces, show lower acid accumulation than those established in shade. Indeed, the Cactaceae family includes the highest number of cactus species protected by nurse plants (Flores and Jurado, 2003), like *Opuntia* spp. seedlings, which establish mostly under the canopy of nurse plants, which provide a less stressful micro-environment, than in open spaces (Yeaton, 1978; Yeaton and Romero-Manzanares, 1986; Vargas-Mendoza and González-Espinoza, 1992; Cody, 1993; Nabhan and Carr, 1994; Reyes-Olivas et al., 2002). Thus, environmental conditions differ between open and under nurse plants microsites, and act in concert to determine plant growth and survival. However, the mechanisms behind the nurse effect on growth response are not enough known. For example, there are studies about the relative growth rate (RGR) and root to shoot ratio (R/S) on cactus seedlings in microhabitats under nurse plants, with lowest RGR (Martínez-Berdeja and Valverde, 2008) and highest R/S at lower soil moisture (González-Monterrubio et al., 2005; Miquelajauregui and Valverde, 2010). Nevertheless, for cactus seedlings there are no studies about the morphological and physiological components of the RGR.

Plants under the canopy of nurse plants are exposed to diminished light, which could limit or improve their photosynthesis and growth. For example, Franco and Nobel (1989) found a higher environmental productivity index (EPI), a measure of the quantitative effects of water, temperature, and photosynthetic photon flux density (PPFD) on net CO_2 uptake, for *Ferocactus acanthodes* seedlings at exposed sites compared to those located under nurse plants. In addition, Hernández and Briones (2007) found higher photosynthetic efficiency of photosystem II ($\Delta F/Fm'$) for seedlings of *Pachycereus weberi* and *Escontria chiotilla* in high light than for shaded seedlings (Hernández and Briones, 2007), and Miquelajauregui and Valverde (2010) found lowest RGR on the shade for *N. macrocephala* and *N. mezcalensis* seedlings. However, other authors have found higher growth for shaded than for exposed columnar cactus seedlings (Gallardo-Vasquez and De la Barrera, 2007; Hernández-González and Briones-Villarreal, 2007), and no differences in photosynthetic efficiency of photosystem II between shaded and exposed columnar cactus seedlings of *Neobuxbaumia tetetzo*, *Ferocactus recurvus*, and *Stenocereus stellatus* (Hernández and Briones, 2007). Thus, photosynthesis and growth under nurse plants are diminished for some species but improved or unaffected for other cactus species.

For *Opuntia* seedlings from semi-arid regions there are no studies about growth or photosynthesis; however, the concentrations of photosynthetic pigments could decrease under full sunlight in *Opuntia* seedlings, as found for adult *Opuntia ficus-indica* (Nobel and Hartsock, 1983), as well as for other adult CAM plants (Adams III et al., 1987; Raveh et al. 1998)). In addition, carotenoids (pigments involved in photoprotection and photorepair) could increase under full sunlight, avoiding changes in the photosystems (Osmond et al., 1994, 1999; Förster et al., 2005, 2009), and even it could be changes in gene expression, transcriptional factors, and metabolism leading to osmotic adjustment, induction of repair and detoxification systems and chaperones under full sunlight and drought (Ingram and Bartels, 1996; Thomashow, 1999; Tunnacliffe and Wise, 2007).

Ecophysiological studies have been realized in *Opuntia* adult plants; however, scarce studies at the molecular level exist in adult and seedlings (Nobel, 1997; Griffith, 2004; Silva-Ortega et al., 2008; Ochoa-Alfaro et al., 2011) and both ecophysiological and molecular mechanisms underlying the nurse effect on *Opuntia* seedlings are unknown. In this study, the effect of the combination of light and watering treatments (shade and watering, shade and non-watering, light and watering, and light and non-watering) has been examined on *O. streptacantha* and *O. leucotricha* seedlings. Both species are perennial arborescent cactus of economic interest which has edible fruit and young pads, and they are distributed throughout the semiarid lands of central Mexico (Britton and Rose, 1963; Bravo-Hollis, 1978). We assessed three types of response variables: ecophysiologycal (chlorophyll fluorescence, ETR, NPQ, Ψ_s, titratable acidity, chlorophylls *a*, *b*, and total, as well as R/S ratio, RGR, NAR, SLA, LWR, and LAR), molecular (*OpsMT*, *OpsLTP*, *OpsDHN* and *OpsAQP*) and environmental (PPFD, air temperature, and soil water condition).

Materials and methods

Study design

Opuntia streptacantha and *Opuntia leucotricha* seeds were collected in Mexquitic de Carmona municipality of San Luis Potosi, Mexico (22º16’ N, 101˚07’ W at 2,020 m asl), at Southern Chihuahuan Desert, in 2007. Seeds were germinated in a greenhouse and four months later, the seedlings were transplanted into pots with soil from *Opuntia* spp. habitat and maintained with constantly watering for 20 days. Four treatments were randomly assigned to 100 seedlings from each species, for a total of 25 replicates after treatment. Treatments included: shade (25% light) with watering (SW), shade without watering (SNW), light (100% light) with watering (LW), and light without watering (LNW). The four treatments simulated dry and wet conditions in site of established of *Opuntia* spp. seedlings. Treatments with high solar radiation simulated solar radiation of open spaces without vegetation, whereas shaded treatments represented the low solar radiation (≈ 50 % of light reduction) found under shrubs in the area. In order to simulate low solar radiation, plants were covered with a plastic film mesh which created shade; for high radiation treatments, plants were not covered. Seedlings in the low moisture treatments were not watered, while those in the high moisture treatments were watered each other day to field capacity during the entire course of the experiment. Two harvests were done at days 30 and 60 for all determinations, except for chlorophyll fluorescence evaluations.

Osmotic potential (ψ_s)

Water status measurements of *O. streptacantha* and *O. leucotricha* seedlings were completed at predawn. The experimental unit was one seedling per pot to obtain enough sap extract. In order to evaluate ψ_s, two harvests (three pots per harvest) were done. *Ex situ* determinations were made of the ψ_s of the sap obtained by mechanical compression (Pearcy *et al.,* 1991). Ψ_s was recorded in C-52 sampling chambers connected to a Wescor HR-33T dew-point microvoltmeter (Wescor Inc., Logan, Utah, U.S.A.). Sampling chambers were previously calibrated with NaCl standard solutions (Rundel and Jarrel, 1991).

Titratable acidity content

The malic acid content of *O. streptacantha* and *O. leucotricha* seedlings was determined at 6:00 a.m. according to Pearcy et al. (1991). We evaluated acidity at this time, when the higher content occurs (Nobel, 2010). Plant tissue was obtained from shoot parts of 0.01 to 0.02 m^2, using a driller. The plant material was cut and preserved in ethanol (80%) in 1.5 ml Eppendorf tubes. Titration was carried to neutrality, with the required volume of 0.01 N NaOH for subsequent calculation of the acid concentration in mmol equiv. H^+ m^{-2} by tissue.

Pigments content

To determine the concentrations of chlorophyll *a*, *b*, and total, as well as carotenoids of *O. streptacantha* and *O. leucotricha* seedlings, samples were collected from three individuals of each treatment at 6:00 a.m. The pigments were extracted according to the procedure of Hendry and Price (1993). Samples of seedlings (50 to 100 mg fresh weight) were taken and transported in cold 2 mL Eppendorf tubes, filling them in the light, the tissue was macerated with 2 mL of acetone to 80% in extracts from cold and the absorbance was determined using a spectrophotometer (Thermo Scientific ®, Model Spectronic Genesys 10 Bio), reading samples for chlorophyll at 645 nm and 663 nm and total carotenoids at 470 nm.

Photosynthetic area

To determine the photosynthetic area of in *O. streptacantha* and *O. leucotricha* stems, three seedlings were collected at 30 and 60 days in the four treatments-watering combination of light (LW, LNW, SW and SNW). Seedlings were scanned with an HP 2400 ScanJet scanner. The images were analyzed with the software ImageJ 1.40g (Wayne Rasband National Institutes of Health, USA).

Growth evaluations

To determine *O. streptacantha* and *O. leucotricha* seedling growth in the four treatments, we measured the relative growth rate (RGR) and its morphological component [the specific leaf area (SLA), which indicates the plant investment in leaves or other photosynthetic structures of the plants, and the leaf weight ratio (LWR), which measures the allocation of biomass to leaves *vs*. other plant parts], and a physiological component, the net assimilation rate (NAR) or plant mass increment per leaf area or photosynthetic area (Poorter 1999; Shipley 2002). We also measured the root to shoot ratio ($\text{Weight}_{root}/\text{Weight}_{shoot}$). Three seedlings were yielded at 15, 30, and 45 days in the four combined treatments. The calculations were realized using the formula Relative growth rate (RGR) = Net assimilation rate (NAR, g cm^{-2} day^{-1}) * Specific leaf area (SLA, cm^{2} g^{-1}) * leaf weight ratio (LWR, g g^{-l}), following Shipley (2000). NAR is a physiological component because it is a measure of whole-plant daily net photosynthetic rate weighted by the rate of change in plant carbon content (Poorter and van der Werf 1998; McKenna and Shipley 1999). SLA is a morphological component because it is determined by leaf dry matter concentration and leaf thickness (Witkowski and Lamont 1991; Shipley 1995, 2002). LWR measures the allocation of biomass to leaves or photosynthetic organs *vs.* other plant parts (Shipley 1995, 2002). Leaf area ratio (LAR, cm^{2} g^{-1}) is a morphological component, and is the result of SLA * LWR (Shipley 2002). In addition, due R/S is a ratio, it does not have units; in this case, a value near unity is an indication that the plant allocates similar amounts of biomass to roots and to aerial parts (Miquelajauregui and Valverde 2010).

Chlorophyll fluorescence evaluations

The measures of chlorophyll fluorescence were made every seven days, until the end of the experiment. During the day, the measures were every three hours (6, 9, 12, 15 and 18 h) in five individuals of *O. streptacantha* and *O. leucotricha* seedlings. The variables of chlorophyll fluorescence that were evaluated: quantum efficiency of photosystem II (Φ_{PSII}), non-photochemical quenching (NPQ), electron transport rate (ETR), photosynthetic photon flux density (PPFD) and temperature (°C) were measured using a portable fluorometer (pulse-amplitude modulated photosynthesis yield analyzer, Mini-PAM; Walz GmbH, Effeltrich, Germany).

Analyses of genes expressed by RT-PCR

To study gene expression of abiotic stress response in in *O. streptacantha* and *O. leucotricha* seedlings subjected to four treatments combined, first used the SuperScript II Kit ™ First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) following the protocol recommendations included in the kit. It was added 1 mL of cDNA in each PCR reaction of 25 mL, using specific primers to amplify the actin gene as an internal control of loading. Specific primers were designed for four genes of *Opuntia* spp. by PrimerSelect program of Lasergene (DNASTAR), these primers were genes for metallothionein (*OpsMT*) Forward 5’-CCGTTACATCTCAGAAAATGTC-3’ and Reverse 5’-TCATTTGCAGTTACATGGGTTG-3’, lipid transfer protein (*OpsLTP*) Forward 5’-CAGAATGGCTAGCTCAGCGGTT-3’ and Reverse 5’-CATCTAGTTCACCTTGGAGCAGTC, dehydrin (*OpsDHN*) Forward 5’-GATCGCGGACTGTTCGACTT-3’ and Reverse 5’-GTCGGAACGATGGAGCTTGT-3’, and aquaporin (*OpsAQP*) Forward 5’-AGAGAGGTCATCATGTCCAAG-3’ and Reverse 5’-

ATGTATGCGCTGGTGCTAGTG-3’ which were previously identified in a cDNA *O. streptacantha* library obtained in our work group. All amplified fragments corresponded to the expected size based on the design of primers. The expression levels of genes in each sample of *O. streptacantha* and *O. leucotricha* seedlings were calculated based on the intensity of the bands by the analysis software Quantity One 1-D 4.5 (BIO-RAD, Hercules, CA.), carrying out a normalization process through the expression of an actin gene. The program for the amplification conditions were: an initial cycle of 95 °C for 5 minutes, followed by 95 °C for 30 seconds (denaturation), 60 °C for 45 seconds (alignment), 72 °C for 1.5 minutes (extension), between 25 or 30 cycles, depending on the transcript analysis. Amplified products were separated by electrophoresis on agarose gels with 1% 1X TAE stained with EtBr and photodocumented.

Statistical analysis

The average values for molecular and ecophysiological variables important for canonical discriminant analysis are shown in Figure 1.

Data were performed using a canonical discriminant analysis that allowed to identify differences among four combined treatments (SW, SNW, LW and LNW) in both species, using the measurements of variables from individuals belonging to each group; ecophysiologycal (chlorophyll fluorescence, ETR, NPQ, Ψ_s, titratable acidity, chlorophyll *a*, *b*, total and carotenoids, root/shoot ratio, RGR, NAR, SLA, LWR, and LAR), molecular (*OpsMT*, *OpsLTP*, *OpsDHN* and *OpsAQP*) and environmental (PPFD, air temperature, and soil water condition); estimating linear functions from the quantified variables, and then separating the groups of individuals maximizing the variance among treatments and minimizing the variance within them. The standardized canonical coefficients show the contribution of the joint variables analyzed for each canonical function, and individually indicate the relative importance of each variable (Pires Da Silva *et al.,* 2001; McGarigal *et al.,* 2000). In addition, a classificatory discriminant analysis was applied to provide the number and percent of entities classified correctly or incorrectly into each group of combined treatments. Both analyses were carried out using PROC CANDISC and PROC DISCRIM procedures.

Subsequently, we performed a generalized canonical correlation. The goal of this correlation is to analyze the relationships among more than two sets of variables, in order to measure the intensity of the relationships and to delineate strategies of simple canonical correlation (McGarigal *et al.,* 2000). In the method of maximum variance, the first vector of canonical variates presents the maximum variance of the first eigenvector, corresponding to maximization of the first eigenvalue of the vector of the canonical variates' correlation matrix (Yáñez-Espinosa *et al.,* 2006). The relationship among the three sets of variables, ecophysiological, molecular, and environmental was determined by the generalized canonical correlation analysis.

From the generalized canonical correlation results, a simple canonical correlation analysis was applied to the original variables more related to their canonical variates (ecophysiological, anatomical, environmental), arranged in two sets, one for plant and the other for environmental variables. The goal of canonical correlation is to analyze the relationships between two sets of variables, and to elucidate the relationship between the two sets of variables of high dimension by generating two sets of low dimension. One set of variables may be response variables and the other set explanatory variables, like in a multiple regression, except that there are several variables in both sides of the equation. Sets of variables on each side are combined to produce a predicted value that has the highest correlation with the predicted value on the other side (McGarigal *et al.,* 2000). A redundancy analysis was also performed to calculate the variance in a set of original variables explained by a canonical variate of another set (McGarigal *et al.,* 2000). All statistical analyses were performed with SAS software (SAS Institute Inc.).

Results

Figures 1-5 show the results for each response variables.

Canonical discriminant analysis

The canonical discriminant analysis identified differences among the four combined treatments (SW, SNW, LW, and LNW) and all of the response variables that were analyzed. It showed that three discriminant functions explained 98 % of the total variation, contributing significantly to the separation among treatments (Wilks' λ: $P < 0.0001$, $n = 48$). The first function (eigenvalue of 204.21; $P < 0.0001$) explained 59 % of the total variation, the second (eigenvalue of 83.38; $P < 0.0001$) explained 24 % and the third (eigenvalue of 50.97; $P < 0.0001$) explained 15% of the variation.

Variables that contributed significantly to the three canonical functions are present in the standardized canonical coefficients (Fig. 6, Table 1), and those indicating the relative importance from each variable were *OpsMT*, *OpsLTP*, *OpsDHN*, *OpsAQP* in the first function, Ψ_s and chlorophylls/carotenoids in the second function, and ETR and titratable acidity in the third one.

The classificatory discriminant analysis showed that centroids of each group were significantly different ($P < 0.0001$). All of the observations were correctly classified for all treatments.

Canonical correlation analysis

The first canonical correlation was 0.96 (88 % of variance, $P < 0.0001$), however the remaining correlations were no significant. Data on the first pair of canonical variables (cross-loadings) appear in Table 2 showing the correlations between the variables and the canonical variables, standardized canonical variable coefficients, with opposite-set variance accounted for by the canonical variable (% of variance), redundancies and canonical correlations. Percentage of variance (76 %), redundancy (0.53), and canonical correlation (0.96) indicate that the first pair of canonical variables was highly related. The cross-loadings showed that environmental conditions were positively associated with *Opuntia streptacantha* variables (Table 2).

With the canonical correlation analysis, we found that plants in the shade – watering treatment at 30 and 60 days had the highest osmotic potential and chlorophylls/carotenoids ratio, as well as acidity, and the lowest expression of *OpsMT*, *OpsLTP*, *OpsDHN*, *OpsAQP*, and ETR, while contrary results were found for both light and shade - drought treatments (Fig. 6).

Discussion

Nurse plant association is a phenomenon that occurs mostly in desert environments where abiotic factors limit plant performance. Nurse plant creates a microenvironment that is less severe for young seedlings growing underneath it to survive in a harsh desert environment. The importance of nurse plant associations ranges from providing shade, humidity and nutrients to providing the only means of seedling establishment (Callaway 1995; Flores and Jurado 2003). However, the ecophysiological and molecular mechanisms behind the nurse effect on responses of *Opuntia* spp. seedlings are not enough known. The present study reveals that molecular variables (the induction in the expression of *OpsMT*, *OpsLTP*, *OpsDHN*, and *OpsAQP*) were the most important responses for *O. streptacantha* seedlings under combined treatments, especially under water and light stress.

The results presented here describe the *OpsMT* up-expression is positively regulated by drought and high-light. Metallothioneins are small cysteine rich proteins that range in size from 4 to 8 kDa and bind several metals like copper, cadmium, zinc, nickel, etc. (Hamer 1986). Recently, several results have indicated MTs play important roles in abiotic stress tolerance as a ROS scavenger (Akashi et al. 2004; Wong et al. 2004; Yang et al. 2009), and act as possible cellular redox sensors (Fabisiak et al. 2002). Severe drought stress impairs the function of cellular ions, transporters and membrane-associated enzymes, leading to generation of ROS subsequently (Mahajan and Tuteja 2005). We found that metallothionein (*OpsMT*) gene is up-expressed under shade – non-watering at 30 day of treatment in *O. leucotricha*, but at 60 day we found in high light and drought in both species the higher up-expression of *OpsMT* than shade – non-watering treatment. We suggest that *OpsMT* is involved in the elimination of ROS caused by water and high light stresses in which *O. streptacantha* seedlings were subjected.

In addition, we found high up-expression of *OpsLTP* under high-light and drought treatments at 60 days. Plant lipid transfer proteins (LTPs) are ubiquitous lipid-binding proteins that are characterized by their ability to act as intracellular lipid transporters between the organelles in *in vitro* plant cells (Jung et al. 2003, 2005). These LTPs are involved in several abiotic stress responses in crop plants (Plant et al. 1991; Hwang et al. 2005; Oshino et al. 2007; Choi et al. 2008), as well as in plants of cold environments as *Bromus inermis* (Wu et al. 2004). For native desert plants there are few research, *i.e.* the non-succulent plants *Prosopis juliflora* and *Tamarix hispida* registered up-regulation of *LTPs* genes under conditions of drought and oxidative stress (George et al. 2007, 2010; Wang et al. 2009). Thus, the participation of *LTP* genes could be involved in the abiotic stress in seedlings of *O. streptacantha* and *O. leucotricha.*

It is widely believed that protection during desiccation is afforded by the accumulation of various proteins, sugars and compatible solutes which serve to replace water and stabilize the sub-cellular environment by vitrification (Crowe et al., 1992). Late embryogenesis abundant (LEA) proteins represent an important group of hydrophilic proteins which accumulate to high levels during the late states of embryogenesis in seeds when desiccation tolerance is acquired or in vegetative and reproductive tissues under dehydration suggesting a role in adaptation during desiccation (Hoekstra et al., 2001). These proteins are predicted to have several protective functions which include protection of DNA, stabilization of cytoskeletal filaments and acting as molecular chaperones to protect protein conformation and activity (Wise and Tunnacliffe, 2004; Goyal et al., 2005). Dehydrin (DHN) protein belong to the group 2 of LEA proteins, and theirs have been descript in crop plants under abiotic stress (Chung et al., 2003; Lee et al., 2005; Shakirova et al., 2009), and their participation in membrane stabilization, water buffering, radical-scavenging, metal binding and cryoprotective activities have been described (Hara et al., 2005; Rorat et al., 2006). We found up-expression of *DHN* genes in both *O. streptacantha* and *O. leucotricha* seedlings under light – non-watering treatment at 60 day; thus, they could be involved in the protection of protein affected by stress. Similar findings in *Anastatica hierochuntica* and *Craterostigma plantagineum* under drought were found and the expression of dehydrin, osmatin and sugars participated in the stabilization of membranes and proteins (Hoekstra et al., 2001; Vicré et al., 2004).

The movement of water between cells is finely regulated, and can be controlled by the regulation of water channels, known as aquaporins (AQPs) (Kaldenhoff and Fischer, 2006). These are ubiquitous in plants and are detected in high amounts at regions of high symplastic water transport, such as the endodermis (Schaffner, 1998). Water channel activity can vary with time of day, with root development, and in response to various stresses such as drought (Tsuda and Tyree, 2000; Hukin et al., 2002; Martre et al., 2002; Siefritz et al., 2002). We found that under drought treatment *OpsAQP* gene is up-expressed at 30 days in *O. leucotricha*, and at 60 days in both *O. streptacantha* and *O. leucotricha* seedlings. However, the major up-expression was found in high light and drought at 60 days of treatment in *O. leucotricha.* Plant AQP phosphorylation status is dependent on environmental parameters, phosphorylation decreases when the apoplastic water potential is reduced, suggesting closure of the channel during osmotic stress (Johansson, 1996). In addition, temperature is also an important factor regulating phosphorylation (Azad et al., 2004), and together drought and temperature create a direct relation between AQP phosphorylation and physiological processes (Hachez et al., 2006). For desert plants, the closure of water channels during drought would help prevent root water loss to a soil that generally has lower water potential than does the plant. Aquaporins are involved in the regulation of hydraulic conductance in roots of *Opuntia acanthocarpa* and *Agave deserti* under different irrigation treatments (Martre et al., 2001; North et al., 2004). However, the relationship between AQP expression and regulation of plant water status is still unclear; little is known about the exact physiological role of AQPs in plants under normal growth conditions or during salt or water stresses (Hachez et al., 2006).

In addition, we found that Ψ_s was also important for ecophysiological variables. Ψ_s was the most important response variable in the second function, which confirms that water availability is the factor controlling the activity and responses of the plant (Cushman, 2001; Cushman and Borland, 2002; Winter et al., 2005; Winter et al., 2011). *Opuntia* plants tolerate drought, displaying water use efficiency in adult plants (Nobel, 1988; North and Nobel, 1992; Pimienta-Barrios et al., 2002, 2003; Zañudo-Hernández et al., 2010), but the information about *Opuntia* seedlings from arid environments under drought is scarce (González-Monterrubio et al., 2005). We found that *O. streptacantha* and *O. leucotricha* seedlings had lower Ψ_s under drought and high solar radiation than under watering and shade.

Thus, *Opuntia* seedlings appear to be adapted to establish under the shade of nurse plants.

Chlorophylls/carotenoids ratio was also important in the second function. Chlorophylls and carotenoids have roles in light harvesting in photosynthesis, excess energy dissipation, and inactivation of stress-related toxic products. Chlorophyll *a* directs energy to the reaction center of photosynthesis, and chlorophyll *b* and carotenoids widen the spectrum used in photosynthesis and transfer energy to chlorophyll *a* (Taiz and Zeiger, 1998). Carotenoids also protect plants against destruction of chlorophyll in high-light by dissipating excess excitation energy (Mathews-Roth, 1997). Some carotenoids stabilize and protect the lipid phase of the thylakoid membrane, and they are UV-absorbing compounds and have roles in the dissipation of harmful UV energy (Havaux, 1998).

Pigment analyses have been used to evaluate plant responses to environmental factors including light levels (Lewandowska and Jarvis, 1977; Demmig-Adams and Adams, 1996). The pigment ratios chlorophylls/carotenoids may be indicators of photosynthetic acclimation and are positively correlated with excess light stress. We found that chlorophylls/carotenoids ratio was lower in high-light and drought than in shade and watering treatment. One of the consequences of prolonged exposure to high-light intensity and drought are reactive oxygen species (ROS) that can damage cells through the oxidation of proteins, lipids and DNA (Apel and Hirt, 2004). Carotenoids make up one class of non-enzymatic compounds that is particularly effective against oxygen radicals (Asada, 2006).

Finally, we found that ETR and acidity content were also important for ecophysiological variables in the third function. ETR decreased under drought and high-light treatments in both *Opuntia* species. Flexas et al. (1999) reported that ETR is significantly dependent of water status on plants. ETR measures showed that water stress and the light incidence strongly affects PSII efficiency in both species of *Opuntia* seedlings. Decreases in ETR suggest a close link between the photosynthetic processes (Foyer et al. 1990). Ours results in *Opuntia* spp. seedlings showed that ETR depression could be caused by an increase in the activity of photoprotective processes such as thermal dissipation at the antenna level (Osmond 1994). This is also reflected in the large increase in NPQ, probably involving the xanthophyll cycle (carotenoids). According to Foyer et al. (1990), the activation of this cycle leads to a full down-regulation of the photosynthetic processes. The main function of this regulation must be the protection of PSII from photodamage in *Opuntia* ssp. seedlings.

The most important diagnostic feature of CAM is the organic acids content for to identify the effect of stress conditions (Winter et al., 2005). We found that the acidity content in both species of *Opuntia* seedlings decreased under drought and high-light treatment. However, the seedlings under shade treatments (simulating the nurse plant effect) are capable of tolerate higher drought periods. In arid lands, where occurs infrequent rainfall patterns, CAM is the most common photosynthesis kind, which is an important survival mechanism of the *Opuntia* plant species for the maintenance of moderate energy levels during periods of moisture stress, possibly by the internal cycling of CO_2 accompanied by complete stomatal closure. Water conservation and maintenance of moderate energy states during periods of drought are important to the survival of succulents.

In conclusion, there are clearly complex interactions between drought and light that involve different molecular and ecophysiological traits, however in *O. streptacantha* and *O. leucotricha* seedlings molecular variables are more sensitive to environmental conditions, and these would be mainly affecting ecophysiological variables which have direct consequences in the seedlings establishment under nurse plants or in open spaces. Up-regulated molecular variables like *OpsMTs*, *OpsLTP*, *OpsDHN*, and *OpsAQP* are the most important stress tolerance responses in *O. streptacantha* and *O. leucotricha* seedlings, followed by ecophysiological changes as Ψ_s, chlorophylls/carotenoids ratio, and finally ETR and titratable acidity. We found that *O. streptacantha* and *O. leucotricha* seedlings establish better with high soil humidity and shade, conditions simulating microhabitat under shade of nurse plants in the rainfall period, having higher Ψ_s and total chlorophylls. In the other hand, seedlings under drought and high solar radiation had up-regulation of *OpsLTP*, *OpsMT*, *OpsDHN* and *OpsAQP* helping to detoxification and to protecting against oxidative stress. Thus, *Opuntia* spp. seedlings appear to be adapted to establish under the shade of nurse plants; however, they have tolerance mechanisms to survive in exposed areas. This is the first time that both gene expressions and ecophysiological responses are studied together to determine mechanisms behind the nurse effect on seedling performance, which is also crucial in predicting the impact of climate change on natural desert regeneration.

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Table 1. Total sample canonical coefficients.

Variables	Function coefficients		
	Function 1	Function 2	Function 3
Total chlorophyll	-0.49	0.59	-0.06
Chlorophyll / Carotenoids (C / C)	-0.66	0.65*	0.30
Titratable acidity	-0.64	0.31	0.66*
Aquaporin (*OpsAQP*)	0.77*	0.29	0.11
Dehydrin (*OpsDHN*)	0.78*	0.47	0.19
Lipid transfer protein (*OpsLTP*)	0.96*	0.06	-0.02
Metallothionein (*OpsMT*)	0.97*	0.04	0.07
Osmotic potential (Ψ_s)	-0.59	0.77*	0.18
Root / Shoot	-0.55	0.16	0.35
Quantum efficiency of photosystem II (ϕ_{PSII})	-0.66	0.50	-0.27
Non-photochemical quenching (NPQ)	0.71	-0.30	0.23
Electron transport rate (ETR)	0.17	0.08	0.97*

* Characteristics with a high contribution to centroid separation among treatments

Table 2. Canonical cross-loadings of the first pair of canonical variables

	Variables	First Pair of Canonical Variates	
		Correlation	Coefficient
Opuntia streptacantha and *Opuntia leucotricha**	Chlorophyll / Carotenoids (C / C)	-0.0020	0.1204
	Titratable acidity	0.2296	0.1435
	Aquaporin (*OpsAQP*)	0.1579	0.0472
	Dehydrin (*OpsDHN*)	0.3333	0.0963
	Lipid transfer protein (*OpsLTP*)	0.2639	-0.0298
	Metallothionein (*OpsMT*)	0.2908	-0.0564
	Osmotic potential (Ψ_s)	-0.1694	-0.5290
	Electron transport rate (ETR)	0.9060	0.9094
Environmental	Watering treatment	0.2574	-0.0621
	PPFD	0.9516	0.8360
	Temperature	0.7911	0.2301

* Molecular and ecophysiological variables

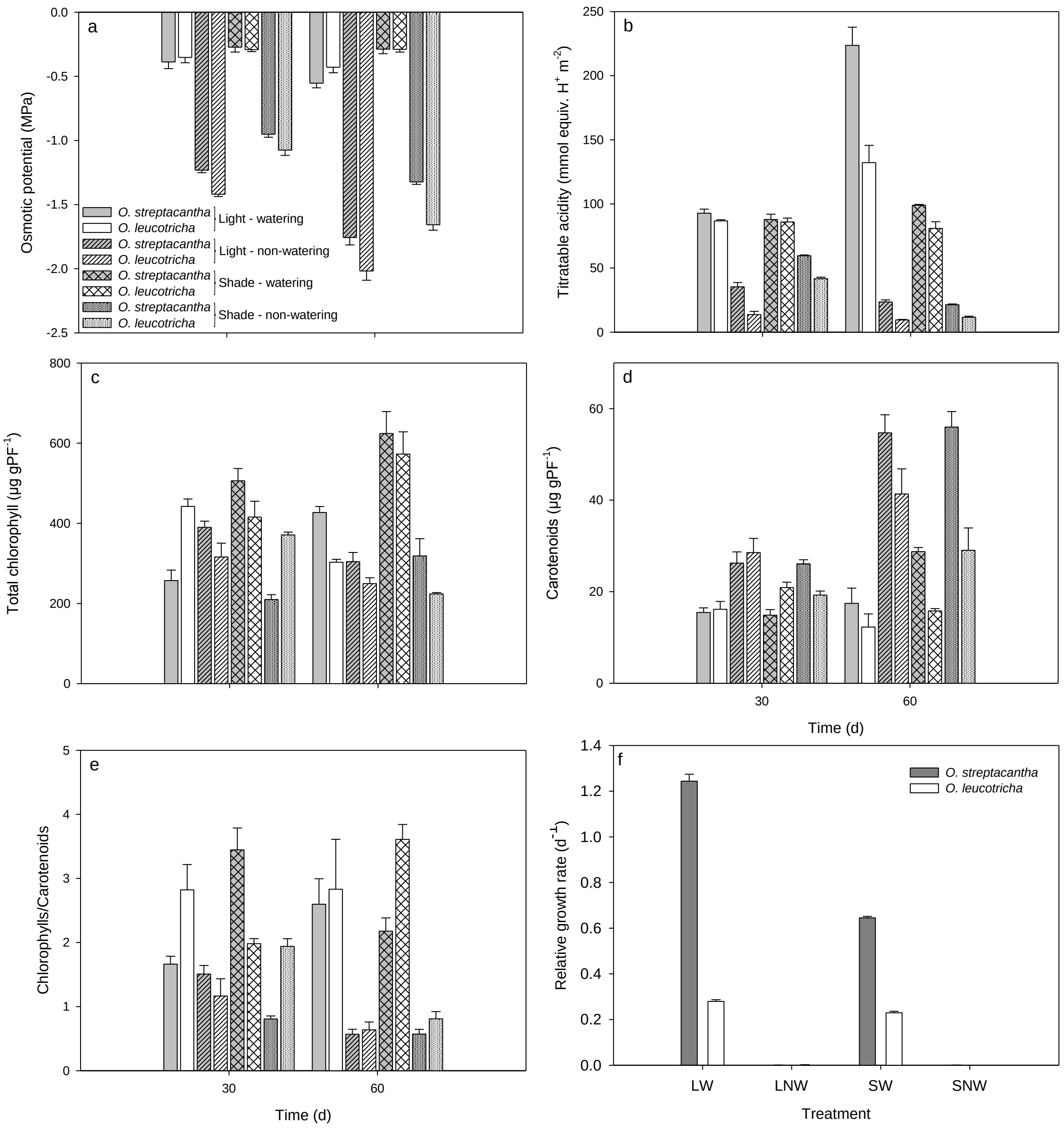

Figure 1. Responses of *Opuntia streptacantha* and *Opuntia leucotricha* seedlings under combined water and light treatments at 30 and 60 days. a) osmotic potential (MPa), b) titratable acidity (mmol equiv. $H^+ \cdot m^{-2}$), c) total chlorophyll content (µg gFW^{-1}), d) total carotenoids content (µg gFW^{-1}), e) chlorophyll/carotenoids ratio, and f) relative growth rate (d^{-1}). Bars represent the means ± SE (n = 3).

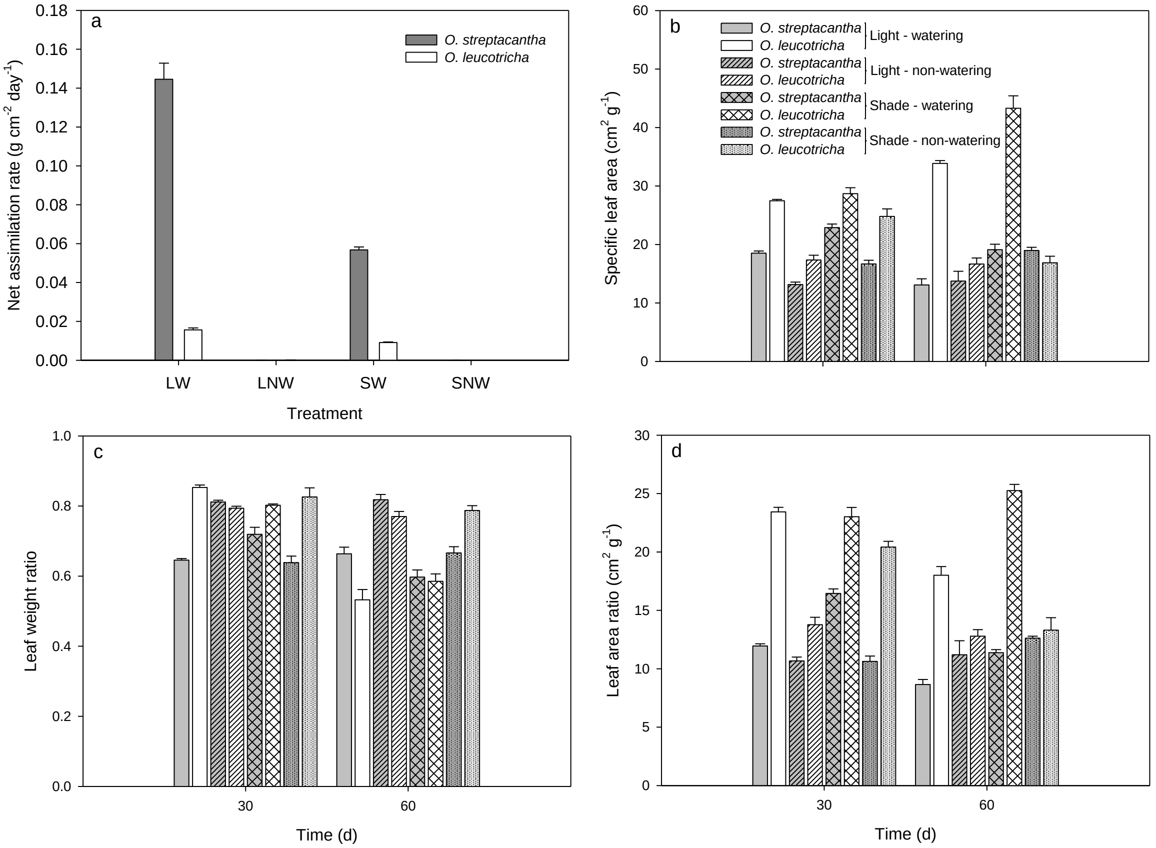

Figure 2. Relative growth rate components of *Opuntia streptacantha* and *Opuntia leucotricha* seedlings under combined water and light treatments at 30 and 45 days. a) net assimilation rate (NAR, g cm^{-2} day^{-1}), b) specific leaf area (SLA, cm^{2} g^{-1}), c) leaf weight ratio (LWR, g g^{-l}), and d) leaf area ratio (LAR, cm^{2} g^{-1}). Bars represent the means ± SE (n = 3).

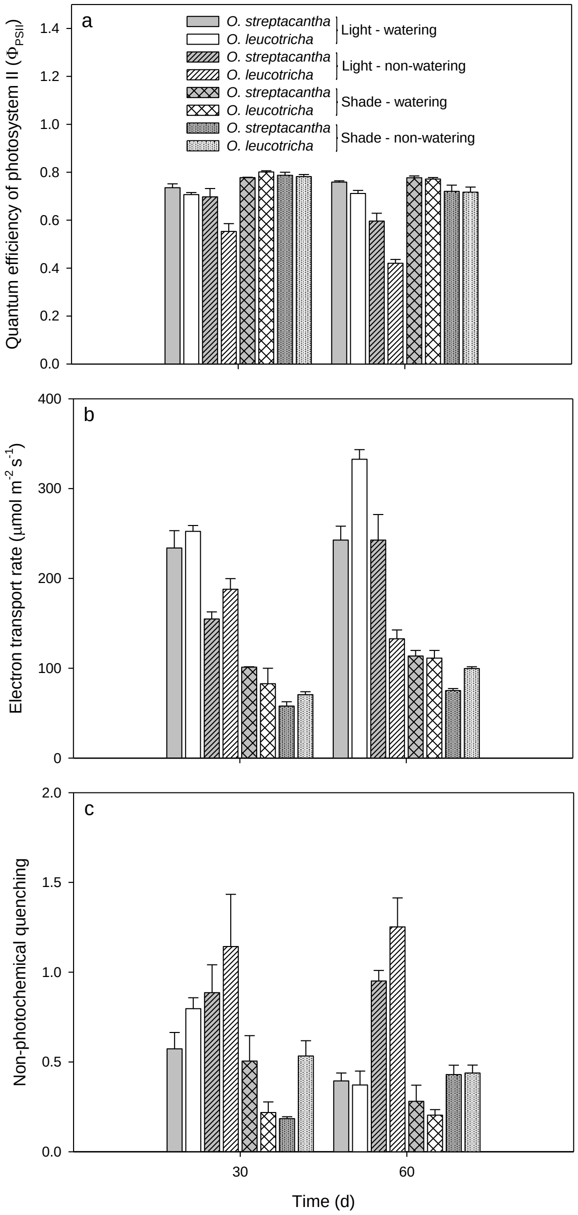

Figure 3. Chlorophyll fluorescence measures in *Opuntia streptacantha* and *Opuntia leucotricha* seedlings under combined water and light treatments at 30 and 45 days. a) quantum efficiency of photosystem II (ϕ_{PSII}), b) electron transport rate (ETR, µmol m^{-2} s^{-1}), and c) non-photochemical quenching (NPQ). Bars represent the means ± SE (n = 5).

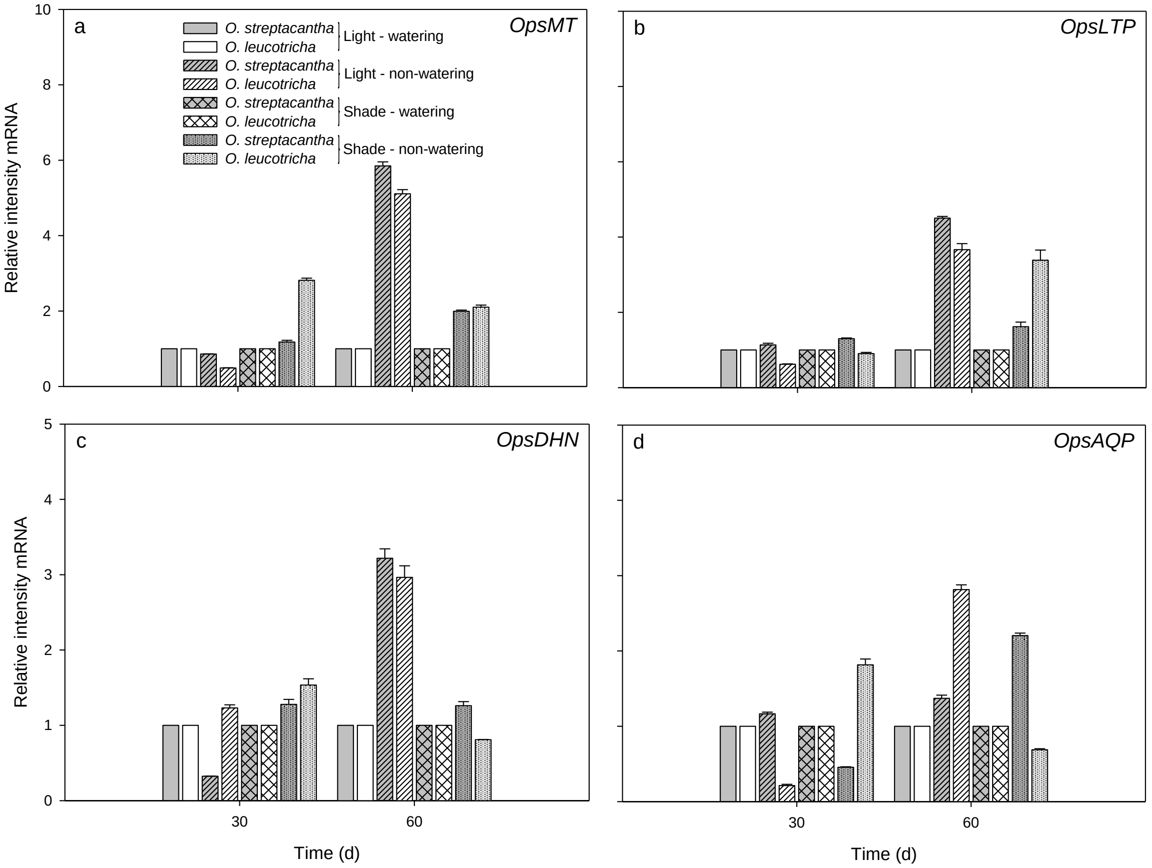

Figure 4. Expression of *Opuntia streptacantha* and *Opuntia leucotricha* genes under four combined treatments of light and watering at 30 and 45 days. a) Metallothionein (*OpsMT*), b) lipid transfer protein (*OpsLTP*), c) dehydrin (*OpsDHN*), and d) aquaporin (*OpsAQP*) genes. Total RNA was isolated from seedlings. The relative amount of each transcript was normalized to that of the actin product. Bars represent the means ± SE (n = 3).

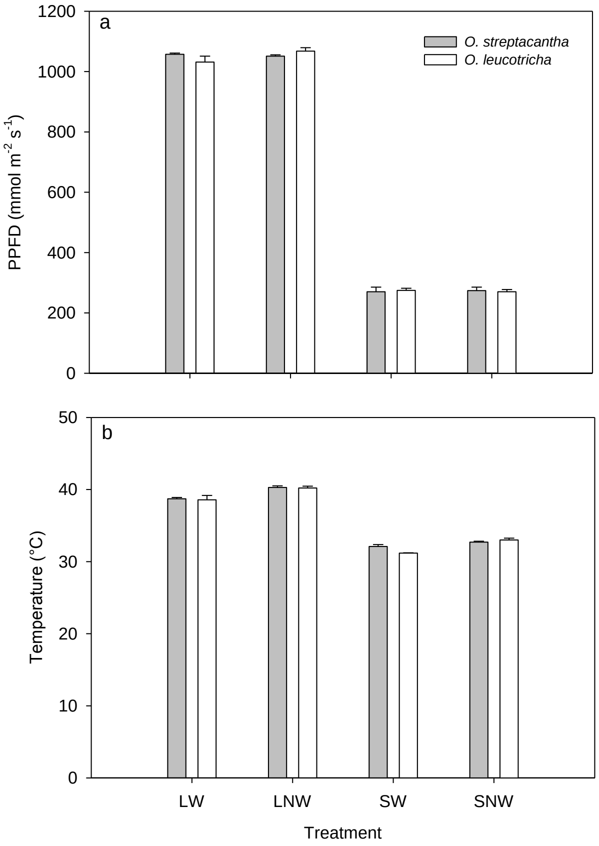

Figure 5. Environmental conditions that receive *Opuntia streptacantha* and *Opuntia lecuotricha* seedlings under combined water and light treatments at 30 and 45 days. a) Photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) and, b) temperature (°C). Bars represent the means ± SE (n = 5).

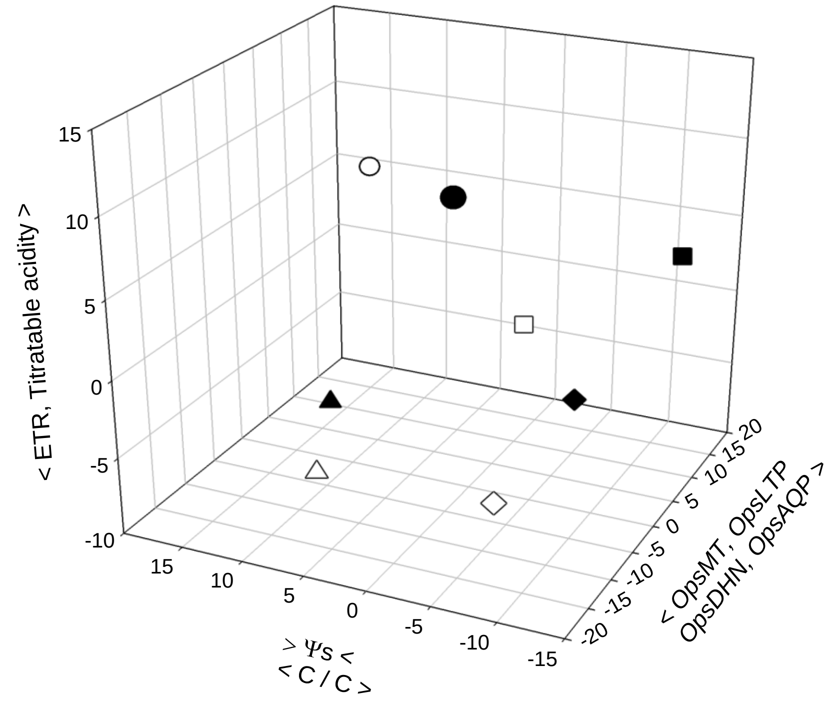

Figure 6. Ordination of treatments using canonical discriminant functions by mean class variables. ○) Light – Watering at 30 d; ●) Light – Watering at 60 d; □) Light – Non-watering at 30 d; ■) Light – Non-watering at 60 d; ∆) Shade – Watering at 30 d; ▲) Shade – Watering at 60 d; ◊) Shade – Non-watering at 30 d; and ♦) Shade – Non-watering at 60 days of treatment. Metallothionein (*OpsMT*), lipid transfer protein (*OpsLTP*), dehydrin (*OpsDHN*), aquaporin (*OpsAQP*), osmotic potential (Ψ_s), chlorophylls/carotenoids ratio (C/C), titratable acidity, and electron transport rate (ETR).

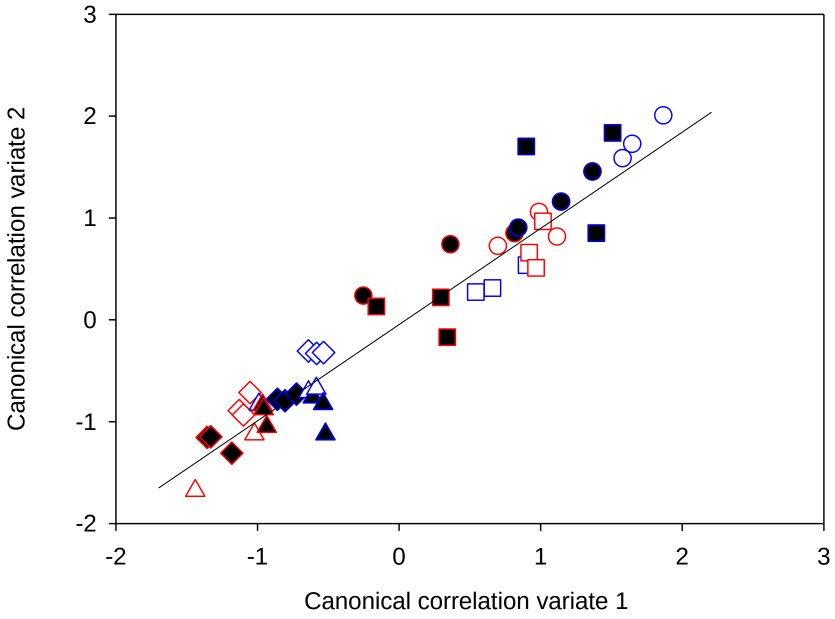

Figure 7. Scatter plot showing the relationship between the first pair of canonical variables canonical correlation. Treatments: Light – Watering (circles); Light – Non-watering (square); Shade – Watering (triangle), and Shade – Non-watering (diamond). Seedlings: *Opuntia leucotricha* (white) and *Opuntia streptacantha* (black). Harvests: 30 days of treatment (red outlines) and 60 days of treatments (blue outlines).

CAPÍTULO VII

Respuestas ecofisiológicas estacionales en tres especies de *Opuntia* del desierto Chihuahuense

Capítulo que será publicado en el libro Fisiología Ecológica en México: Estrategias de respuesta al ambiente, diversidad funcional y cambio climático.

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Resumen

Se realizaron evaluaciones del contenido de acidez, de pigmentos (clorofilas a y b, clorofila total, proporción clorofila a/b, carotenoides) y de potencial osmótico (Ψ_S) durante las cuatro estaciones del año 2009, en *Opuntia streptacantha*, *O. leucotricha* y *O. robusta*, en el sur del desierto chihuahuense. Además, en las caras Este y Oeste de los cladodios se midió la eficiencia cuántica del PSII (Φ_{PSII}), la tasa de transporte de electrones (ETR) y la disipación de energía no fotoquímica (NPQ). Mediante un análisis discriminante canónico se observó que las variables más importantes fueron el Ψ_S al pre-amanecer y el NPQ en las distintas horas del día (6 a 18 h) y la acidez (función 1, 69.9% de la varianza explicada), así como la Φ_{PSII} a las 9 horas (función 2, 13% de la varianza explicada). Las especies responden de manera diferente durante las estaciones del año dependiendo de la orientación del cladodio; en otoño, época de lluvias, se mostraron menos estresadas. En cambio, mostraron mayor estrés en invierno y primavera, cuando las heladas son frecuentes y la sequía es común. Las especies fueron negativamente afectadas por la estacionalidad para las variables potencial osmótico, disipación no fotoquímica y acidez. Se encontraron valores más bajos de Ψ_S (mayor estrés hídrico) en primavera, siendo *O. leucotricha* la especie más afectada, seguida de *O. streptacantha* y *O. robusta*. Verano y otoño fueron las estaciones en las que las plantas tuvieron mayor disponibilidad de agua. La acidez mostró un comportamiento similar al potencial osmótico, con mayor acidez en verano y otoño en las tres especies, pero principalmente en *O. streptacantha.* Además, en primavera se presentó mayor disipación no fotoquímica o disipación de calor (NPQ). En cambio, en otoño se registraron los valores más bajos de NPQ, siendo esta estación donde la disponibilidad de agua es mayor y existe una menor incidencia de radiación solar. Además, en la cara Oeste de los cladodios de *O. leucotricha* y *O. robusta* hubo mayor NPQ que en la cara Este. En general para las tres especies, las caras Oeste de los cladodios presentaron menor Φ_{PSII} en Otoño, Verano e Invierno que las caras Este. En cambio, en primavera las caras Oeste mostraron mayor Φ_{PSII} que las caras Este. Todas las especies mostraron fotoinhibición al mediodía pero se recuperaron al final del día. De manera general, se encontró que al aumentar la temperatura en el día disminuye el potencial osmótico y la eficiencia cuántica del fotosistema II (Φ_{PSII}), pero aumenta la disipación no fotoquímica (NPQ) a lo largo del día. En general para las tres especies, el potencial osmótico, la acidez titulable y la eficiencia cuántica del fotosistema II (Φ_{PSII}) disminuyen en la época de sequía, pero aumenta la disipación no fotoquímica (NPQ) como mecanismo para tolerar el estrés.

Palabras clave: acidez, eficiencia cuántica, nopaleras, pigmentos, plantas CAM

Introducción

El Altiplano Potosino-Zacatecano comprende gran parte de los estados mexicanos de San Luis Potosí y Zacatecas, así como porciones de los estados de Aguascalientes, Durango, Guanajuato, Jalisco y Querétaro, en la parte sur del desierto chihuahuense. Esta región se caracteriza por la presencia de extensas áreas en las que la comunidad vegetal está constituida por especies del género *Opuntia* (Marroquín *et al.*, 1964), constituyendo formaciones conocidas como “nopaleras” (Miranda y Hernández-Xolocotzi, 1963; Janzen, 1986). Las principales especies son *O. streptacantha* Lem, *O. robusta* H.L. Wendl. y *O. leucotricha* D.C., con una densidad de 596 individuos - 1,852 individuos ha$^{-1,}$ 138 individuos ha^{-1} y 112 individuos ha^{-1}, respectivamente (Muñoz-Urías *et al.*, 2008).

La región del Altiplano Potosino-Zacatecano se caracteriza por sus condiciones típicas de ambientes semiáridos: temperaturas extremas, baja precipitación y alta radiación solar. En esta zona la estación seca puede tener una duración de hasta 6 - 8 meses y es interrumpida por lluvias durante el verano (Muñoz-Urías *et al.*, 2008). Así, las especies de *Opuntia* nativas deben presentar adaptaciones ecofisiológicas para tolerar diferentes tipos de estrés a lo largo de las estaciones del año; sin embargo, no existe evidencia sobre esto en la región.

Se ha realizado investigación sobre respuestas estacionales en especies del género *Opuntia* en desiertos estadounidenses principalmente. Por ejemplo, para *Opuntia humifusa* en Iowa, se encontró actividad CAM solamente de abril a septiembre, sin importar los cambios de temperatura o precipitación que se tuvieron en ese tiempo (Koch y Kennedy, 1980) y para *Opuntia erinacea*, en el sureste del estado de Washington, USA, se encontraron niveles menores de acidez en invierno que en verano (Littlejohn y Williams, 1983).

Dentro del género, la especie más estudiada bajo condiciones de estrés es *O. basilaris*. Para esta especie, Szarek y Ting (1974) encontraron mayor estrés hídrico y menor acumulación de acidez en verano y otoño cerca de Palm Desert, California (33˚ 30' N; 116˚ 07' W; 305 m. Por otra parte, para la misma especie pero en Death Valley, California (36˚59'34"N; 117°21'59"W; 750 m), Adams *et al.* (1987) encontraron que los niveles de acumulación nocturna ácida y de eficiencia cuántica máxima fueron bajos a lo largo del año, indicativo de fotoinhibición, pero los niveles de la última variable aumentaron después de lluvias de verano. Además, encontraron que en el otoño la eficiencia cuántica de la orientación sur fue menor que la de la orientación norte. Finalmente, reportaron que las concentraciones de clorofila cambiaron con las estaciones del año, disminuyendo en el verano. Así, se puede sugerir que la respuesta de las especies está determinada por el ambiente en el que se desarrollan.

En México se ha investigado muy poco sobre el comportamiento estacional e incluso diario de especies de *Opuntia* silvestres. Lerdau *et al.* (1992) encontraron que las plantas de *O. excelsa*, en Chamela, Jal., presentaron menores niveles de acidez en la estación seca que en la estación húmeda, lo que sugiere menor eficiencia fotosintética de esta especie en la época de sequía. Pimienta-Barrios *et al.* (2000) encontraron valores menores de toma de CO_2 neto diario positivo en el verano y mayores en otoño e invierno para *Opuntia ficus-indica*. Además, encontraron que en primavera, cuando la sequía fue mayor, la toma de CO_2 neto diario fue negativa. Sortibrán *et al.* (2005) evaluaron el comportamiento diario, pero no estacional, de *Opuntia puberula* en el Valle de Tehuacán. Dichos autores encontraron que la acumulación ácida nocturna incrementó con la densidad de flujo de fotones para la fotosíntesis diario interceptado.

En las nopaleras del Altiplano Potosino-Zacatecano no se han realizado estudios ecofisiológicos de este tipo. Así, se realizó un experimento con el fin de determinar las adaptaciones ecofisiológicas que poseen tres de las especies de *Opuntia* más comunes del sur del desierto chihuahuense para tolerar diferentes tipos de estrés a lo largo de las estaciones del año. Se realizaron evaluaciones del contenido de ácido málico, de pigmentos (clorofilas a y b, clorofila total, carotenoides) y de potencial osmótico (Ψ_S) durante las cuatro estaciones del año, en *Opuntia streptacantha*, *O. leucotricha* y *O. robusta*. Además, en las caras Este y Oeste de los cladodios se midió la eficiencia cuántica del PSII (Φ_{PSII}), la tasa de transporte de electrones (ETR, por sus siglas en inglés) y la disipación de energía no fotoquímica (NPQ, por sus siglas en inglés). Se escogió evaluar las caras planas con exposición Este y Oeste con base en Adams *et al.* (1989), quienes sugirieron tomar en cuenta las caras que reciben la luz directa del sol por la mañana y por la tarde, en este caso las caras Este y Oeste, respectivamente. También se evaluaron variables de respuesta ambientales, como la densidad de flujo de fotones para la fotosíntesis (DFFF) y la temperatura ambiental (°C).

Materiales y métodos

Especies de estudio

Opuntia leucotricha, O. robusta y *O. streptacantha* son las especies más comunes en las nopaleras del Altiplano Potosino-Zacatecano. Además, son importantes debido a sus usos alimenticio, medicinal y forrajero (Bravo-Hollis, 1978).

Las tres especies se distinguen fácilmente por sus características morfológicas. *Opuntia leucotricha* es una planta arborescente de 3 a 5 m de altura, con tronco más o menos bien definido y ramoso, cubierto con pelos setosos, blancos y flexibles; sus cladodios son de forma oblonga, de 15 a 30 cm de largo y de epidermis pubescente. *Opuntia robusta* es arbustiva, muy ramificada, de 1 a 2 m de altura; su tronco está más o menos definido, con ramas como de 1.5 m de largo y cladodios orbiculares o algo oblongos, de 15 a 40 cm de longitud o más. *Opuntia streptacantha* es arborescente, muy ramificada, hasta de 5 m de altura, con tronco bien definido, a veces hasta 45 cm de diámetro; sus cladodios tienen formas obovados hasta orbiculares, de 25 a 30 cm de longitud (Britton y Rose, 1963; Bravo-Hollis, 1978).

Área de estudio

El área de estudio está localizada en un predio privado del municipio de Mexquitic de Carmona, San Luis Potosí (22°16' 20.37''N; 101°06' 41.70''O), en el cual se encuentran las tres especies de *Opuntia*. Debido a su ubicación, posee un clima definido como seco estepario templado en el sistema de Köppen (Pimienta-Barrios, 1990). La temperatura media anual es de 16.7°C, la máxima extrema se registra en el mes de mayo (40°C) y la mínima extrema se registra en diciembre (-8.5°C). Las lluvias ocurren entre los meses de abril y septiembre, con un promedio de 422.7 mm (Medina-García et al., 2005) (Figura 1). En esta localidad se seleccionaron cinco plantas adultas de cada una de las tres especies (*Opuntia leucotricha, O. robusta* y *O. streptacantha*) presentes, y se marcaron las caras Este y Oeste de un cladodio por planta. La cara Este recibe la luz directa del sol por la mañana y la cara Oeste durante la tarde.

Evaluación de fluorescencia de la clorofila

Se realizaron cuatro evaluaciones, una en cada estación del año (primavera, verano, otoño e invierno). Durante el día se hicieron registros de las variables cada tres horas (6, 9, 12, 15 y 18 h) en cinco individuos de cada especie. Las variables de fluorescencia de la clorofila evaluadas fueron la eficiencia cuántica de fotosistema II (Φ_{PSII}), la disipación no fotoquímica (NPQ, por sus siglas en inglés) y la tasa de transporte de electrones (ETR, por sus siglas en inglés) con un analizador de cosecha de fotosíntesis MINI-PAM (Heinz Walz GmbH®, Germany). Con el mismo esquipo se midió la densidad de flujo de fotones para la fotosíntesis (FFF) y la temperatura ambiental (T°C).

Determinación de ácido málico

La fluctuación diurna de pH (∆ acidez) se determinó a las 18 h y 6 h del día en cada estación del año. Se tomaron muestras de cinco plantas de cada especie con ayuda de un sacabocados y se congelaron en N_2 líquido, la determinación se realizó mediante el método empleado por Zotz y Andrade (1998), el pH fue medido con un potenciómetro HORIBA® Modelo pH Meter F-52. Se empleó una solución de NaOH 0.01 N para realizar la titulación y calcular la concentración de mmol/H^+ m^{-2} de tejido.

Cuantificación de pigmentos

Para determinar la concentración de clorofila *a*, *b*, total y carotenoides totales se colectaron muestras en cada estación del año de cinco individuos de cada especie a las 6 h del día, las muestras fueron cubiertas para evitar la degradación de los pigmentos por la exposición a la luz y se transportaron en frío en tubos Eppendorf de 2 ml. Los pigmentos fueron extraídos de acuerdo al procedimiento de Hendry y Price (1993), la absorbancia fue determinada con un espectrofotómetro (Thermo Scientific®, Modelo Spectronic Genesys 10 Bio), leyendo las muestras para clorofila a 645 nm y 663 nm, y para carotenoides totales a 470 nm.

Determinación del potencial osmótico (Ψ_S)

En cada estación del año se tomaron cinco muestras de cladodio para cada especie con un saca bocados a las 6 h del día. Las muestras fueron congeladas en nitrógeno líquido, transportadas al laboratorio para hacer las mediciones con un microvoltímetro de punto de rocío (Modelo HT-33T; Wescor®, Inc.; Logan, Utah) y cámaras psicrométricas (modelo C-52-SF; Wescor, Inc.; Logan, Utah). Las muestras fueron maceradas, posteriormente se humedeció un disco de papel filtro con la muestra macerada y se colocó en las cámaras psicrométricas. Las unidades de medición se expresaron en valores negativos de MPa.

Análisis estadísticos

Los datos registrados se analizaron mediante un análisis canónico discriminante que permite identificar las diferencias entre las interacciones de las dos caras del cladodio (este y oeste) con las diferentes estaciones del año (primavera, verano, otoño e invierno) en las tres especies (*O. leucotricha*, *O. robusta* y *O. streptacantha*). Este análisis emplea las mediciones de las variables ecofisiológicas de los individuos (Δ acidez, clorofila *a*, clorofila *b*, clorofila total, proporción clorofila *a*/*b*, carotenoides, Ψ_S, Φ_{PSII}, ETR y NPQ) y estima las funciones lineales de las variables cuantificadas, separando los grupos de individuos al maximizar la varianza entre los grupos y reducir al mínimo la varianza dentro de ellos.

Los coeficientes canónicos estandarizados muestran la contribución de las variables conjuntas analizadas para cada función canónica, e indican individualmente la importancia relativa de cada variable (Pires Da Silva et al., 2001; McGarigal *et al.*, 2000). También se aplicó un análisis discriminante de clasificación para proporcionar el número y porcentaje de entidades clasificadas correctamente o incorrectamente en cada grupo combinado. Los comandos utilizados para estos análisis fueron PROC CANDISC y PROC DISCRIM. Para analizar las relaciones entre los dos grupos de variables ecofisiológicas y ambientales se aplicó un análisis de correlación canónica simple PROC CANCORR (McGarigal *et al.*, 2000). Todos los análisis estadísticos fueron realizados con el software SAS (SAS Institute Inc.).

Resultados

Análisis canónico discriminante

El análisis canónico discriminante mostró que tres funciones explican el 88.5% de la variación total, contribuyendo significativamente a la separación entre las interacciones de las dos orientaciones del cladodio con las diferentes estaciones del año de las tres especies de *Opuntia* spp. (Wilks' λ: $P < 0.0001$, $n = 120$). La primera función explica el 69.9 % de la variación total (valor característico de 83.23; $P < 0.0001$), la segunda función explica el 13 % (valor característico de 15.77; $P < 0.0001$) y la tercera función explica el 5 % de la variación (valor característico de 6.39; $P < 0.0001$). Las variables que contribuyen significativamente a las primeras dos funciones canónicas, las más importantes, se muestran en las Figuras 2-6. Las variables de la tercera función no se consideraron debido a que explican un porcentaje muy bajo de la variación. Además, en la Tabla 1 se muestran los coeficientes canónicos estandarizados, los cuales indican la importancia relativa de cada variable. Para la primera función las variables más importantes fueron el Ψ_S al pre-amanecer y el NPQ en las distintas horas del día (6 a 18 h), así como la acidez, y para la segunda función fue la eficiencia cuántica (Φ_{PSII}) a las 9 horas. Las medias de clase para cada variable se muestran en la Figura 7.

De acuerdo con el análisis, en la primera función que explica la mayor parte de la variación, las especies se ven afectadas por la estacionalidad para las variables potencial osmótico, disipación no fotoquímica y acidez. Se encontraron valores más bajos de Ψ_S (mayor estrés hídrico) en primavera, siendo *O. leucotricha* (-1.59 ± 0.02 MPa) la especie más afectada, seguida de *O. streptacantha* (-1.42 ± 0.03 MPa) y *O. robusta* (-1.22 ± 0.05 MPa). Verano y otoño fueron las estaciones en las que las plantas tuvieron mayor disponibilidad de agua, mostrando Ψ_S de -0.5 a -0.55 MPa (Figura 2). La acidez mostró un comportamiento similar al potencial osmótico, con mayor acidez en verano y otoño en las tres especies, pero principalmente en *O. streptacantha.* Además, en primavera se presentó mayor disipación no fotoquímica o disipación de calor (NPQ). En cambio, en otoño se registraron los valores más bajos de NPQ (Figura 5), siendo esta estación donde la disponibilidad de agua es mayor y existe una menor incidencia de radiación solar. Además, en la cara Oeste de los cladodios de *O. leucotricha* y *O. robusta* hubo mayor NPQ que en la cara Este.

En la segunda función, las especies se ven afectadas en la eficiencia cuántica del fotosistema II (Φ_{PSII}) por la estacionalidad. Esta variable fue más afectada a las 9 h, en las estaciones de primavera, verano e invierno. En general para las tres especies, las caras Oeste de los cladodios fueron más afectadas en Otoño, Verano e Invierno que las caras Este. En cambio, en primavera las caras Oeste mostraron mayor Φ_{PSII} que las caras Este.

El análisis de clasificación discriminante mostró que los centroides de cada grupo fueron significativamente diferentes ($P < 0.0001$), en donde las interacciones (especie/estación/cara) se clasificaron cinco al 100 %, nueve al 80 %, siete al 60%, uno al 40%y dos al 20%. Las cinco clasificadas al 100% son las interacciones *O. streptacantha*/primavera/cara Este, *O. leucotricha*/primavera/cara Este y Oeste, *O. streptacantha*/invierno/cara Este y *O. robusta*/otoño/cara Este.

