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# Cold Stress Tolerance during Early Growth Stages of Naturalized *Helianthus petiolaris* Populations

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**Abstract:** In the last 10 years the sunflower crop area of Argentina has shifted towards the Southwest as a result of the expansion of soybean cultivation. Early sowings require greater tolerance to low temperatures in order to optimize the use of water resources by the crop, especially in the seedling stage, when cold stress limits growth and productivity. *Helianthus petiolaris* is a wild genetic resource that has contributed to the production of sunflower cultivars. The goal of this study was to evaluate the tolerance to low temperatures in *H. petiolaris* plants. Morphological traits (plant height, leaf width and leaf length) and physiological traits (electrolyte leakage, chlorophyll content, and glucose content) were compared in eight accessions having an early germination in the field and one with normal life cycle. Acclimated and non-acclimated plants were subjected to cold stress followed by a recovery period. Physiological traits during early growth stages showed differences in cold tolerance and for acclimation ability between accessions, suggesting the existence of heritable components. Four accessions were the most cold-tolerant among the studied populations, suggesting a natural adaptation related to the geographical site. Results point out that *H. petiolaris* is a useful genetic resource to contribute to sunflower breeding in the search of cold tolerant cultivars.

**Keywords:** acclimation, chlorophyll content, electrolyte leakage, glucose content, morphological traits

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## Introduction

The sunflower is a source of high quality edible oil and Argentina is one of the major producing countries in the world. In recent years the crop area underwent a shift towards the Southwest as a result of the expansion of soybean cultivation in the humid pampas (De la Vega *et al.*, 2007). Consequently, there is a need for early sowings to optimize the use of water resources, which in turn requires greater tolerance to low temperatures by the crop, especially in the seedling stage. The low temperature is a major abiotic stress that limits growth, productivity, and geographical distribution since vital metabolic processes are influenced by temperature. Wild species are a major source of stress tolerance genes, whose transfer to the crop can markedly increase commercial production (Seiler, 1992; Jan and Seiler, 2007). Two annual *Helianthus* species that naturalized during the last 60 years in Argentine constitute a genetic resource of high potential value. *H. annuus* and *H. petiolaris* have contributed to the production of modern cultivars (Seiler and Rieseberg, 1997) being a reservoir of genetic diversity easily transferable to the crop (Maxted *et al.*, 2006). The adaptation to the colonized environment under natural selection could have generated new features for cold tolerance traits (Ureta *et al.*, 2008; Gutierrez *et al.*, 2010a). However, there are no studies on cold tolerance in wild *Helianthus* spp. *H. petiolaris* seedlings that grow earlier than normal under natural conditions of extreme habitats may have some tolerance to low temperatures during germination. Plants have inducible tolerance mechanisms that extend the temperature range for survival during acute stress. Low temperatures define a climatic border for the biogeography of some species (Hoffmann, 2002), making temperature an interesting candidate for a habitat parameter that influences the genetic structure of local populations through natural selection (Hannah *et al.*, 2006). In cold acclimation, highly complex processes operate, including perception and signal transduction at low temperatures. Physical consequences on cellular components program broad physiological and biochemical changes. