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# LICENCIATURA EN CIENCIAS AMBIENTALES

Centro de Investigaciones en Ecosistemas

Respuesta Fisiológica del Abono Verde,  
*Vicia sativa*, a la sequía

# TESIS

QUE PARA OBTENER EL TÍTULO DE  
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P R E S E N T A

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## I. Resumen

Los abonos verdes y cultivos de cobertura han sido adoptados en la agricultura de temporal debido a que son económicos, a que incrementan la protección del suelo entre periodos de cultivo y aumentan la fertilidad y el contenido de materia orgánica. Se realizó un experimento de invernadero sobre la respuesta fisiológica del abono verde *Vicia sativa* a la sequía. El contenido gravimétrico de agua de 80% no disminuyó durante los 24 días que duró el experimento. El intercambio de gases fue muy sensible, v. g., la asimilación de CO<sub>2</sub> de  $7.8 \pm 0.17 \mu\text{mol m}^{-2} \text{s}^{-1}$ , disminuyó significativamente desde los 8 días de suspensión del riego. Mientras que los pigmentos fotosintéticos no se degradaron en las plantas sin riego, estas acumularon 1,000 veces más prolina que los  $0.41 \pm 0.09 \mu\text{mol g}^{-1}$  del control. Los resultados sugieren que *V. sativa* puede tolerar periodos de sequía de casi un mes y puede ser una alternativa útil en terrenos en proceso de aridificación.

## II. Abstract

Cover crops and green manures have been adopted in rainfed agriculture owing to their low input cost, by their ability to protect the soil between cultivation seasons, and to increase soil fertility and organic matter content. A greenhouse experiment was conducted to evaluate the physiological response of the green manure *Vicia sativa* to drought. The gravimetric water content of 80% did not decrease during the 24 days of the experiment. Gas exchange was very sensitive, *e. g.*, the net CO<sub>2</sub> uptake of  $7.8 \pm 0.17 \mu\text{mol m}^{-2} \text{s}^{-1}$  decreased significantly from 8 days of water withholding until the plants were rewatered. While the photosynthetic pigments were not degraded for the droughted plants, these individuals accumulated 1,000-fold the proline of the control, which amounted to  $0.41 \pm 0.09 \mu\text{mol g}^{-1}$ . The results suggest that *V. sativa* can tolerate month long drought periods and constitutes useful alternative for increasingly arid locations.

### III. Introducción

Es ampliamente reconocido que la concentración de CO<sub>2</sub> en la atmosfera se está elevando y que esto tiene como resultado incrementos en la temperatura del aire y alteraciones en los patrones de precipitación (IPCC, 2001). Uno de los principales factores que impulsan el cambio climático es el cambio de uso de suelo (Nobel, 2009). Por ejemplo, la conversión de bosque a terrenos de cultivo produjo 18% de las emisiones globales de gases efecto invernadero (Terceiro, 2009). Además, la labranza, la quema de biomasa y el uso excesivo de agroquímicos generó el 16% de las emisiones globales de gases de efecto invernadero, incluyendo metano, CO<sub>2</sub> y, óxidos de nitrógeno (Terceiro, 2009; Ellis y Pontius, 2010). En México, la conversión de bosques a agricultura generó el 14% de las emisiones de gases de efecto invernadero en 2006 y la labranza, la quema de biomasa y el uso excesivo de agroquímicos generó el 7% (Instituto Nacional de Ecología, 2010). Finalmente, a nivel de parcela los cambios en la cobertura vegetal incrementan la vulnerabilidad del suelo a la erosión por viento y agua, especialmente en terrenos escarpados, reduciendo la fertilidad del suelo y otras cualidades para la agricultura.

Una manera de mantener cubiertos los suelos durante los periodos entre cosechas es el uso de cultivos de cobertura, que muchas veces funcionan como abonos verdes, cuando estos se incorporan al suelo aportando nutrientes y materia orgánica que genera una mejor estructura del suelo y una mejor protección a la erosión (Brady and Weil, 1996). Adicionalmente, los cultivos de leguminosas incrementan el contenido de nitrógeno del suelo por la fijación biológica a través de la asociación con bacterias del genero *Rhizobium*, tambien favorecen el reciclaje de nutrientes como fósforo y potasio y evitan que los nutrientes se lixivien. Esto puede contribuir a reducir el consumo de fertilizantes nitrogenados y fosfatados, que incrementan los consumos de energía y los costos



económicos de la producción de alimentos y la contaminación del ambiente, como puede ser la eutrofización de los cuerpos de agua (Ellis and Pontius, 2010).

*Vicia sativa* L. es una planta fijadora de nitrógeno que se usa ampliamente como cultivo de cobertura y abono verde (Uzum *et al.*, 2011) en la agricultura de temporal y se siembra en los meses de secano con el agua residual en el suelo y las lluvias tardías. Su uso como abono verde reduce los consumos de los fertilizantes químicos sin afectar el rendimiento de los cultivos y reduciendo los lixiviados contaminantes (Salmerón *et al.*, 2011). Otra consecuencia de su uso es la reducción de los costos asociados al cultivo, por lo que se ha adoptado en la agricultura de pequeña escala, que en México por lo general es de temporal y de autoconsumo (Orozco-Martinez *et al.*, 2011)

La sequía reduce la capacidad de las plantas para tomar agua del suelo reduciendo la disponibilidad de agua para su crecimiento y productividad (Munns, 2002; Chaves *et al.*, 2003; Hamdy *et al.*, 2003; Flexas *et al.*, 2006). Esta limitación se debe a los balances entre la respiración y la fotosíntesis. En sequía severa la fotosíntesis se puede disminuir completamente, pero la respiración no es afectada totalmente (Flexas *et al.*, 2005; Flexas *et al.*, 2006). La reducción en la respiración está regulada por la capacidad de los estomas de cerrarse y por la reducción de la conductancia del mesófilo (Flexas *et al.*, 2006). La acumulación de prolina en respuesta a la sequía puede jugar roles positivos en situaciones de estrés por sequía actuando como un osmoregulador (Xue *et al.*, 2008) y se sugiere que existe una relación positiva entre la acumulación de prolina y la resistencia a la sequía (Hanson, 1976).

Debido a los efectos del cambio climático en los patrones de lluvia, las alteraciones en la humedad del suelo y las posibles consecuencias en la producción de alimentos,

(especialmente sobre la agricultura de temporal), además de la necesidad de desarrollar prácticas agrícolas con un menor impacto ambiental. Se evaluaron las respuestas fisiológicas de *Vicia sativa* a la sequía en un experimento de invernadero. En particular se exploró como afecta la sequía al uso de agua de esta especie en términos de su conductancia estomática y su transpiración; se exploró la forma en la que la sequía afecta la fotosíntesis de *V. sativa* reflejándose en la absorción total de CO<sub>2</sub> y en la degradación de pigmentos fotosintéticos y la forma en la que *V. sativa* acumula osmolitos como prolina en respuesta a la sequía.

#### **IV. Physiological responses of the green manure, *Vicia sativa*, to drought**

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##### *a. Introduction*

It is widely recognized that the atmospheric CO<sub>2</sub> concentration is increasing and this will result in increases in air temperature and alterations in precipitation patterns (Intergovernmental Panel on Climate Change, 2001). A main driver of this climate change is the change in land-use (Nobel, 2009). For example, conversion of forest to cropland that has a lower amount of standing biomass results in 18% of the global greenhouse gas emissions (Terceiro, 2009). In addition, tillage, biomass burning, and an excessive use of agrochemicals generate 16% of greenhouse gas emissions including methane, CO<sub>2</sub>, and nitrous oxides (Terceiro, 2009; Ellis and Pontius, 2010). In Mexico, forest conversion to agriculture generated 14% of the country's greenhouse gas emissions in 2006, while tillage, biomass burning, and the excessive use of agrochemicals accounted for 7% of such emissions (Instituto Nacional de Ecología, 2010). Furthermore, at the plot level changes in

plant cover increase the vulnerability of soil to water and wind erosion, especially in steep terrains, reducing fertility and agricultural qualities.

A way to maintain plant cover in agricultural lands during intercrop periods, thus avoiding soil erosion, is the use of cover crops that in many cases also work as green manures that incorporate nutrients and organic matter, leading to a better soil structure and conferring soil protection to erosion (Brady and Weil, 1996). In addition, legume cover crops can increase soil nitrogen content via biological fixation by the symbiotic bacteria *Rhizobium*, and recycle nutrients like phosphorus and potassium and reduce the nutrient leaching. This can contribute to reduce the consumption of nitrogen and phosphorus fertilizers, which increase the economic and energetic costs of food production and environmental pollution, for example through eutrophication of water bodies (Ellis and Pontius, 2010).

*Vicia sativa* L. is a nitrogen-fixing legume that is widely used as green manure and cover crop (Uzum *et al.*, 2011). It is utilized in rainfed agriculture, where it is planted during the dry months sustained by residual soil humidity and late rains. Its use as green manure reduces the consumption of chemical fertilizers without affecting crop yield and reduces fertilizer leaching (Salmerón *et al.*, 2011). Another consequence of its use is the reduction of cultivation associated costs, so it has been adopted in small scale agricultural operations, which in Mexico are usually rainfed and for self-consumption agriculture (Orozco-Martinez *et al.*, 2011).

Drought reduces the capacity of plants to take up water from the soil (Munns, 2002; Chaves *et al.*, 2003; Hamdy *et al.*, 2003; Felax *et al.*, 2006). This hydric limitation leads to reductions in the balance between photosynthesis and respiration. Under severe drought

photosynthesis can become totally impaired (Flexas *et al.*, 2005, 2006). The reduction in gas exchange is controlled by the stomatal closure and a reduced mesophyll conductance (Flexas *et al.*, 2006). In addition, proline accumulation in response to drought stress acts as an osmoprotectant suggesting that there is a positive correlation between proline-accumulating potential and drought resistance (Hanson, 1976; Xue *et al.*, 2008).

Due to climate change effects on precipitation patterns, alterations in soil humidity and possible consequences in food production in rainfed agriculture, in addition to a need to develop agricultural practices with a lower environmental impact, some physiological responses of *Vicia sativa* to drought were evaluated through a greenhouse experiment. In particular, we explored whether drought i) will affect water use by this species, in terms of stomatal conductance and transpiration; ii) the photosynthetic capacity of *V. sativa* as reflected by net CO<sub>2</sub> uptake and the degradation of photosynthetic pigments; and iii) the accumulation of osmolites, such as proline, in response to drought.

## *b. Materials and methods*

### Plant material and experimental treatments

Seeds of *Vicia sativa* L. (Fabaceae) were obtained from a local vendor (Casa Treviño, Guadalajara, Jalisco). A group of ten seeds were sown in ten different nursery bags (volume of 3.8 l) on January 2011 containing a commercial growth medium CreciRoot® (Sistemas Agrotec, Uruapan, Michoacán, México) composed by agrolite, shredded bark, coal and coir, *i.e.*, which were kept in a greenhouse without environmental controlled conditions at Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Morelia, Michoacán (19° 38' 55.9" N; 13° 13' 43.7" W; 1967 masl).

Bags were watered every four days to field capacity, in particular, field capacity was determined by the technique of free drainage. Once the plants reached an average size of  $60 \pm 20$  cm, a two treatment (water withholding and control) drought experiment was established. After 50 days irrigation was suspended for a group of five randomly selected individuals and continued on a five randomly selected control group. Various physiological parameters were monitored periodically over 24 days to determine the response of the experimental group, and the measurements were conducted every four days for the entire variable. On 20 April 2011 irrigation was resumed to determine the kinetics of recovery by *V. sativa*. Finally the statistical analysis consisted on a one way repeated measures ANOVA test.

#### Gravimetric water content

Gravimetric water content in the leaf was determined by collect a randomly selected leaf and weighed to obtain the fresh weigh, finally the leaf was dried to constant weight and weighed to obtain the dry weigh and calculate the percent of water amount in the leafs

#### Gas exchange

Gas exchange, *i.e.*, stomatal conductance, transpiration, and net CO<sub>2</sub> uptake, were measured with a CI-340 Hand-Held photosynthesis system (CID Instruments, Camas, Washington, U.S.A.). Measurements were conducted at 16:00 h. The time of measurement was determined after a set of preliminary measurements conducted during the day for which the times for gas exchange maxima were at 10:00 h and at 16:00 h. the measurements were conducted in a randomly selected leaf, in a random order for all the plants (water withholding and control treatments).

### Photosynthetic pigments

Changes in the tissular concentration of total chlorophyll during drought and recovery were measured according to Lichtenthaler (1987). A randomly selected leaflet was weighed and macerated with a cold (3 °C) aqueous solution of acetone (80% v/v) and brought up to a final volume of 3.0 ml. Absorbance at 665 nm and 645 nm was measured with an EZ 301 Spectrometer (Perkin Elmer, Waltham, Massachusetts, U.S.A.).

### Accumulation of solutes

Proline accumulation in leaf tissue of *V. sativa* during drought and recovery was measured by spectrophotometry according to Bates (1973). In particular, 1 g of randomly selected fresh leaf tissue was frozen with liquid nitrogen and homogenized in a porcelain mortar with 10 ml of sulfosalicylic acid (3% v/v) and filtered in a funnel with No. 1 filter paper. From the filtrate 2 ml were taken and were placed in a test tube with 2 ml of glacial acetic acid and reactive solution of ninhydrin. The reactive solution of ninhydrin was obtained by mixing 2.5 g of ninhydrin and 60 ml of glacial acetic acid (99% v/v). The solution was incubated in a thermoregulatory bath for 15 minutes at 50 °C, and finally 40 ml of orthophosphoric acid were added to the solution. The mixture was stirred in a vortex for 15 seconds, incubated in a thermoregulatory bath for 1 h at 100 °C, and quickly cooled in ice. Finally, 4 ml of toluene were added and stirred in a vortex for 30 seconds. Absorbance at 520 nm was measured for the resulting colored compound.

c. Results

Tissue water content for both treatments remained constant over the course of the experiment, averaging 80% by mass for both the well watered control and the droughted individuals ( $p = 0.256$ ; Fig. 1).

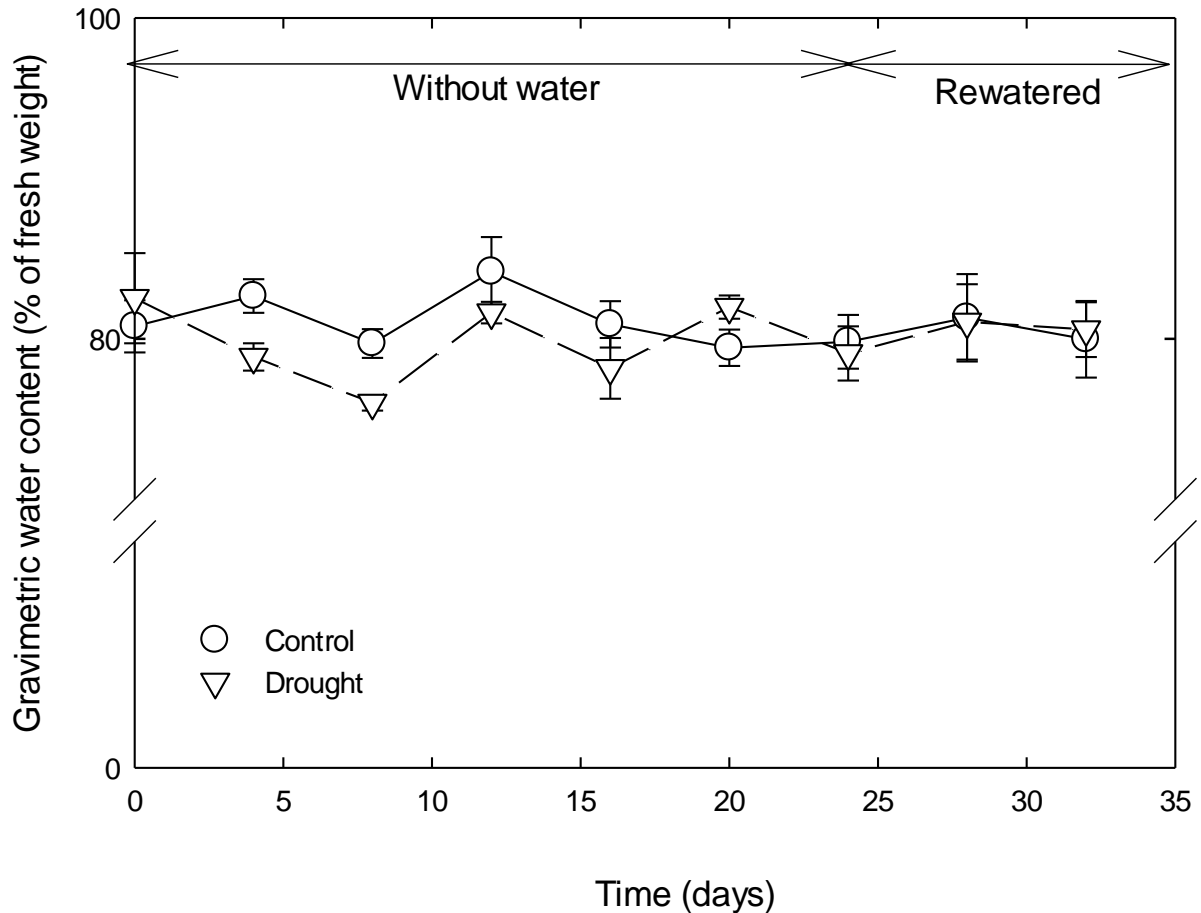


Figure 1. Tissue water content for *Vicia sativa* in response to drought. Plants were irrigated to field capacity every four days (O) or had irrigation withheld ( $\Delta$ ). The arrows indicate the time (day 24) when irrigation was resumed.



Stomatal conductance averaged  $80.33 \pm 7.08 \text{ mmol m}^{-2} \text{ s}^{-1}$  over the course of the experiment for plants of *Vicia sativa* that were well watered (Fig. 2). For the droughted plants, stomatal conductance was lower than for the control ( $p < 0.001$ ), ranging from 41% ( $45.56 \pm 4.95 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) lower at 12 days without irrigation to 55% ( $38.33 \pm 8.22 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) lower at 24 days without irrigation. Stomatal conductance recovered to rates similar to those of the control at 8 days after resuming irrigation.

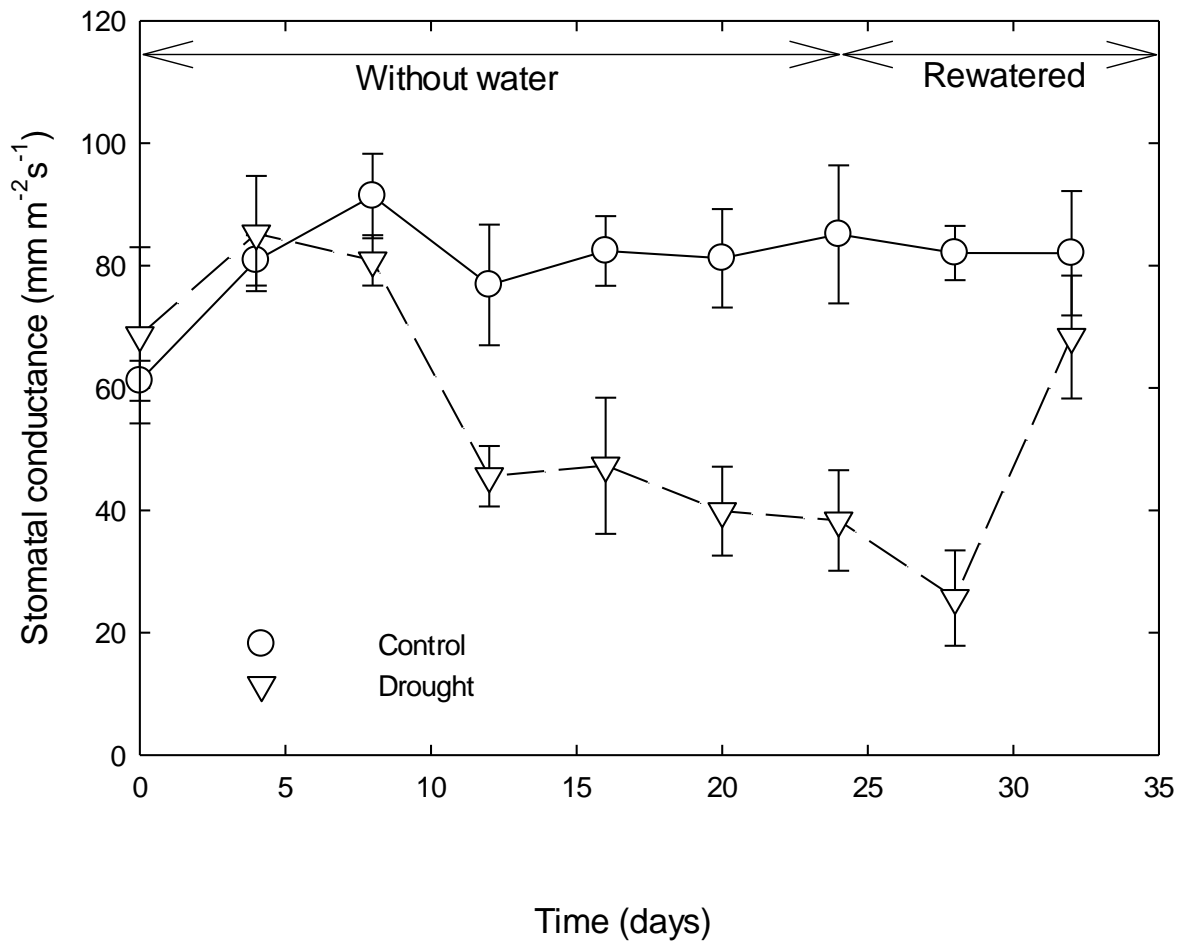


Figure 2. Stomatal conductance of *V. sativa* in response to drought. Plants were irrigated to field capacity every four days (O) or had

Transpiration averaged  $5.07 \pm 0.53 \mu\text{mol m}^{-2} \text{s}^{-1}$  over the course of the experiment for plants of *V. sativa* that were well watered (Fig. 3). For the droughted plants, transpiration was lower than the control ( $p < 0.001$ ), ranging from 44% ( $2.81 \pm 0.63 \text{ mmol m}^{-2} \text{s}^{-1}$ ) lower at 8 days without irrigation to 59% ( $2.28 \pm 0.37 \text{ mmol m}^{-2} \text{s}^{-1}$ ) lower at 24 days without irrigation. Transpiration recovered to rates similar to those of the control at 8 days after resuming irrigation.

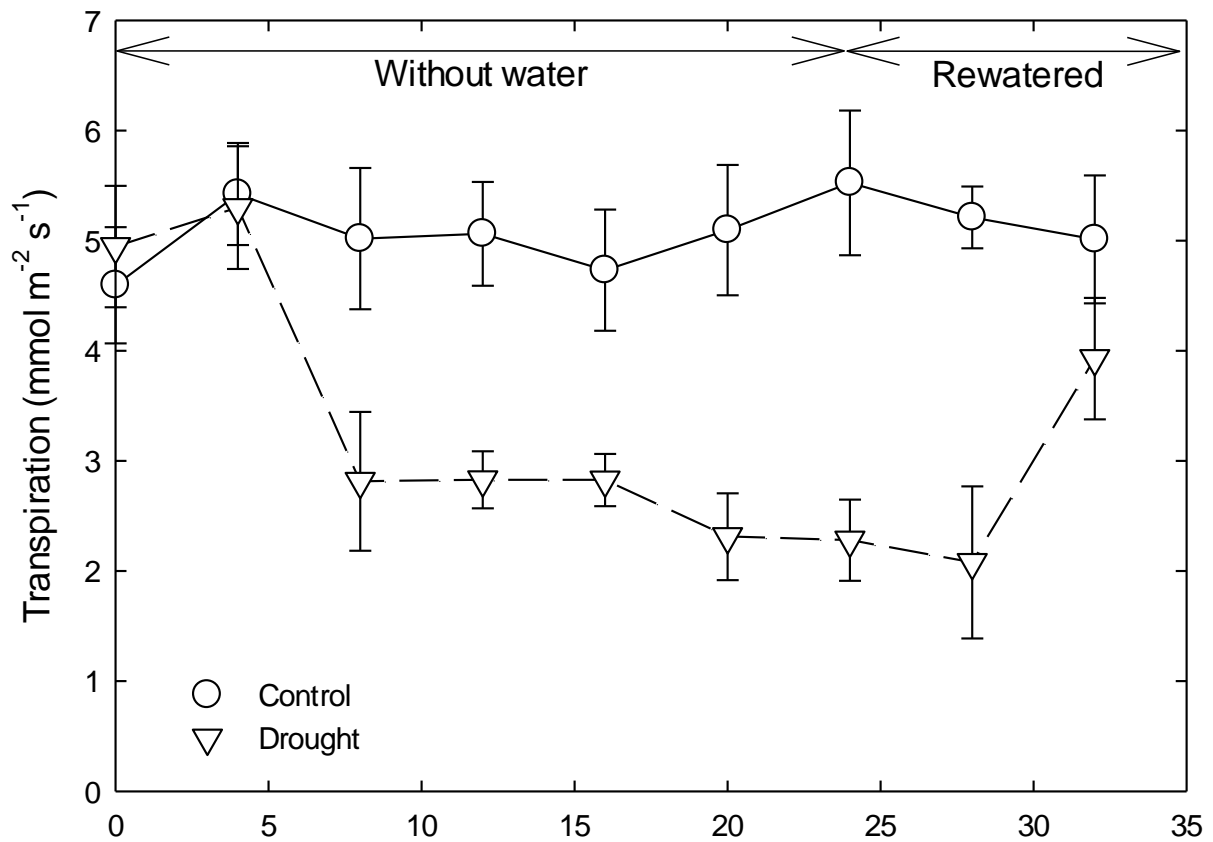


Figure 3. Transpiration of *V. sativa* in response to drought. Plants were irrigated to field capacity every four days (O) or had irrigation withheld (Δ). Error bars represent standard error.

Total CO<sub>2</sub> uptake averaged  $7.82 \pm 0.44 \mu\text{mol m}^{-2} \text{s}^{-1}$  over the course of the experiment for plants of *V. sativa* that were well watered (Fig. 4). For the droughted plants, total CO<sub>2</sub> uptake was lower than for the control ( $p < 0.001$ ), ranging from 36% ( $5.34 \pm 0.64 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) lower at 4 days without irrigation to 66% ( $2.75 \pm 0.56 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) lower at 24 days without irrigation. At 4 days after resuming irrigation, the total CO<sub>2</sub> uptake started to increase, returning to levels similar to those of the control by 8 days after resuming irrigation.

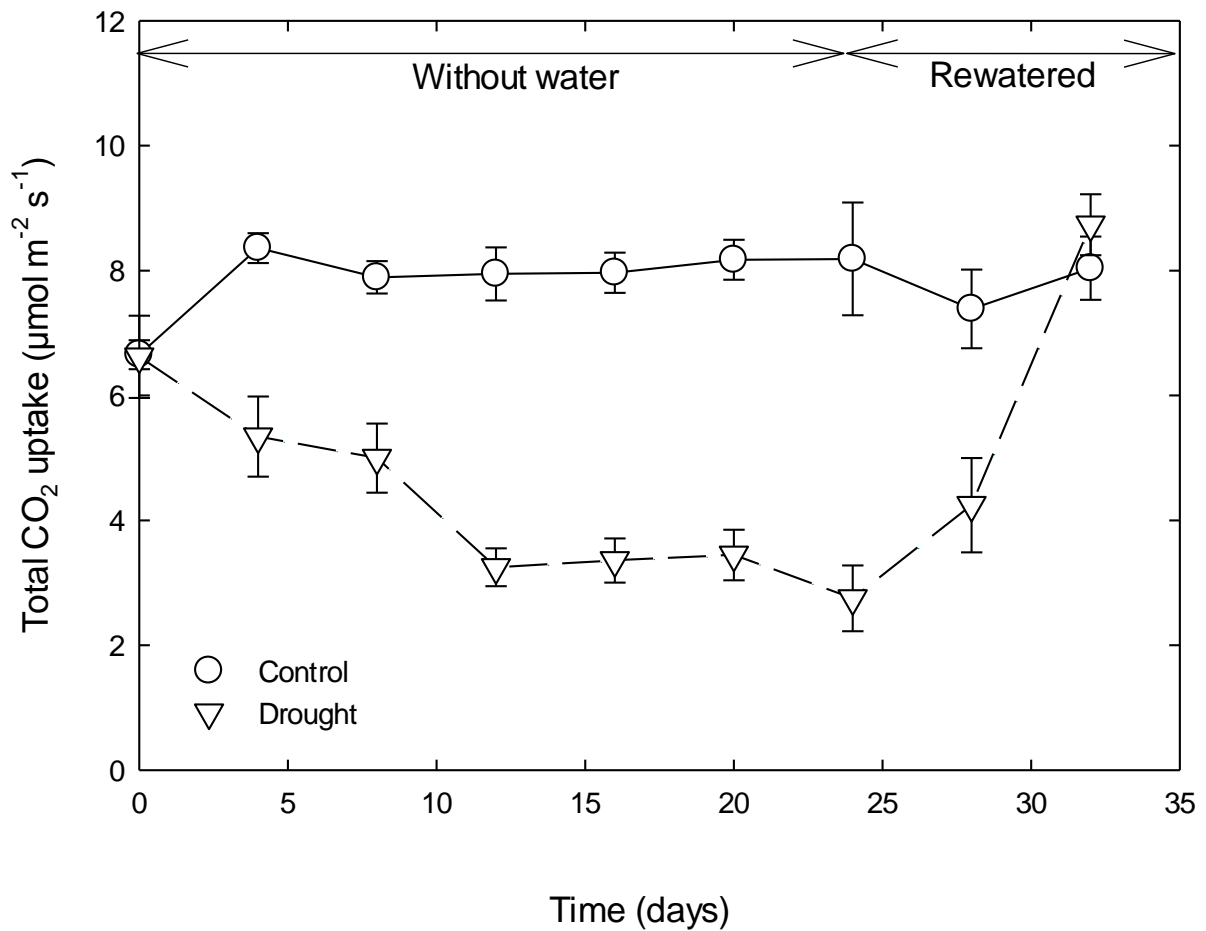


Figure 4. Total CO<sub>2</sub> uptake of *V. sativa* in response to drought. Plants were irrigated to field capacity every four days (○) or had

Total chlorophyll content averaged  $6.74 \pm 0.95 \text{ mg m}^{-2}$  over the course of the experiment for plants of *V. sativa* that were well watered (Fig. 5). In this case, although the droughted plants had lower chlorophyll contents, no statistical difference was found between treatments ( $p = 0.472$ )

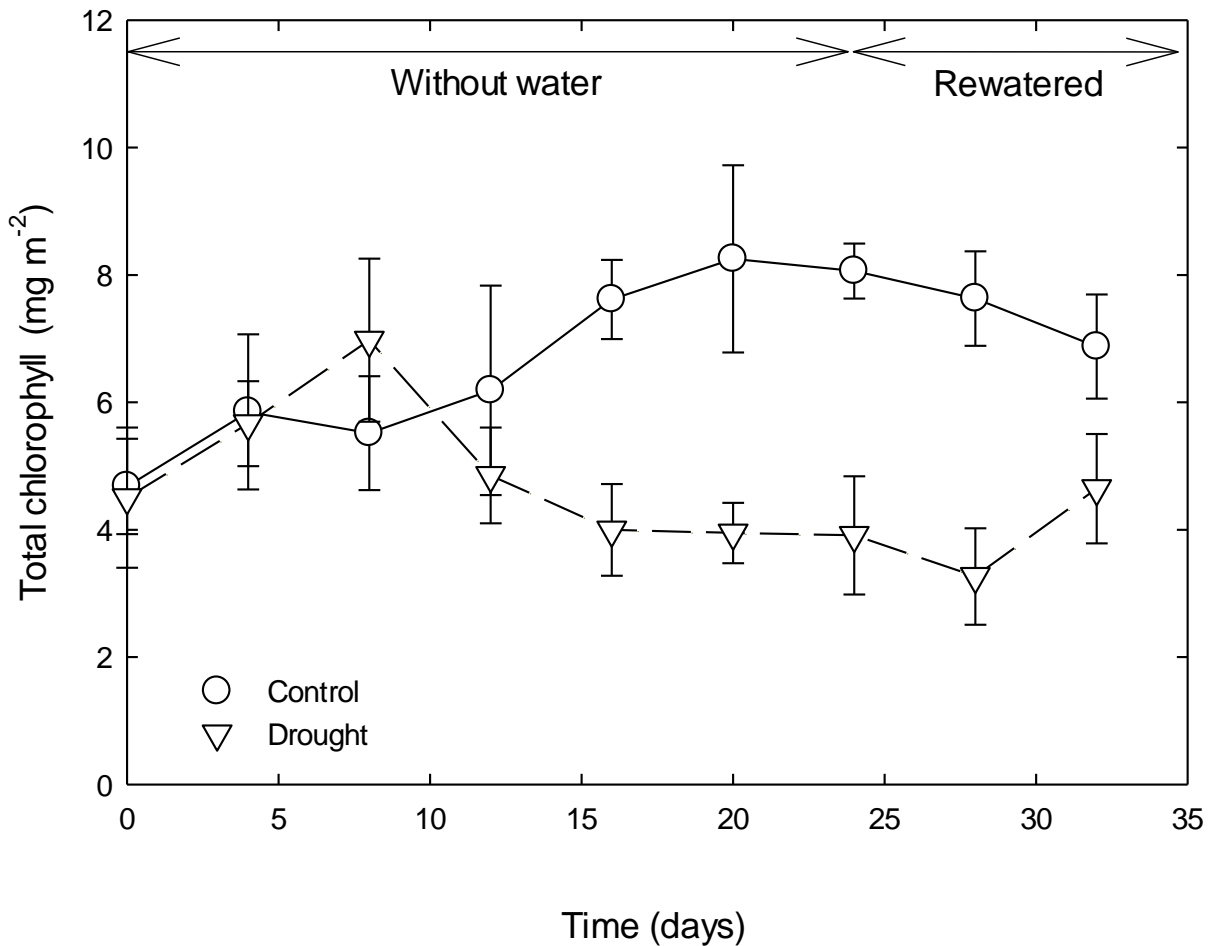


Figure 5. Total chlorophyll of *V. sativa* in response to drought. Plants were irrigated to field capacity every four days (O) or had

Proline concentration averaged  $0.64 \pm 0.37 \mu\text{mol g}^{-1}$  (fresh weight) over the course of the experiment for plants of *V. sativa* that were well watered (Fig. 6). For the case of the droughted plants, proline concentration was extremely higher than for the control ( $p < 0.001$ ). For instance, it was 100-fold higher at 12 days without irrigation and remained high while water was withheld. At 4 days after resuming irrigation, the tissue concentration of proline started to decrease, returning to levels similar to those of the control by 8 days after resuming irrigation.

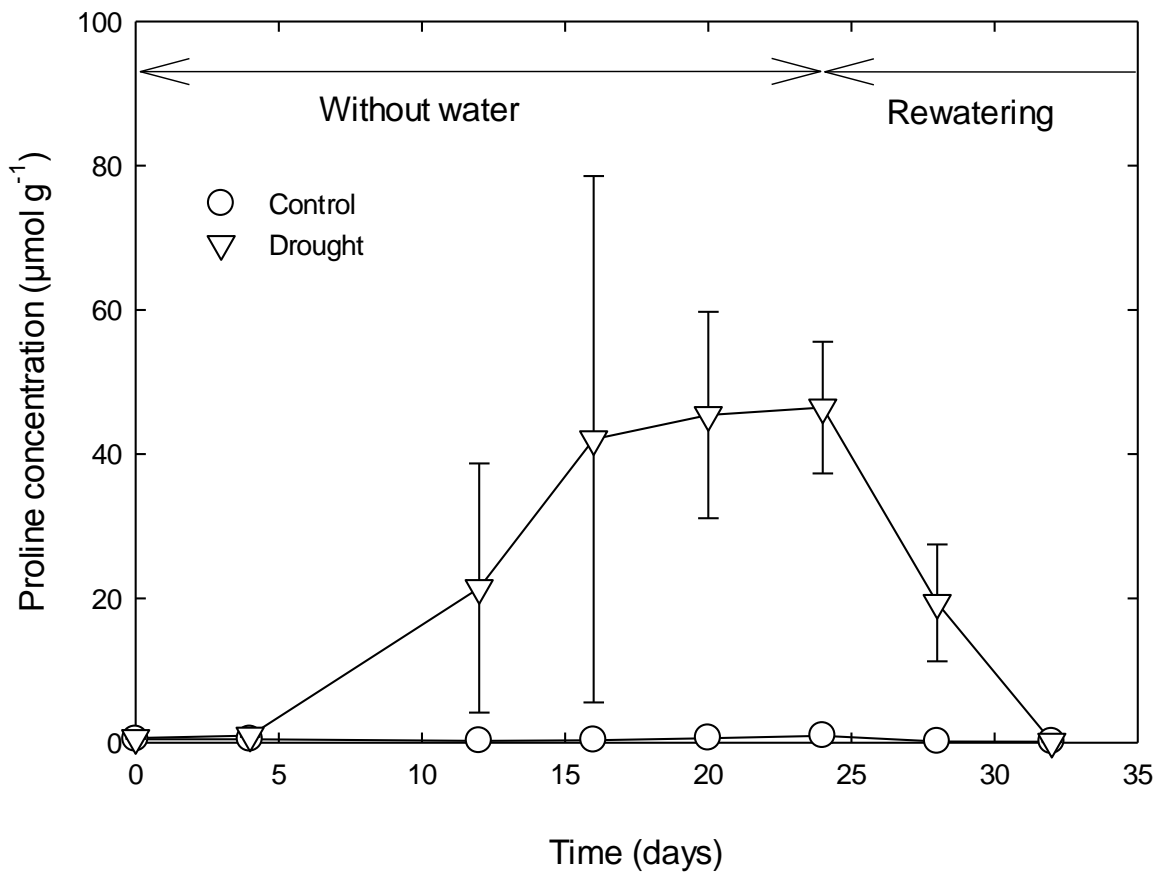


Figure 6. Proline concentration of *V. sativa* in response to drought. Plants were irrigated to field capacity every four days (O) or had

#### *d. Discussion*

Climate change models estimate, for Mexico and other subtropical locations, that a reduction in precipitation is impending and that the regularity of such rainfall will become less predictable (Intergovernmental Panel on Climate Change, 2007; Conde *et al.*, 2008). Considering that most of the agricultural operations in Mexico are rainfed, understanding the responses of crops to drier conditions is of paramount importance, especially because this small scale agricultural production is for self-sustenance (Comisión Nacional del Agua, 2008; Orozco-Martinez *et al.*, 2011). A reduction in residual soil humidity has already been detected in some locations. For instance, along a precipitation gradient from west-central Mexico, maize cultivation in higher sites with a greater annual precipitation started earlier than at drier sites because it relied on residual soil humidity to sustain plant growth before the rainy season. However, because enough water is no longer retained in the soil, producers have been forced to delay sowing until the first few rains occur (unpublished observations).

Here, for *Vicia sativa*, a cover crop that is also utilized as green-manure that relies on residual soil humidity, drought led to a reduction in gas exchange and to an accumulation of osmotically active solutes such as proline. However, over the course of the 24 days spanning water withholding, tissue water content did not decrease. This results suggest that the behavior of *V. sativa* is consistent with the behavior of an “isohydric” species, for which the leaf water status does not correlate with the soil water status (Lambers *et al.*, 1998). In this case, the roots respond to a decreasing water potential by producing abscisic acid, which in turn closes stomata before a substantial water loss occurs, thus helping the plant resist drought (DaCosta and Huang, 2009). For other crops such as *Zea mays* and *Vigna sinensis* an interruption of irrigation does not affect leaf water

potential because the stomatal conductance declines before any adverse effects on water shortage reaches the leaves (Lambers *et al.*, 1998).

Also consistent with isohydric behavior, gas exchange for *V. sativa* decreased after water withholding. In particular, transpiration halved at 8 days of drought, while stomatal conductance decreased by 1/3 at 12 days of drought. This is consistent with a response to drying soil. For example, for silica sand of similar drainage properties as the substrate utilized here, the water potential of a 10 cm-deep column reaches the permanent wilting point of -1.5 MPa at 5 days after water withholding, leading to a decrease of 1/3 in the stomatal conductance of a succulent crop (Nobel and de la Barrera, 2002; Nobel, 2009). Here, the substrate depth was 25 cm, explaining the delay in the reduction of gas exchange rates.

The first effect of drought on plant productivity is a reduction of the net CO<sub>2</sub> uptake rate (Taiz and Zeiger, 2002). Indeed, such assimilation was the most sensitive parameter of gas exchange to drought for *V. sativa*. The fact that a significant reduction of carbon assimilation occurred four days earlier than a reduction of transpiration could be reflecting the physiological water requirements of photosynthesis, *i.e.*, water is the source of electrons for photosynthesis (Taiz and Zeiger, 2002). A faster reduction of photosynthesis than of transpiration also occurs for *Phaseolus vulgaris*, *Gossypium hirsutum*, and *Dorycnium hirsutum* (Samarakoon and Gifford, 1996; Mayashita *et al.*, 2004; Moreno *et al.*, 2008). While the assimilation rates of droughted plants were less than half of those of the control individuals, no permanent damage to the photosynthetic apparatus of *V. sativa* appeared to occur over the duration of the experiment, as suggested by the recovery of the carbon assimilation rate within one week of resuming irrigation. In addition, no significant changes occurred during drought for the tissular content of chlorophyll, although a decreasing trend

was observed. It is likely that a longer drought could lead to permanent photosynthetic damage, as it occurs for *Poa pratensis* (14 days of drought until permanent damage; Jiang and Huang, 2001), *Festuca arundinacea* (24 days of drought until death; Wang and Huang, 2004). In any case, for *V. sativa*, whose lifecycle spans a mere 120 days, the lethal effects of a drought of over one month of duration may be overpowered by the onset of senescence (Özhan *et al.*, 2008). In this case, the crop can be utilized in increasingly arid locations as long as an initial water pulse of sufficient magnitude is available.

While transpiration was reduced substantially following water withholding, stomatal water loss did not cease, this suggest that water from the substrate might have been extracted by the plant by further reducing its osmotic potential, *e. g.*, via the accumulation of proline and other osmolites, in order to maintain constant gravimetric tissue water content. Water deficit induced a dramatic increase in the proline concentration in response osmotic stress because proline has the ability to mediate osmotic adjustments, to reduce stress-induced cellular acidification, to provide energy for recovery from drought via prime oxidative respiration (Hare and Cress, 1997). Here, proline was detected the same day that stomatal conductance started to decrease, confirming for *V. sativa*, as it occurs for other plant species, that the proline concentration can be utilized as an indicator of tolerance to water stress that reduces leaf osmotic potential, which drives water into the plant to maintain sufficient water in the leaves and avoid permanent damage in the photosynthetic pigments and allow a fast recovery when the water withhold stops.

*Vicia sativa* was able to withstand a month-long drought by reducing its transpirational water loss and accumulating osmotically active solutes, which enabled this plant to quickly recover its physiological processes once irrigation was resumed. The velocity of the recovery depends on the degree of the photosynthetic recovery (Flexas *et al.*,



2006). In this case, the photosynthetic pigments did not decrease significantly, which allowed the plants to have a fast recovery of physiological processes. This suggests that this species can be useful in increasingly arid locations, especially when its short lifespan is considered. Future research should validate these findings in the field and explore variations such as the developmental time for the onset of drought, the maximum duration of drought that still allows the development of the crop, and the minimum initial amount of soil humidity required by this crop.

## V. Discusión

Los modelos de cambio climático para México y otras regiones subtropicales indican que una reducción en la precipitación es inminente y que la lluvia se volverá aún menos predecible (IPCC, 2007; Conde *et al.*, 2008). Considerando que la mayoría de las prácticas agrícolas de México son de temporal, entender la respuesta de los cultivos a condiciones de sequía es de suma importancia, especialmente porque la agricultura a pequeña escala es de autoconsumo (CNA, 2008; Orozco-Martinez *et al.*, 2011). Una reducción en la humedad residual del suelo ya ha sido detectada en algunas localidades. Por ejemplo, a lo largo del gradiente de precipitación del centro-oeste de México, los cultivos de maíz en sitios elevados y con gran precipitación comienzan más temprano que en sitios secos debido a que se sostienen de la humedad residual acumulada en el suelo para mantener el crecimiento de las plantas antes de la temporada de lluvias. Sin embargo, como el suelo ya no podrá retener suficiente agua, los productores han sido forzados a retrasar la siembra hasta las primeras lluvias (observaciones no publicadas).

Para *Vicia sativa*, (usada como cultivo de cobertura y abono verde que utiliza la humedad residual del suelo), la sequía genera una reducción en el intercambio de gases y una acumulación de solutos osmolíticamente activos como la prolina. Sin embargo, durante el curso de 24 días en los que se suspendió el riego la cantidad de agua en las hojas no se redujo. Este resultado sugiere que el comportamiento de *V. sativa* es consistente con el comportamiento de una planta “isohídrica”, para las cuales la cantidad de agua en las hojas no está relacionada con la cantidad de agua en el suelo (Lambers *et al.*, 1998). En este caso, las raíces responden al decremento en el potencial osmótico produciendo ácido abscísico, que cierra los estomas antes de una pérdida sustancial de agua, lo que le permite a la planta resistir la sequía (DaCosta and Huang 2009). Para otros cultivos como *Zea mays* y *Vigna*

*sinensis* una interrupción en el riego no afecta el potencial de agua en las hojas porque la conductancia estomática se reduce antes de que ocurran efectos adversos en las hojas (Lambers *et al.*, 1998).

También consistentemente con una planta isohídrica, el intercambio de gases de *V. sativa* decrece durante la interrupción del riego. En particular, la transpiración se redujo a la mitad a los ocho días de sequía, mientras la conductancia estomática se redujo a 1/3 en 12 días de sequía. Esto es consistente con una respuesta a un suelo que se seca. Por ejemplo, para la arena de sílice de propiedades de drenado similares al sustrato utilizado en este experimento, el potencial de agua de una columna de 10 cm de profundidad llega al punto de marchitamiento de 1.5 MPa en 5 días después de que se detiene el riego, llevando a un decremento de 1/3 en la conductancia estomática de un cultivo suculento (Nobel and de la Barrera 2002; Nobel 2009). En este caso, el sustrato tenía una profundidad de 25 cm, lo que explica el retraso en la reducción de las tasas en el intercambio de gases.

El primer efecto en la reducción de la productividad de la planta es una reducción en la asimilación neta de CO<sub>2</sub> (Taiz and Zeiger, 2002). En realidad, dicha asimilación fue el parámetro de intercambio de gases más sensible a la sequía para *V. sativa*. El hecho de una reducción significativa en la asimilación de carbono ocurrida cuatro días antes de la reducción en la transpiración puede reflejar los requerimientos fisiológicos de agua para la fotosíntesis, ya que el agua es la fuente de electrones para la fotosíntesis (Taiz and Zeiger, 2002). Una rápida reducción tanto en la fotosíntesis como en la transpiración también ocurre para *Phaseolus vulgaris*, *Gossypium hirsutum* y para *Dorycnium hirsutum* (Samarakoon and Gifford 1996; Mayashita *et al.*, 2004; Moreno *et al.* 2008). Mientras que la tasa de asimilación de las plantas en sequía fueron menores a la mitad que la de las plantas testigo, no se presentó ningún daño en el aparato fotosintético de *V. sativa* a lo largo

del experimento, como sugiere la rápida recuperación de la tasa de asimilación de carbono que se midió en una semana después de reanudar el riego. Adicionalmente, no ocurrieron cambios significativos en el contenido de clorofila durante el periodo de sequía, a pesar de que se logró observar una tendencia a la disminución. Es posible que un largo periodo de sequía pueda generar un daño permanente en los pigmentos fotosintéticos, como ocurre para *Poa pratensis* (14 días de sequía generan daño permanente; Jiang and Huang, 2001), y para *Festuca arundinacea* (24 de sequía generan daño permanente; Wang and Huang 2004). En cualquier caso, para *V. sativa*, cuyo ciclo de vida es de apenas 120 días, los efectos letales de la sequía durante un mes de duración pudieron haber sido superados por el inicio de la senescencia (Özhan 2008). En este caso, el cultivo puede ser utilizado en zonas cada vez más áridas siempre y cuando exista un pulso de suficiente agua para las plantas.

Mientras que la transpiración se redujo sustancialmente durante la interrupción del riego, la pérdida de agua por los estomas no se detuvo, esto sugiere que el agua del sustrato debió de ser extraída por la planta mediante una continua reducción del potencial osmótico, generada por acumulación de prolina y otros osmolitos, de manera que se mantenga un contenido gravimétrico de agua constante. El déficit de agua induce un incremento dramático en el contenido de prolina debido a un estrés osmótico ya que la prolina tiene la capacidad de mediar los ajustes osmóticos, la prolina también reduce el estrés inducido por la acidificación celular, brinda energía para la recuperación por medio la respiración oxidativa principal y mantiene al metabolismo de la planta en estándares inferiores a los de las condiciones normales (Hare and Cress, 1997). En este caso, la prolina se detectó el mismo día en el que la conductancia estomática comenzó a decrecer, confirmando que para *V. sativa*, como para otras especies, la concentración de prolina puede ser utilizado como

un indicador de tolerancia al estrés hídrico que reduce el potencial osmótico de las hojas, lo que dirige agua a la planta para mantener suficiente agua en las hojas y evitar el daño permanente en los pigmentos fotosintéticos y permitir una recuperación rápida cuando se reanuda el riego.

*Vicia sativa* es capaz de mantener un mes de sequía reduciendo sus tasas de transpiración y acumulando solutos osmolíticamente activos, lo que le permitió a recuperar rápidamente sus procesos fisiológicos una vez que el riego se reanudó. La velocidad de recuperación depende del grado de la recuperación de la fotosíntesis (Felxas *et al.*, 2006), en este caso los pigmentos fotosintéticos no decrecieron significativamente, lo que permitió a las plantas una rápida recuperación en todos sus procesos fisiológicos al terminar el periodo de sequía pero no hubo una recuperación total de los pigmentos fotosintéticos a un estado similar a los del testigo. Esto sugiere que esta especie puede ser usada en zonas donde la aridez se incrementa, especialmente considerando su corto periodo de vida. Para futuros estudios se deben validar estos descubrimientos en campo y explorar si dichas variaciones como el desarrollo durante el tiempo de sequía, la duración máxima de la sequía, si esta permite el desarrollo del cultivo y la cantidad mínima inicial de humedad residual del suelo requerida por este cultivo.

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