Análisis de correlación canónica

Los datos sobre el primer par de variables canónicas (cargas canónicas cruzadas) aparecen en la Tabla 2, mostrando las correlaciones entre las variables originales y las variables canónicas y los coeficientes estandarizados de las variables canónicas. La proporción de la varianza y redundancia indican que el primer par de variables está muy relacionado. La primera correlación canónica para las tres especies agrupadas mostró que una correlación canónica de 0.90 ($\lambda < 0.0001$), redundancia de 0.40 y el 57.25% de la variabilidad en NPQ, potencial osmótico, acidez titulable y eficiencia fotosintética a las 9:00 horas es explicado por las características de DFFF y temperatura ambiental a lo largo del día; para *O. leucotricha* fue de 0.42 ($\lambda < 0.0001$), 0.27 y el 35.7%; para *O. robusta* de 0.94 ($\lambda < 0.0001$), 0.50 y el 65.5% y para *O. streptacanthta* de 0.93 ($\lambda < 0.0001$), 0.26, el 35.0 %. Para las especies agrupadas se encontró que al aumentar la temperatura en el día disminuye el potencial osmótico y la eficiencia cuántica del fotosistema II (Φ_{PSII}), pero aumenta la disipación no fotoquímica (NPQ) a lo largo del día. Al analizar las especies por separado se encontraron resultados distintos. Para *O. leucotricha* y *O. robusta* únicamente se correlacionaron las variables de disipación de calor con la temperatura a las distintas horas del día, excepto a las 9:00 horas. *Opuntia streptacanthta* mostró correlación negativa entre potencial osmótico y las variables disipación de calor y temperatura a las distintas horas del día.

Las especies responden de manera diferente durante las estaciones del año dependiendo de la orientación del cladodio, siendo menos afectadas en otoño, época de lluvias, que en invierno y primavera, cuando se presentan heladas y sequía. Las tres especies responden de manera similar al gradiente de la DFFF y la temperatura en el transcurso del día y a lo largo del año, aunque *O. robusta* presenta correlación positiva débil entre acidez y las variables canónicas ambientales.

Discusión

Se sabe que las especies de *Opuntia* toleran la sequía al poseer un alto uso eficiente de agua (Nobel, 1988; North y Nobel, 1992; Pimienta-Barrios *et al.*, 2002; 2003; Zañudo-Hernández *et al.*, 2010). En este estudio se evaluó el potencial osmótico de los cladodios como una estimación del estrés hídrico y fue la variable más importante de la primera función del análisis estadístico. En verano y otoño, las estaciones del año con mayor precipitación, las tres especies de *Opuntia* mantuvieron altos potenciales osmóticos, a la vez que mostraron mayor contenido de acidez. Sin embargo; durante la sequía del invierno y principalmente de la primavera disminuyó la acidez y el potencial osmótico de las tres especies. El comportamiento de las especies fue distinto, la especie menos afectada en el potencial osmótico fue *O. robusta*, seguida de *O. streptacantha* y *O. leucotricha*. El aumento de la acidez y la disminución del potencial osmótico en la época de sequía en las tres especies es similar a lo encontrado para *O. basilaris* (Szarek y Ting, 1974; Hanscom y Ting, 1978). Para *O. erinacea*, en el sureste del estado de Washington, USA, se encontraron niveles menores de acidez en invierno que en verano, estaciones del año que no muestran diferencias en precipitación pero sí en temperatura, dado que hay nevadas que provocan estrés en las plantas (Littlejohn y Williams 1983). Además, Lerdau et al. (1992) encontraron que las plantas de *O. excelsa*, en Chamela, Jal., presentaron menores niveles de acidez en la estación seca que en la estación húmeda, lo que sugiere menor eficiencia fotosintética de esta especie en la época de sequía.

Otra variable que disminuyó en primavera, la época de mayor sequía, es el contenido de pigmentos; sin embargo, no fue tan importante en el análisis realizado. Adams *et al.* (1987) reportaron que las concentraciones de clorofila cambiaron con las estaciones del año, disminuyendo en la sequía del verano. También se ha encontrado disminución de pigmentos en *Opuntia* spp. por alta intensidad de luz (Barker y Adams, 1997), estos mismos autores realizaron un estudio comparativo entre pigmentos (clorofilas totales y carotenoides) y encontraron que los últimos son los más importantes y que además están correlacionados con la disipación no fotoquímica (NPQ), siendo un mecanismo que utilizan estas especies para tolerar condiciones de estrés por alta radiación solar. Precisamente la NPQ a lo largo del día también fue importante para la primera función del análisis de nuestros resultados, con valores más altos en Primavera (sequía) y más bajos en otoño, estación en la que ocurren la mayor parte de las lluvias y en la cual se tiene menor incidencia de radiación solar. Además, en la cara Oeste de los cladodios de *O. leucotricha* y *O. robusta*, en donde se recibe mayor luz del sol por la tarde, hubo mayor NPQ que en la cara Este. Es decir, que en la cara Oeste hubo mayor radiación solar y las plantas mostraron mayor disipación de calor como mecanismo para tolerar la alta radiación solar. Este resultado es similar al obtenido por Adams III *et al.* (1989) para *Nopalea cochenillifera*, *Opuntia ficus-indica* y *Opuntia wentiana*, en el norte de Venezuela. Esto autores mencionan que la disipación no fotoquímica es un proceso importante en plantas CAM bajo condiciones naturales, y que puede reflejar un mecanismo de protección contra el potencial daño de los efectos de la acumulación de energía de fotones absorbida por los cladodios, particularmente bajo condiciones en las cuales la disponibilidad de CO_2 se encuentra restringida, como en la época de sequía y mayor radiación solar.

La variable más importante para la segunda función del análisis fue la eficiencia cuántica del fotosistema II (Φ_{PSII}) a lo largo del día. La Φ_{PSII} fue mayor en otoño (la época de lluvias) y menor en primavera, verano e invierno. En general para las tres especies, las caras Oeste de los cladodios fueron más afectadas en Otoño, Verano e Invierno que las caras Este. En cambio, en Primavera las caras Oeste mostraron mayor Φ_{PSII} que las caras Este. Lo anterior coincide con Adams *et al.* (1987) quienes encontraron que los niveles de eficiencia cuántica de *O. basilaris* fueron bajos a lo largo del año, indicativo de fotoinhibición, pero aumentaron después de lluvias de verano. También encontraron que en el otoño la eficiencia cuántica en la orientación Oeste fue menor que en las demás orientaciones. Así, se puede decir que la respuesta de las especies está determinada por el ambiente en el que se desarrollan.

En general para las tres especies agrupadas, al aumentar la temperatura en el día disminuyó el potencial osmótico y la eficiencia cuántica del fotosistema II (Φ_{PSII}), pero aumentó la disipación de calor a lo largo del día como mecanismo para tolerar el estrés. Al analizar las especies por separado los resultados fueron distintos. En las especies *O. leucotricha* y *O. robusta* la disipación no fotoquímica (NPQ) se correlacionó con la temperatura a las distintas horas del día, excepto a las 9:00 horas, lo cual implica que a esa hora las plantas no necesitan disipar calor porque no están estresadas. Para *O. streptacanthta* hubo correlación negativa entre potencial osmótico y las variables disipación de calor y temperatura a las distintas horas del día. Es decir, que al estar las plantas más estresadas por la sequía y las altas temperaturas, presentaban mayor disipación de calor.

En conclusión, en el análisis encontramos que las variables de respuesta más importante fueron el potencial osmótico y las relacionadas con fluorescencia de la clorofila (la eficiencia cuántica del fotosistema II (Φ_{PSII}) y la disipación no fotoquímica (NPQ) a lo largo del día), así como la acidez titulable. En general para las tres especies, el potencial osmótico, la acidez titulable y la eficiencia cuántica del fotosistema II (Φ_{PSII}) disminuyen en la época de sequía, pero aumenta la disipación no fotoquímica (NPQ) como mecanismo para tolerar el estrés.

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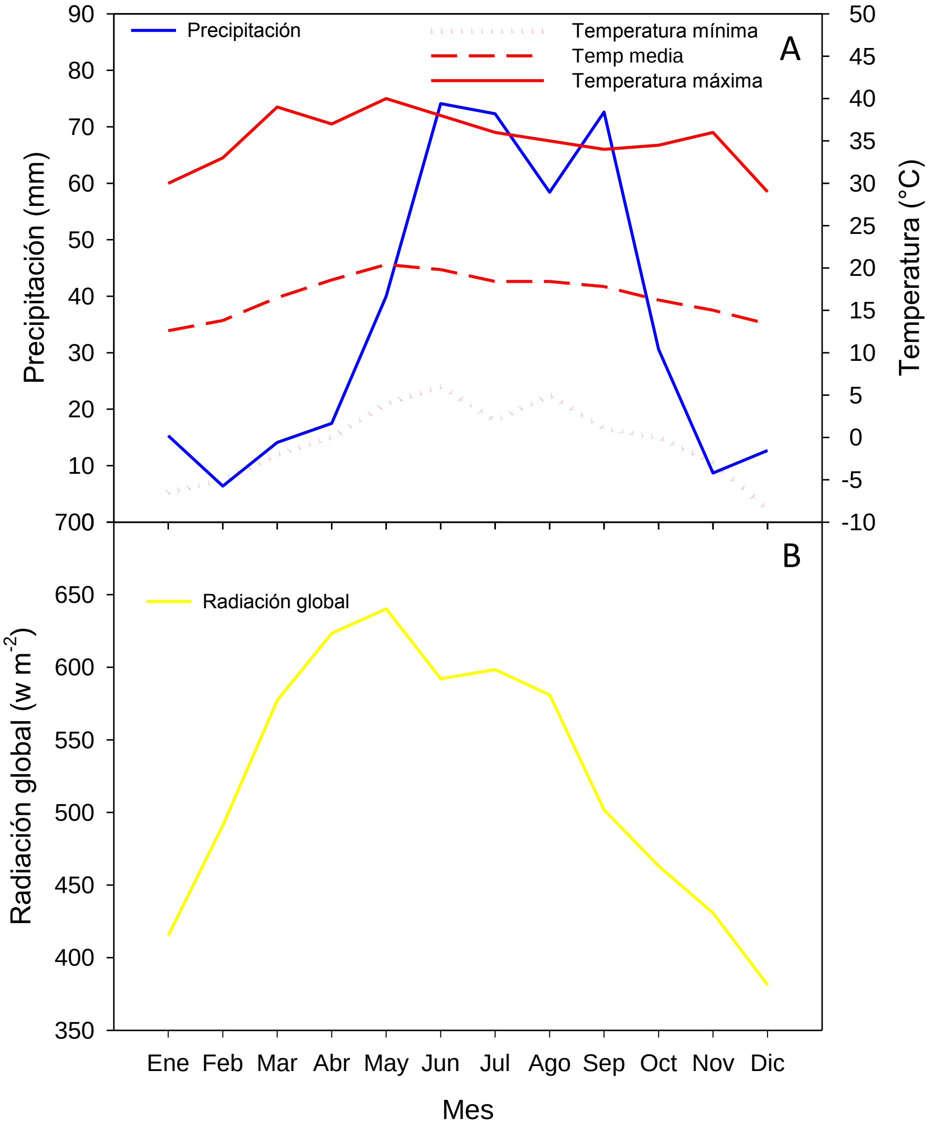

Figura 1. Condiciones ambientales durante el año. A) Precipitación total (mm) y Temperatura mínima, media y máxima (°C) y B) Radiación global (w m^{-2}).

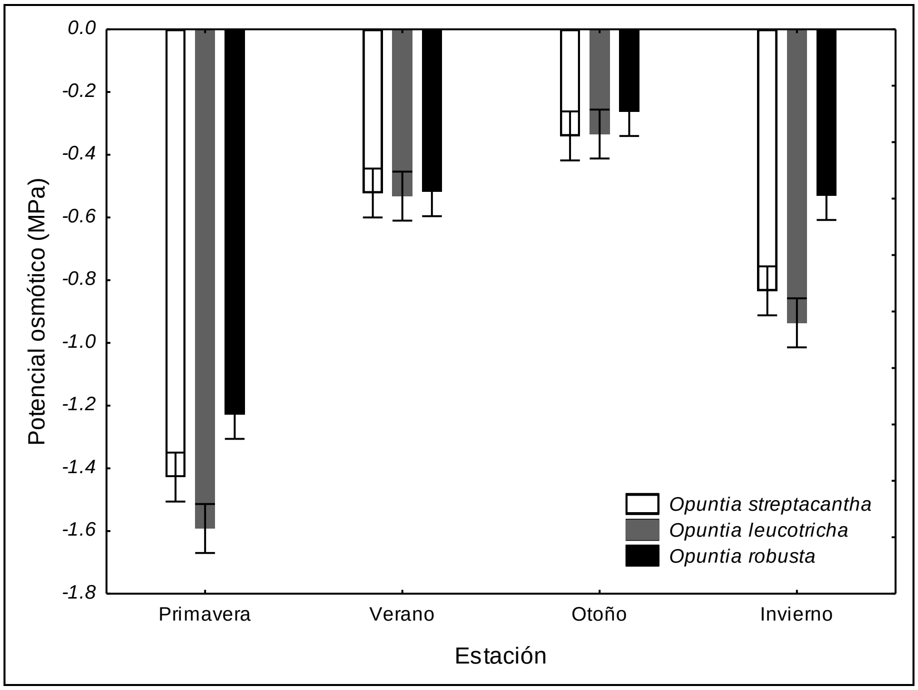

Figura 2. Evaluación estacional del potencial osmótico para tres especies de *Opuntia* en las cuatro estaciones del año.

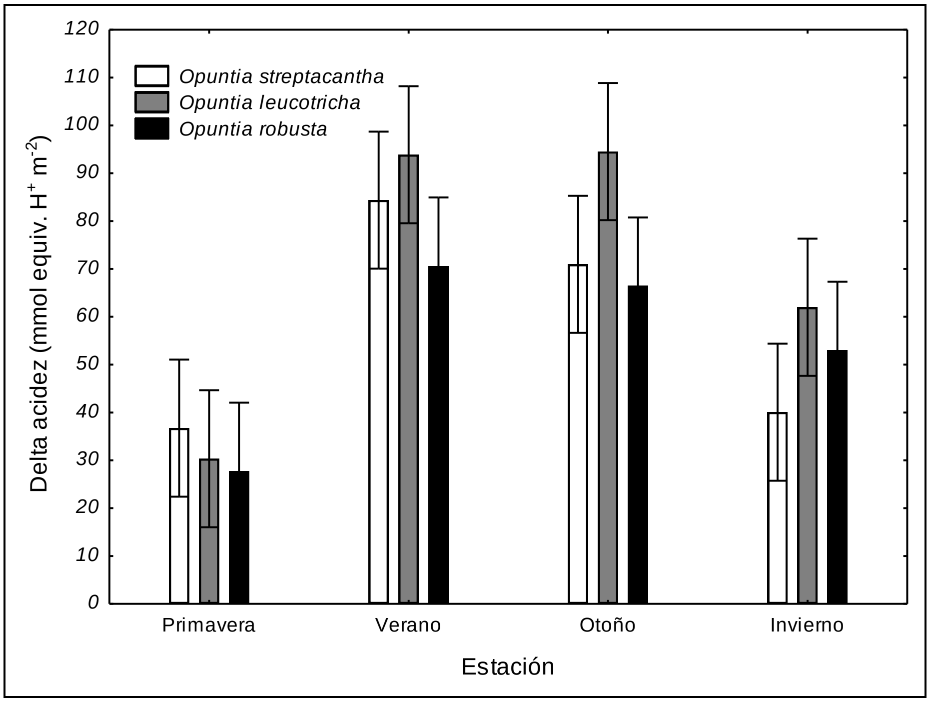

Figura 3. Cuantificación del ∆ acidez para tres especies de *Opuntia* en las cuatro estaciones del año (*n* = 5).

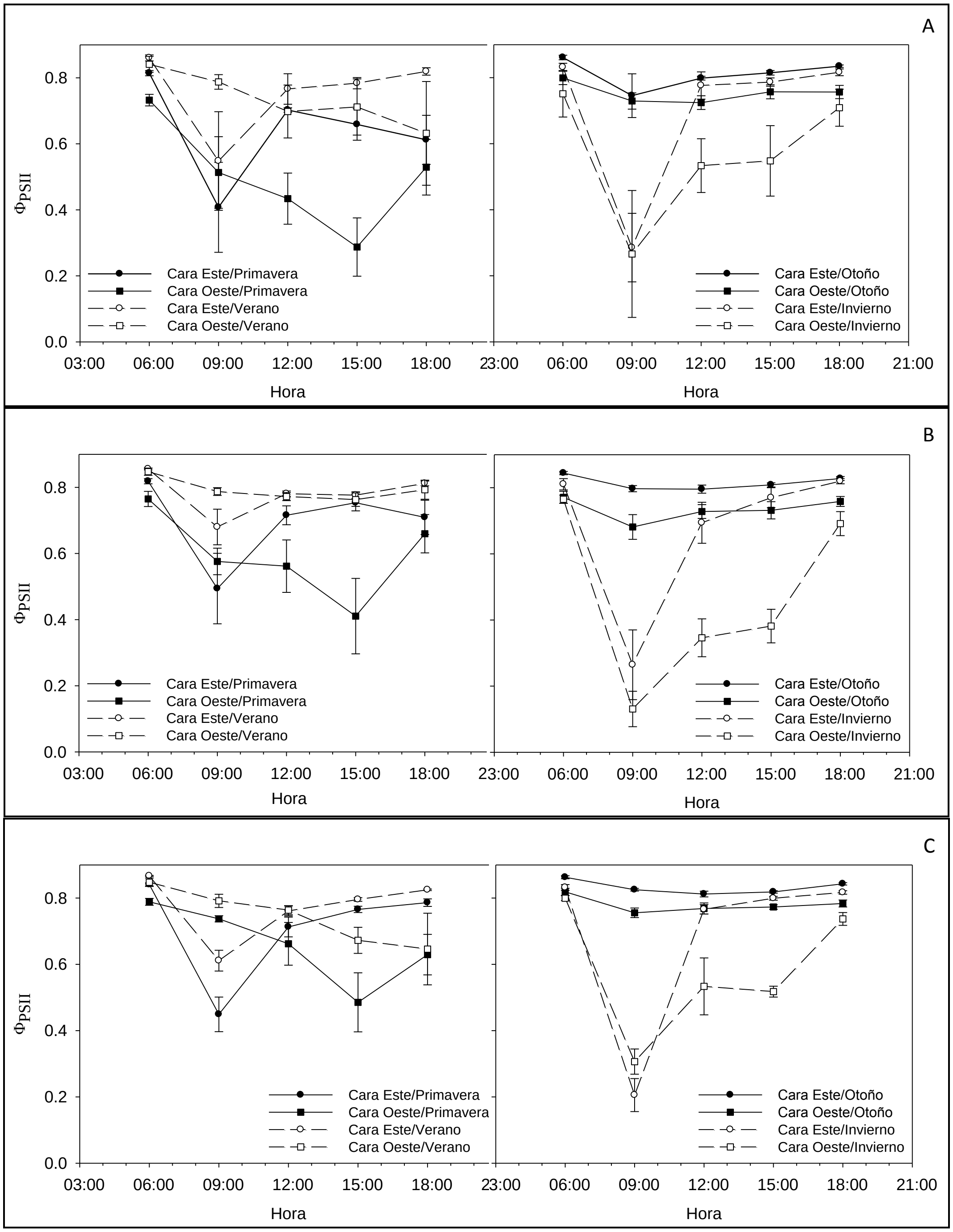

Figura 4. Curso estacional de la eficiencia cuántica del fotosistema II (Φ_{PSII}) en ambas caras planas del cladodio de tres especies: A) *Opuntia leucotricha*; B) *O. robusta* y C) *O. streptacantha*.

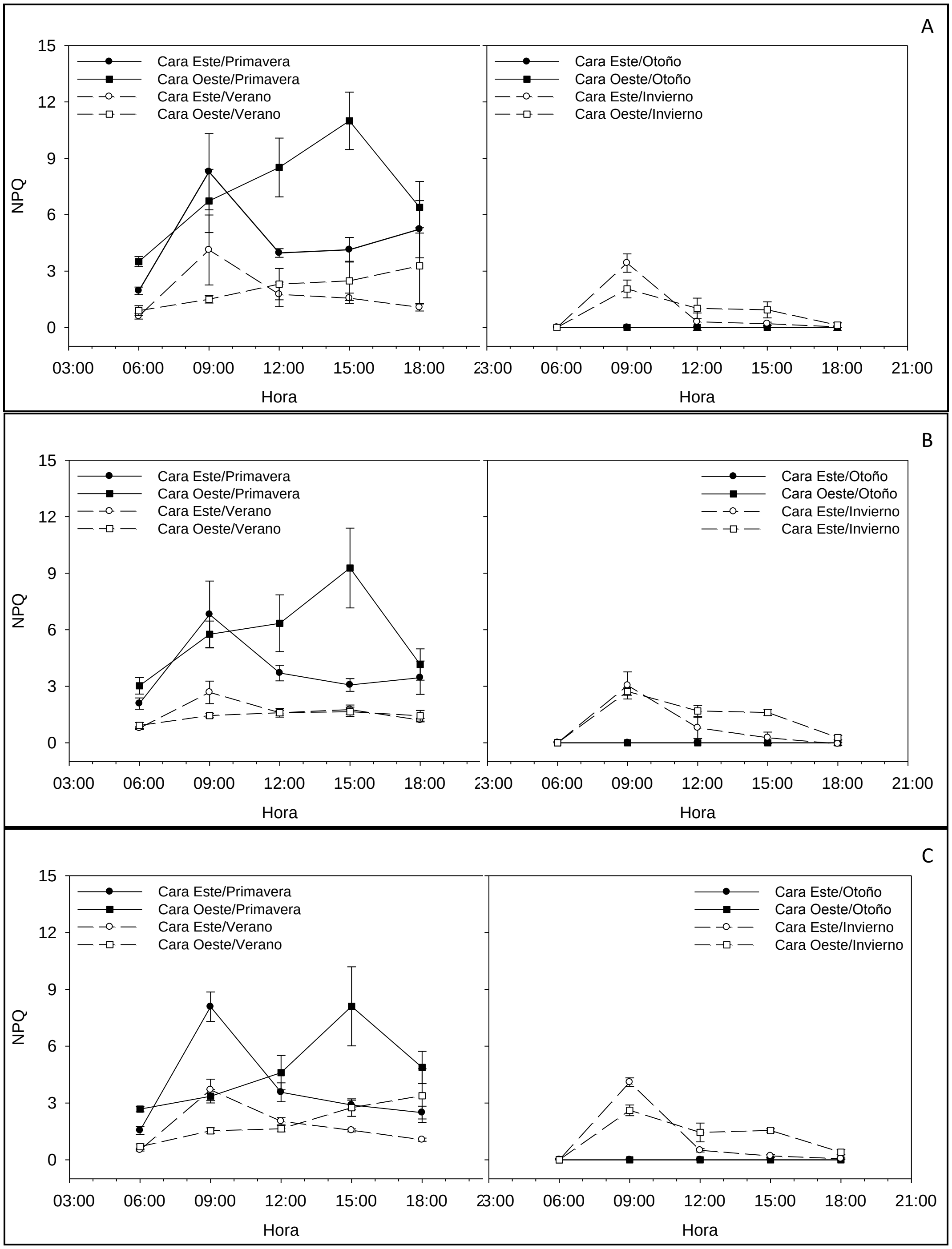

Figura 5. Evaluación estacional de la disipación no fotoquímica (NPQ, por sus siglas en inglés) en ambas caras planas del cladodio de tres especies: A) *Opuntia leucotricha*; B) *O. robusta* y C) *O. streptacantha*.

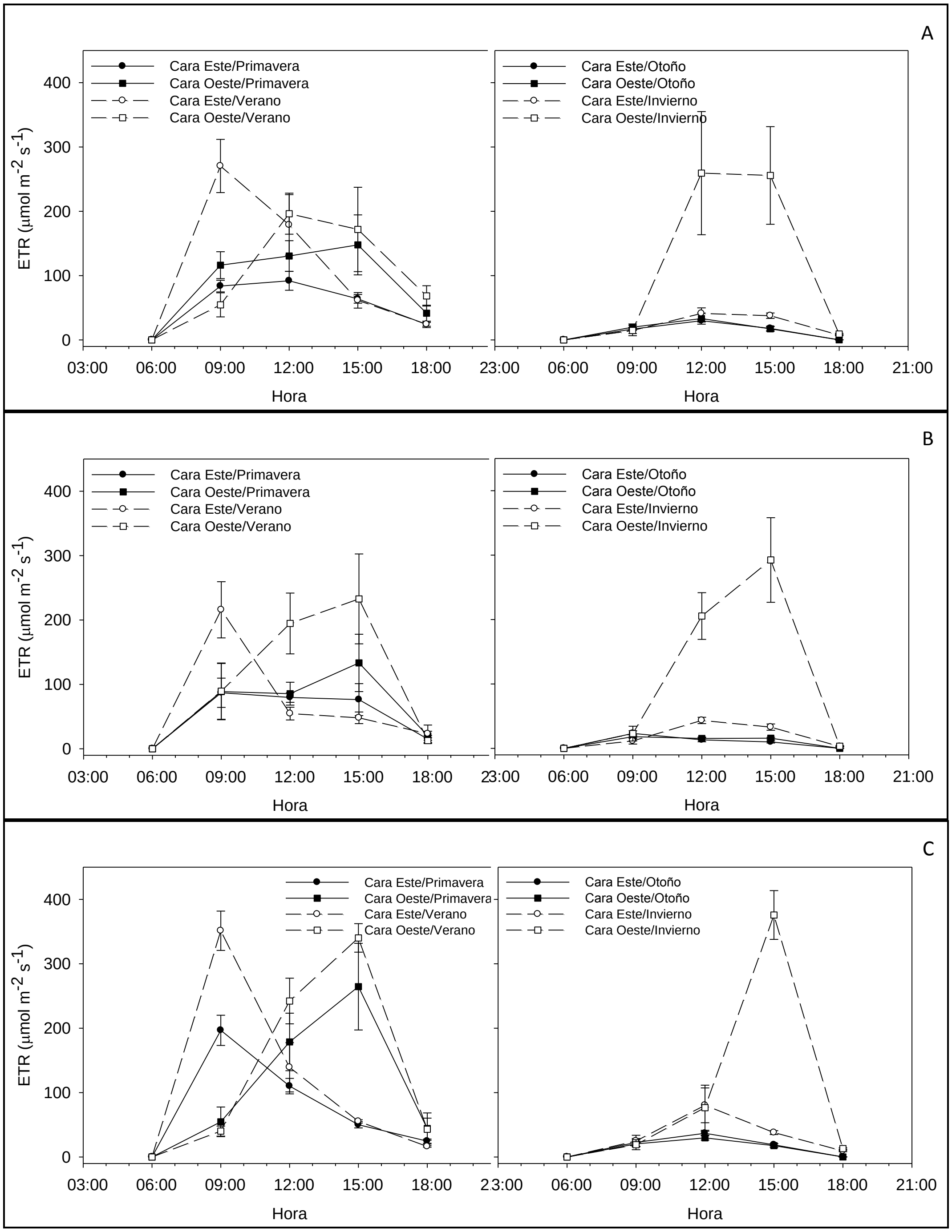

Figura 6. Evaluación estacional de la tasa de transporte de electrones (ETR, por sus siglas en inglés) en ambas caras planas del cladodio de tres especies: A) *Opuntia leucotricha*; B) *O. robusta* y C) *O. streptacantha*.

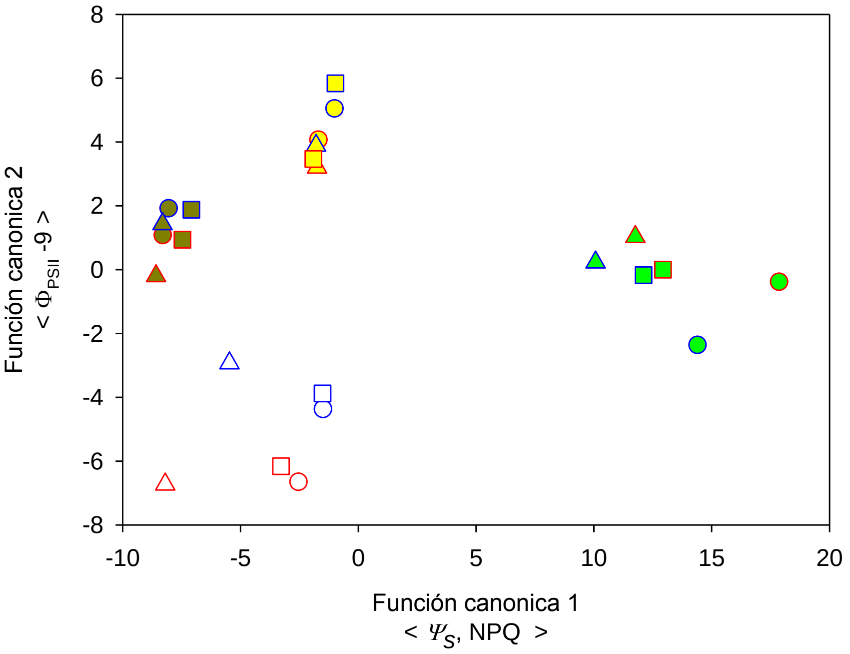

Figura 7. Ordenación usando dos funciones canónicas discriminantes para las medias de clase. *Opuntia leucotricha* (○); *O. robusta* (Δ) y *O. streptacantha* (□). Primavera (verde); Verano (amarillo); Otoño (café) e Invierno (blanco). Caras planas del cladodio con exposición Este (contorno azul) y con exposición Oeste (contorno rojo).

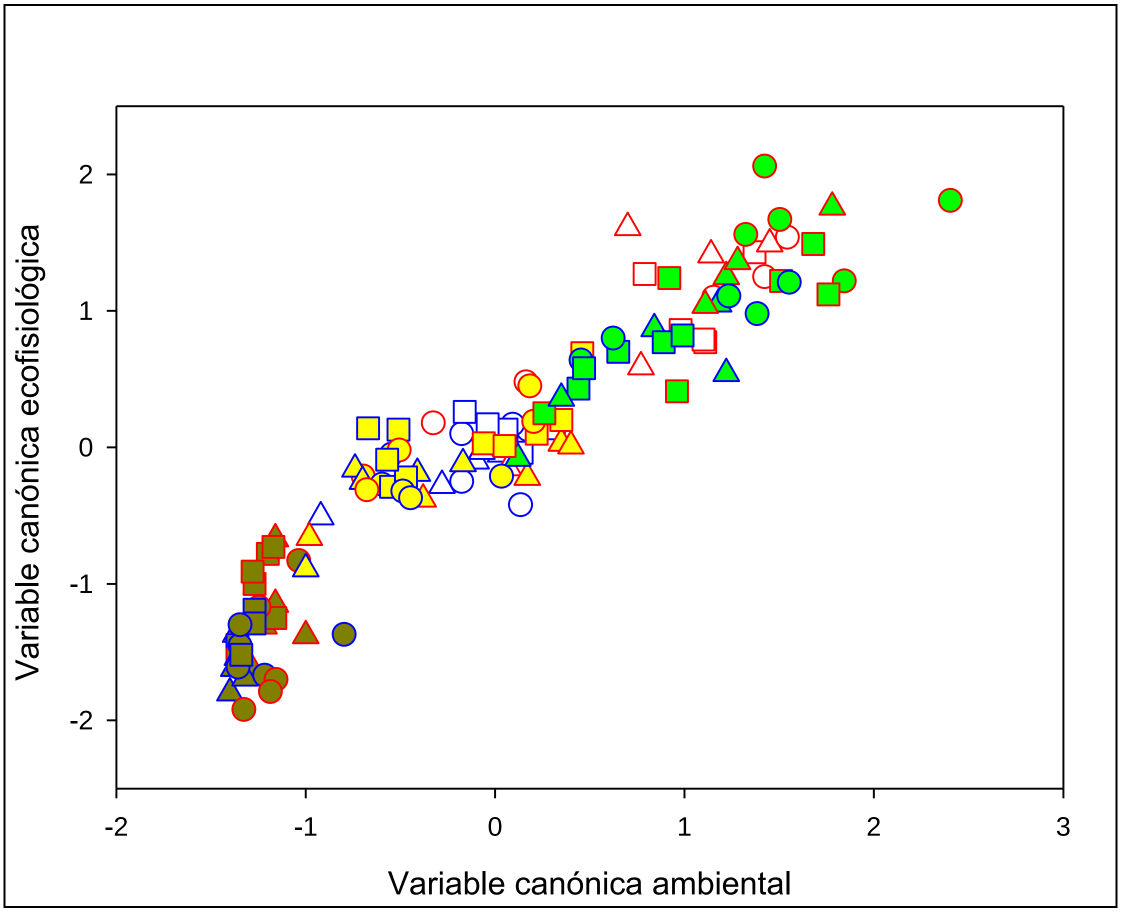

Figura 8. Correlación entre el primer par de variables canónicas. *Opuntia leucotricha* (○); *O. robusta* (Δ) y *O. streptacantha* (□). Primavera (verde); Verano (amarillo); Otoño (café) e Invierno (blanco). Caras planas del cladodio con exposición Este (contorno azul) y con exposición Oeste (contorno rojo).

Tabla 1. Coeficientes canónicos de la muestra total.

	Coeficientes de Función	
Variables	Función 1	Función 2
Potencial osmótico (Ψ_s)	-0.94*	0.31
Acidez titulable	-0.69*	0.54
Eficiencia cuántica del PSII (Φ_{PSII})- 9 horas	-0.13	0.81*
Eficiencia fotosintética - 12 horas	-0.28	0.58
Eficiencia fotosintética - 15 horas	-0.47	0.41
ETR-9 horas	0.32	0.56
ETR-12 horas	0.22	-0.01
ETR-15 horas	0.13	-0.30
NPQ-6 horas	0.93*	0.16
NPQ-9 horas	0.87*	-0.16
NPQ-12 horas	0.91*	0.04
NPQ-15 horas	0.85*	0.05
NPQ-18 horas	0.89*	0.17

* Variables con una alta contribución a la separación de los centroides entre muestras. ETR = Siglas en inglés de tasa de transporte de electrones; NPQ = Siglas en inglés de disipación no fotoquímica.

Tabla 2. Cargas canónicas cruzadas del primer par de variables canónicas para las tres especies agrupadas y para cada especie por separado.

Variables	**Tres especies**		***O. leucotricha***		***O. robusta***		***O. streptacantha***	
Ecofisiológicas	Correlación	Coeficientes estandarizados	Correlación	Coeficientes estandarizados	Correlación	Coeficientes estandarizados	Correlación	Coeficientes estandarizados
Acidez titulable	-0.276	0.183	-0.170	0.087	0.203	0.218	-0.492	0.243
Ψ_s	-0.663*	-0.141	-0.285	0.546	-0.316	0.208	-0.856*	-0.501
Φ_{PSII}-9 h	0.193	0.446	0.412	0.653	0.555*	0.693	0.151	0.297
NPQ-6 h	0.843*	0.294	0.728*	-0.439	0.706*	0.381	0.895*	0.371
NPQ-9 h	0.592*	0.643	0.373	1.090	0.292	0.641	0.687*	0.410
NPQ-12 h	0.686*	-0.043	0.554*	-0.099	0.593*	0.254	0.714*	-0.026
NPQ-15 h	0.672*	-0.103	0.653*	0.591	0.506*	-0.209	0.679*	-0.214
NPQ-18 h	0.801*	0.360	0.702*	0.634	0.662*	0.153	0.850*	0.194
Ambientales								
DFFF-9 h	0.076	-0.020	0.177	0.128	0.160	0.005	-0.079	-0.068
DFFF-12 h	-0.154	0.033	-0.246	-0.282	0.263	0.090	-0.278	-0.001
DFFF-15 h	-0.041	-0.010	0.028	0.123	-0.114	-0.061	-0.090	0.042
DFFF-18 h	0.305	0.074	0.447	0.151	0.289	0.097	0.283	-0.030
T-6 h	0.354	0.235	0.666*	0.779	0.599*	0.669	0.269	-0.020
T-9 h	0.791*	0.042	0.815*	-0.429	0.835*	0.021	0.806*	0.258
T-12 h	0.707*	-0.779	0.683*	-0.366	0.713*	-0.891	0.680*	-0.866
T-15 h	0.704*	-0.639	0.587*	0.492	0.584*	-0.230	0.779*	-0.950
T-18 h	0.837*	2.168	0.719*	0.821	0.697*	1.804	0.877*	2.396

Ψ_S = Potencial osmótico; Φ_{PSII} = Eficiencia cuántica del PSII; NPQ = Siglas en inglés de disipación no fotoquímica; DFFF = Densidad de flujo de fotones para la fotosíntesis; T = Temperatura del ambiente.

CAPÍTULO VIII

Discusión general

En zonas semiáridas y áridas existen factores ambientales estresantes como son sequía, alta radiación solar y temperaturas extremas, los cuales limitan el crecimiento y desarrollo de las plantas (Wickens, 1998; Rzedowski, 2006). En estas regiones son abundantes las plantas de la familia Cactaceae, las cuales presentan adaptaciones como el tipo de fotosíntesis conocida como CAM, por las siglas en inglés de “Crassulacean Acid Metabolism”, que se caracteriza por realizar la captura y almacenamiento de dióxido de carbono (CO_2) durante la noche, con el fin de evitar la pérdida de agua al abrir los estomas en el día, y este CO_2 es utilizado durante el día para realizar la fotosíntesis (Wickens, 1998; Dodd et al., 2002; Pimienta-Barrios et al., 2002).

Otras adaptaciones son la acumulación de pigmentos como los carotenoides, los cuales ayudan a la disipación de la radiación solar por medio de calor, y así evitar daños a los fotosistemas (Winter y Lesch, 1992); la alta cantidad de mucílago, compuesto por aproximadamente 47% de arabinosa, 23% de xylosa, 18% de galactosa, 7% de ramnosa y 5% de ácido galacturónico, el cual ayuda a reducir los efectos de la deshidratación en la época seca (Nobel et al., 1992; Cárdenas et al.,1997) y la cutícula cerosa que recubre las pencas y evita la pérdida de agua (Silva et al., 2001). Todas estas adaptaciones, en conjunto, hacen que las suculentas sean exitosas en los ambientes secos.

En el continente americano son comunes las plantas suculentas de la familia Cactaceae (Barthlott y Hunt, 1993; Stevens, 2001). El género *Opuntia* se distribuye desde Canadá hasta la Patagonia (Anderson, 2001; Cota-Sánchez, 2008) y es de los más frecuentes en regiones áridas y semiáridas (Wickens, 1998; Dodd et al., 2002). La forma más exitosa de propagación de plantas de nopal a corto plazo es la vegetativa, puesto que los cladodios tienen la capacidad de desarrollar tanto raíces como brotes a partir de las areolas, proveyendo diversas ventajas a los nuevos brotes para poder crecer y desarrollarse (Mandujano et al., 1996).

Por otro lado, la reproducción por semilla se da en periodos más largos, debido a que las semillas de *Opuntia* presentan latencia, formando bancos de semillas en el suelo. Esta es una adaptación que permite que con el tiempo las semillas pierdan la latencia y den origen a la aparición de nuevos individuos, sin embargo se conoce que el número de plántulas que puedan establecerse y llegar a la madurez es limitado (Mandujano et al., 1996). Por otra parte, las plántulas de *Opuntia* que llegan a emerger se enfrentan con los factores abióticos (sequía y alta radiación solar) que condicionan su desarrollo, por lo que su establecimiento se da con mayor frecuencia bajo plantas nodriza, las cuales proveen un microambiente menos estresante que en espacios abiertos (Yeaton, 1978; Yeaton y Romero-Manzanares, 1986; Vargas-Mendoza y González-Espinoza, 1992; Cody, 1993; Nabhan y Carr, 1994; Reyes-Olivas et al., 2002).

Aun cuando se ha reportado que las plántulas de *Opuntia* se pueden establecer bajo la protección de plantas nodriza, a la fecha no se sabe si las plántulas provenientes de semillas presentan las mismas respuestas de tolerancia bajo condiciones de estrés abiótico como lo hacen en estado adulto, en donde las pencas son grandes reservorios de agua (Stintzing y Carle, 2005). Pimienta-Barrios et al. (2007) analizaron el efecto de la sequía en cladodios jóvenes de plantas adultas de *Opuntia*, donde el cladodio madre (penca) asigna recursos y agua al nuevo brote, amortiguando los efectos del estrés.

Respecto a las plántulas de *Opuntia* provenientes de semillas, los efectos del ambiente pueden afectar de manera más directa la fisiología de las plántulas. Es por eso que en esta investigación el objetivo fue evaluar los mecanismos de respuesta a estrés por sequía, agregando una variable muy importante del ambiente, la alta incidencia de radiación solar, por lo que se incluyó en los tratamientos plántulas bajo sombra, simulando la sombra dada por las plantas nodriza. Los cuatro tratamientos fueron: 1) sombra con riego, 2) sombra sin riego, 3) luz con riego y 4) luz sin riego.

Con base en los resultados obtenidos, se puede concluir que las plántulas de *O. leucotricha* y *O. streptacantha* se establecen mejor bajo la sombra, puesto que junto con la activación de los mecanismos de tolerancia a la sequía de las plántulas, mitigan los efectos del estrés y pueden sobrevivir durante tiempos más prolongados. De manera contraria, las plántulas que se encuentran sin la protección de la sombra muestran mayor daño en los tres diferentes niveles evaluados (anatómicos, ecofisiológicos y moleculares), sin embargo las plántulas que no estaban bajo sombra toleraron hasta los 60 días de estrés, mediante la alta expresión de genes con confieren tolerancia al estrés, así como mayor tasa de transporte de electrones y alta disipación no fotoquímica (disipación de calor), siendo esto último una respuesta para ayudar a que la planta no presente fotoinhibición (Adams III et al., 1989).

En el caso de la evaluación anatómica, encontramos que las células de plántulas de *O. streptacantha* llevan a cabo un agrupamiento de cloroplastos hacia el centro de las células bajo condiciones de estrés hídrico y lumínico. Este es probablemente un mecanismo de protección para reducir el área de exposición a la luz solar y evitar asi el fotodaño por la alta incidencia de luz. Tal comportamiento se ha reportado en plantas suculentas como *Kalanchoë* spp. y *Zygocactus truncates*, una cactácea epifita (Kondo et al., 2004). En esta investigación, en los tratamientos bajo sombra, con y sin riego, el agrupamiento de cloroplastos no es tan evidente como en el caso de las plántulas que se encuentran expuestas a una combinación de alta incidencia de luz solar y sequía.

De todas las variables evaluadas (ecofisiológicas, moleculares y ambientales) en el estudio comparativo entre *O. leucotricha* y *O. streptacantha*, las más importantes y sensibles a los factores combinados de sequía y luz, fueron las moleculares, en donde los cuatro genes evaluados que codifican para una metalotioneina (*OpsMTs*), una proteína de transporte de lípidos (*OpsLTP*), una dehidrina SK_3 (*OpsDHN*) y una acuaporina (*OpsAQP*) presentaron mayor expresión en comparación con las plántulas que se encontraban bajo condiciones de riego. Estos genes se ha reportado que están involucrados en la tolerancia ante diversos tipos de estrés abiótico. Por ejemplo, en el caso de la metalotioneína y la proteína de transporte de lípidos, se ha reportado que ayudan a la eliminación de especies reactivas de oxígeno que se generan bajo estrés por sequía (Wong et al., 2004; George et al., 2007, 2010; Wang et al., 2009; Yang et al., 2009). Con respecto a las dehidrinas, se ha sugerido que están implicadas en la protección de proteínas y que evitan que se degraden (Shakirova et al., 2009). En un estudio reciente del grupo de investigación al que pertenezco, reportamos que esta dehidrina de *O. streptacantha* confiere tolerancia al estrés por frío (Ochoa-Alfaro et al., 2011). Para el caso de la acuaporina, se ha reportado que se induce en condiciones de estrés por sequía ayudando a una mayor captación de agua (Martre et al., 2002; Siefritz et al., 2002). Por lo tanto, es probable que los genes analizados en este estudio tengan una participación en la protección de moléculas esenciales y además participen en la destoxificación de compuestos dañinos que se generan por el estrés.

Las siguientes variables que presentaron también una importancia en la tolerancia de las plántulas de *Opuntia* fueron el Ψ_s, la proporción clorofila/carotenoides y, finalmente, la tasa de transporte de electrones y el contenido de acidez. De esta forma, se puede concluir que las plántulas de ambas especies se establecen mejor bajo la sombra, condiciones que simulan el microhábitat que se crea bajo plantas nodriza, aunque con diferencias entre especies, pues las de *O. streptacantha* presentaron mayor tolerancia a las condiciones de estrés por sequía y alta radiación solar. Este estudio es pionero al evaluar los mecanismos de tolerancia al estrés abiótico de plántulas de *Opuntia*, mediante mediciones ecofisiológicas y moleculares con el fin de profundizar en el conocimiento de los mecanismos implicados en las respuestas de plántulas del género *Opuntia*.

Además, durante los experimentos de germinación para la obtención de plántulas se encontró que la latencia de semillas de *O. streptacantha* y *O.*

leucotricha se rompe por medio de la acción de hongos como *Penicillium chrysogenum*, *Phoma* sp. y *Trichoderma* spp. Estos resultados son muy interesantes, debido a que los porcentajes de germinación logrados con el efecto de los hongos son muy altos incluso en colectas de semillas frescas, en donde el porciento de germinación sin la ayuda de estos hongos es casi nula. El rompimiento de latencia de semillas de *Opuntia* ha sido un tema recurrente y estudiado por varios grupos, en donde han empleando diferentes métodos físicos y químicos pero han obtenido porcentajes de germinación muy bajos (Mandujano et al., 2005, 2007; Orozco-Segovia et al., 2007; Ochoa-Alfaro et al., 2008). El modo de acción de los hongos sobre el rompimiento de la latencia de las semillas podría ser debido a la degradación de la testa por medio de enzimas, reduciendo la resistencia mecánica y de esta manera permitiendo la entrada de agua y la emergencia del embrión.

Estos resultados muestran que los hongos juegan un papel importante en el rompimiento de la latencia de semillas de especies de *Opuntia*, y contribuyen al entendimiento de la biología de la germinación de especies de cactáceas, abriendo también nuevas perspectivas sobre el efecto de los hongos en el rompimiento de la latencia de semillas de especies de zonas áridas y semiáridas. Recientemente, Sánchez-Coronado et al. (2011) reportaron la identificación de hongos del género *Penicillium*, *Phoma* y *Trichoderma* aislados en semillas de *O. tomentosa*. Lo anterior está en concordancia con los resultados de esta investigación, puesto que en este trabajo se aislaron los hongos de los géneros *Penicillium* y *Phoma* de semillas de *O. streptacantha*, los cuales aceleran el rompimeinto de la latencia de estas semillas.

Además de estudiar las plántulas de *Opuntia* bajo condiciones de estrés hídrico y lumínico, también se investigó durante el año 2009 la respuesta de plantas de individuos adultos de tres especies (*O. leucotricha*, *O. streptacantha* y *O. robusta*), bajo condiciones naturales en campo durante las diferentes estaciones del año 2009*.* En este estudio, el potencial osmótico de los cladodios, una estimación del estrés hídrico de las plantas, fue la variable más importante de la primera función del análisis estadístico. En verano y otoño, las estaciones del año con mayor precipitación, las tres especies de *Opuntia* mantuvieron altos potenciales osmóticos, a la vez que mostraron mayor contenido de acidez. Sin embargo; durante la sequía del invierno y principalmente de la primavera disminuyó la acidez y el potencial osmótico de las tres especies. El comportamiento de las especies fue distinto, la especie menos afectada en el potencial osmótico fue *O. robusta*, seguida de *O. streptacantha* y *O. leucotricha*. El aumento de la acidez y la disminución del potencial osmótico en la época de sequía en las tres especies es similar a lo encontrado para *O. basilaris* (Szarek y Ting, 1974; Hanscom y Ting, 1978). Además, Lerdau et al. (1992) encontraron que las plantas de *O. excelsa*, en Chamela, Jal., presentaron menores niveles de acidez en la estación seca que en la estación húmeda, lo que sugiere menor eficiencia fotosintética de esta especie en la época de sequía.

La disipación no fotoquímica (disipación de calor) a lo largo del día también fue importante para la primera función del análisis de nuestros resultados, con valores más altos en Primavera (sequía) y más bajos en Otoño, estación en la que ocurren la mayor parte de las lluvias y en la cual se tiene menor incidencia de radiación solar. Además, en la cara Oeste de los cladodios de *O. leucotricha* y *O.*

robusta, en donde se recibe mayor luz del sol por la tarde, hubo mayor disipación de calor que en la cara Este. Es decir, que en la cara Oeste hubo mayor radiación solar y las plantas mostraron mayor disipación de calor como mecanismo para tolerar la alta radiación solar. Este resultado es similar al obtenido por Adams III *et al.* (1989) para *Nopalea cochenillifera*, *Opuntia ficus-indica* y *Opuntia wentiana*, en el norte de Venezuela. Tales autores mencionan que la disipación no fotoquímica es un proceso importante en plantas CAM bajo condiciones naturales, lo cual puede reflejar un mecanismo de protección contra el potencial daño de los efectos de la acumulación de energía de fotones absorbida por los cladodios, particularmente bajo condiciones en las cuales la disponibilidad de CO_2 se encuentra restringida, como en la época de sequía y mayor radiación solar.

La variable más importante para la segunda función del análisis fue la eficiencia cuántica del fotosistema II (Φ_{PSII}) a lo largo del día. La Φ_{PSII} fue mayor en otoño (la época de lluvias) y menor en primavera, verano e invierno. En general para las tres especies, las caras Oeste de los cladodios fueron más afectadas en Otoño, Verano e Invierno que las caras Este. En cambio, en Primavera las caras Oeste mostraron mayor Φ_{PSII} que las caras Este. Lo anterior coincide con Adams *et al.* (1987) quienes encontraron que los niveles de eficiencia cuántica de *O. basilaris* fueron bajos a lo largo del año, indicativo de fotoinhibición, pero aumentaron después de lluvias de verano. También encontraron que en el otoño la eficiencia cuántica en la orientación Oeste fue menor que en las demás orientaciones. Así, se puede decir que la respuesta de las especies está determinada por el ambiente en el que se desarrollan.

En general para las tres especies agrupadas, al aumentar la temperatura en el día disminuyó el potencial osmótico y la eficiencia cuántica del fotosistema II (Φ_{PSII}), pero aumentó la disipación de calor a lo largo del día como mecanismo para tolerar el estrés. Al analizar las especies por separado los resultados fueron distintos. En las especies *O. leucotricha* y *O. robusta* la disipación no fotoquímica (NPQ) se correlacionó con la temperatura a las distintas horas del día, excepto a las 9:00 horas, lo cual implica que a esa hora las plantas no necesitan disipar calor porque no están estresadas. Para *O. streptacanthta* hubo correlación negativa entre potencial osmótico y las variables disipación de calor y temperatura a las distintas horas del día. Es decir, que al estar las plantas más estresadas por la sequía y las altas temperaturas, presentaban mayor disipación de calor.

En conclusión para este estudio en campo, las variables de respuesta más importante fueron el potencial osmótico y las relacionadas con fluorescencia de la clorofila (la eficiencia cuántica del fotosistema II (Φ_{PSII}) y la disipación no fotoquímica (NPQ) a lo largo del día), así como la acidez titulable. En general para las tres especies, el potencial osmótico, la acidez titulable y la eficiencia cuántica del fotosistema II (Φ_{PSII}) disminuyen en la época de sequía, pero aumenta la disipación no fotoquímica (NPQ) como mecanismo para tolerar el estrés.

Finalmente, el estudio de las respuestas de plantas de zonas desérticas a condiciones de estrés por sequía y alta radiación solar, condiciones ambientales a las cuales están expuestas ya sean plántulas o plantas adultas, nos aporta información muy valiosa sobre la comprensión de los diferentes mecanismos ecofisiologicos, anatómicos y moleculares que confieren dicha tolerancia al estrés abiótico.

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ANEXOS

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RESEARCH PAPER

Are fungi important for breaking seed dormancy in desert species? Experimental evidence in *Opuntia streptacantha* (Cactaceae)

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Keywords
Binucleate *Rhizoctonia*; *Penicillium chrysogenum*; *Phoma* sp.; physiological dormancy; prickly pear; seed germination; *Trichoderma koningii*.

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ABSTRACT

Seeds of *Opuntia* spp. have physiological dormancy; they need a period of after-ripening to break dormancy, and the embryos have low growth potential. We evaluated the combined effects of seed age and presence of fungi on the testa on germination of *Opuntia streptacantha*, an abundant species in the Chihuahuan Desert (Mexico), assuming that older seeds have broken seed dormancy and fungi can reduce mechanical resistance to germination. In a preliminary experiment, we found no germination of 9-year-old (1998) and freshly collected (2007) seeds. However, we obtained 67% and 27% germination from 9-year-old and fresh non-sterilized seeds, respectively, and found fungi growing on the testa of all germinated seeds. Two fungal strains were isolated and identified using ribosomal internal transcribed spacer (ITS) sequence analysis: *Penicillium chrysogenum* and *Phoma* sp. In a second experiment, we inoculated seeds with strains of *P. chrysogenum* and *Phoma* sp., as well as *Trichoderma koningii* and binucleate *Rhizoctonia* (Gto17S2), to evaluate their ability to break seed dormancy. Seeds inoculated with *P. chrysogenum*, *Phoma* sp. and *T. koningii* had higher germination than controls for both seed ages, but germination was higher in older seeds. Scanning electron microscopy showed that these fungi eroded the funiculus, reducing its resistance. Binucleate *Rhizoctonia* did not lead to germination and controls had almost no germination. Our results strongly indicate that fungi are involved in breaking seed dormancy of *O. streptacantha*, and that the effect of fungi on seeds is species-specific.

INTRODUCTION

Seed dormancy is a very common adaptive plant strategy in unpredictable and harsh environments, such as arid and semi-arid landscapes (Jurado & Moles 2003; Jurado & Flores 2005) and is common in the Cactaceae (Rojas-Aréchiga & Vázquez-Yanes 2000; Flores *et al.* 2005, 2006, 2008), especially in the genus *Opuntia* (Mandujano *et al.* 2005, 2007; Orozco-Segovia *et al.* 2007; Ochoa-Alfaro *et al.* 2008). The first *Opuntia* species found to have dormant seeds was *O. auranthiaca* (Archibald 1939), and currently seed dormancy has been found in more than 20 species (Ochoa-Alfaro *et al.* 2008). In most cases, different germination responses have been achieved using several treatments (Mandujano *et al.* 2005, 2007; Orozco-Segovia *et al.* 2007; Ochoa-Alfaro *et al.* 2008), but although seed dormancy in *Opuntia* spp. can be broken by scarification, seeds also have physiological dormancy, *i.e.*, the embryo has low growth potential and thus needs a period of after-ripening to break dormancy (Mandujano *et al.* 2005, 2007; Orozco-Segovia *et al.* 2007). If the seed coat is scarified, mechanical restriction is decreased, and the embryo is able to emerge. Moreover, when physiological dormancy is broken in intact seeds, the embryo has sufficient growth potential to overcome the restraint of the seed coat (Orozco-Segovia *et al.* 2007).

Seeds in the soil interact with microorganisms that could help to break dormancy. Fungi grow on the testa, eroding or cracking the hard/stony endocarp, and thus can potentially reduce mechanical resistance to germination in seeds with physiological dormancy (Morpeth & Hall 2000). In arid environments, some fungi have a role in seedling establishment, growth, water relations and nutrition of cacti (González-Monterrubio *et al.* 2005), but the effects of fungi on breaking seed dormancy in cacti have received very little attention. The previous studies are by Arredondo *et al.* (2007), who found that *Rhizopus* sp. has a moderate effect in breaking seed dormancy of *Thelocactus hexahedrophorus*, another cactus from the Chihuahuan Desert, and by Olvera-Carrillo *et al.* (2009), who found that 7-month-old exhumed seeds of *O. tomentosa* had fungal hyphae penetrating the funicular envelope through the openings, favouring germination of a weak embryo (*i.e.*, an embryo with low growth potential).

In this study, we assumed that old seeds have broken seed dormancy and that fungi can reduce mechanical resistance to germination, and present experiments in which we evaluated: (i) the combined effects of seed age (seeds collected in 1998 and 2007) and natural fungi on seed testa, and (ii) the combined effects of seed age and fungal inoculation with each of four fungi species (two isolated from *O. streptacantha* testa)

ARTICLE ADDENDUM

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Further evidence from the effect of fungi on breaking Opuntia seed dormancy

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Recently, we found that fungi are involved in breaking seed dormancy of *Opuntia streptacantha*, and that the effect of fungi on seeds is species-specific. However, the effect of fungi on seed germination from other Opuntia spp. has not been evaluated. Thus, we evaluated the effect of four fungal species (*Penicillium chrysogenum*, Phoma sp., *Trichoderma harzianum*, *Trichoderma koningii*) on the germination of *Opuntia leucotricha*, an abundant species in the Chihuahuan Desert, Mexico. We found that seeds inoculated with the four fungal species had higher germination than control seeds. Trichoderma spp. were the most effective. Our results strongly indicate that fungi are involved in breaking seed dormancy of *O. leucotricha*. Thus, we suggest that these fungi could promote seed germination from other Opuntia species.

Seeds in the soil interact with microorganisms that could help them break seed dormancy. Fungi attack the testa, eroding or cracking the hard/stony endocarp, and could reduce the mechanical resistance to germination in seeds with physiological dormancy.[1] In arid environments, the effects of fungi on breaking seed dormancy in cacti have received very little attention. Recently, our work group found that Phoma sp. and *Trichoderma koningii*, and in less proportion *Penicillium chrysogenum*, help break seed dormancy of *Opuntia streptacantha*, maybe by the action of enzymes that degrade the testa.[2] However, the effect of fungi on seed germination from other Opuntia species has not been evaluated.

In this study, we test the effects of four fungal species (two isolated from *O. streptacantha* testa) in breaking seed dormancy of *Opuntia leucotricha*; a perennial arborescent cactus of economic interest distributed on the semiarid lands of central Mexico.

Since seeds of Opuntia spp. have physiological dormancy, they need a period of after-ripening to break dormancy, and the embryos have low growth potential; we used 2-year-old seeds, assuming that old seeds have broken physiological seed dormancy and that fungi can reduce mechanical resistance to germination.[2] *O. leucotricha* seeds were collected from mature fruits, in 2008, and stored in paper bags at room temperature during two years.

Penicillium chrysogenum, Phoma sp., *Trichoderma harzianum* and *T. koningii* were grown on PDA plates at 28°C for 3 days. The spores (*P. chrysogenum*, *T. harzianum* and *T. koningii*) and mycelia (Phoma sp.) were collected in sterile distilled water and counted in a Neubauer chamber for later inoculation of *O. leucotricha* seeds. Sterilized seeds were grown on water-agar plates and inoculated with 2 μl of spore solution or mycelium (6 x 10^7 ml^{-1}) from each fungus. Seeds were incubated in water-agar plates for 35 days in an automatic germination chamber with a 16-h light and 8-h dark photoperiod at 25°C ± 2°C. There were five replicates per treatment and 20 seeds per replicate.

Table 1. Effect of fungal species on germination of *Opuntia leucotricha* seeds

Treatment	Germination percentage (±S.E.)
Control	0%^{c}
Penicillium chrysogenum	15% (±3.35)^{b}
Phoma sp.	10% (±2.23)^{b}
Trichoderma harzianum	40% (±8.94)^{a}
Trichoderma koningii	37% (±8.27)^{a}

Significant differences ($P < 0.0001$) between fungal species are indicated by different lower-case letters.

After one-way ANOVA, we found a significant effect of fungal species ($F = 52.198$, $p < 0.0001$) on *O. leucotricha* seed germination. Seeds inoculated with the four fungal species had higher germination than control, although Trichoderma spp. promoted higher seed germination than the other fungi examined (**Table 1**).

Opuntia species have hard to break dormancy in their seeds.[2-8] Seed dormancy is a common plant strategy in arid and semiarid environments, which has been found in several plant families,[9-11] including Cactaceae.[12-16]

In our experiment, we found that four fungal species erode the endocarp and break seed dormancy of *O. leucotricha*. Since seeds of Opuntia are known to have physiological dormancy, i.e., the embryo has low growth potential,[6] it is possible that fungal attack of the tests would reduce the mechanical resistance of the testa, thus promoting embryo growth.

Arredondo et al.[16] found that Rhizopus sp. moderately breaks seed dormancy of *Thelocactus hexahedrophorus*, another cactus species from the Chihuahuan Desert. Olvera-Carrillo et al.[8] found that 7-month-old exhumed seeds from *O. tomentosa* showed fungal hyphae penetrating the funicular envelope through the openings, favoring germination but with a weak embryo (an embryo with low growth potential).

Conclusions

We found that *O. leucotricha* seeds inoculated with the four fungal species had higher germination than control seeds, similar to findings for *O. streptacantha*.[2] These results show that fungi play an important role in breaking seed dormancy of Opuntia species; they contribute to understanding germination biology of cactus species, opening new insights regarding the effect of fungi on breaking seed dormancy of arid and semiarid plants.

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El nopal, una planta adaptada para afrontar el cambio climático del planeta

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26-julio-2011

Por Pablo Delgado Sánchez, Joel Flores Rivas y Juan Francisco Jiménez Bremont (IPICYT)*

La vida en nuestro planeta ha evolucionado durante millones de años para tener la gran riqueza de organismos que podemos encontrar en la actualidad. Esta biodiversidad provee de diversos beneficios (oxígeno, alimento, ropa, medicina, vivienda, etcétera) y permite disfrutar de múltiples paisajes en cada rincón del planeta.

Sin embargo, ¿por qué es importante la biodiversidad en los ecosistemas de nuestro planeta? ¿Cómo podría ser el futuro si seguimos perdiendo biodiversidad? ¿Pueden algunas especies estar adaptadas para enfrentar cambios ambientales y superar las consecuencias del cambio climático global? Preguntas como éstas son inquietantes y requieren ser contestadas mediante investigaciones científicas que nos ayuden a entender las respuestas de los organismos a estos cambios.

A lo largo de la historia del planeta, han existido cambios en el clima; sin embargo, a partir de la década de los años ochenta del siglo XX, ha comenzado a surgir evidencia científica que el cambio climático del último siglo se ha intensificado por las actividades humanas. Se puede afirmar que estamos viviendo en una época de cambio climático acelerado y las consecuencias en el planeta pueden ser muy severas: modificaciones de temperaturas (más cálidas y más frías); mayor cantidad e intensidad de eventos meteorológicos extremos y, con ello, mayores riesgos de inundaciones y sequías; reducción de glaciares; aumento del nivel del mar; sustitución de tipos de vegetación; desplazamiento de áreas de distribución de especies, así como extinción de flora y fauna.

Se estima que estos cambios serán demasiado rápidos como para permitir que muchas especies se adapten y (o, además) se desplacen, lo que causará cambios significativos en la biodiversidad, así como la disminución de zonas agrícolas.

En México, aproximadamente, 60% del territorio tiene ambientes áridos y semiáridos, como son los desiertos Sonorense y Chihuahuense, donde las plantas predominantes son las suculentas. El nopal (Opuntia sp.) es ejemplo de ellas; pertenece a la familia Cactácea, originaria del continente americano; se distribuye desde Canadá hasta la Patagonia. A la fecha se conocen entre 163 y 188 especies de nopal, de las cuales de 58 a 78 se encuentran en México. Después de la llegada de los españoles a América, esta planta comenzó a ser llevada a otros continentes, por lo que ahora se le puede encontrar en el Mediterráneo, en África, Australia y Asia.

El nopal durante millones de años ha evolucionado y posee diversas adaptaciones que le permiten afrontar el cambio climático acelerado del planeta. Las especies de nopal son ecológicamente exitosas en diversos ambientes debido a que toleran condiciones ambientales especiales, como sucede en los desiertos, donde en todos los años existen pocas semanas con lluvia, varios meses secos (sequía), alta radiación solar, temperaturas que pueden oscilar por debajo de los 0°C hasta arriba de los 50°C, por mencionar algunos de los factores a los que las especies de nopal están adaptadas. Además, estas plantas ayudan a disminuir los efectos de la desertificación y la conservación de los suelos.

En estas condiciones, los nopales presentan adaptaciones fisiológicas y morfológicas como:

1) el tipo de fotosíntesis conocida como CAM (Metabolismo Ácido de las Crasuláceas), el cual se caracteriza por realizar la captura y almacenamiento de dióxido de carbono (CO2) durante la noche, para evitar la pérdida de agua al abrir los estomas – pequeños poros – (por cierto, ese CO2 obtenido de manera nocturna es utilizado durante el día para realizar la fotosíntesis);

2) el uso de pigmentos, diferentes al verde, los cuales ayudan a las pencas, a protegerse de la radiación solar;

3) alta cantidad de baba (mucílago), una adaptación para reducir los efectos de la deshidratación en la época seca;

4) espinas que disminuyen la intensidad de los rayos solares que inciden en las pencas y generan micro-turbulencias que disipan el calor, y

5) la cutícula cerosa que recubren las pencas y evita la pérdida de agua.

Estas son algunas otras adaptaciones que, en conjunto, hacen que los nopales sean exitosos en los ambientes secos.

Estas plantas son utilizadas en áreas agrícolas con baja disponibilidad de agua; se cultivan por sus frutos; las tunas que aportan carbohidratos, vitaminas y minerales, y por sus renuevos o nopalitos, que son consumidos como verdura. La producción mundial de tunas se estima en 400,000 toneladas, siendo México e Italia los mayores productores; son utilizadas para la fabricación de mermeladas y jaleas, néctar, en almíbar, alcoholes, vinos y colorantes. También esta planta es muy importante en la medicina tradicional y en la ganadería como forraje.

El nopal como modelo de investigación científica

Con todo lo anterior, una necesidad es desarrollar estudios sobre los mecanismos de respuesta del nopal a diversos factores desfavorables como la sequía, la salinidad, las temperaturas extremas, entre otros, que pueden agudizarse por el cambio climático. Por ello, esta planta ha sido motivo de estudios ecológicos y fisiológicos desde hace más de 100 años.

Investigadores del Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), la Universidad Autónoma de San Luis Potosí (UASLP) y Centro de Investigaciones Biológicas del Noroeste (Cibnor) formamos un grupo de investigación enfocado en estudiar las respuestas de las plantas en condiciones de estrés desde un enfoque multidisciplinario e integral mediante evaluaciones ecológicas, ecofisiológicas, bioquímicas, anatómicas, moleculares y evolutivas, siendo el nopal uno de los modelos de estudio, puesto que es una planta muy interesante pero, a pesar de los múltiples estudios realizados, aún poco explorada.

Además, el Altiplano Potosino y su vecino Zacatecano son los lugares del mundo con la mayor riqueza de especies y variantes cultivadas de nopal, por lo que se tiene una gama de ambientes naturales y son ecosistemas ideales para analizar las respuestas de aclimatación de esta planta, debido a las presiones que sobre ellos han ejercido las actividades humanas. Las adaptaciones que a lo largo de la evolución ha desarrollado el nopal pueden ser una valiosa fuente de información para la comprensión integral de las respuestas de estas plantas a los factores ambientales a los cuales están expuestas; nos interesa encontrar posibles soluciones, no sólo, a los problemas que estamos viviendo, sino también a los más complicados que se avecinan, productos del cambio climático acelerado que está sufriendo nuestro planeta.

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Grupo de Investigación

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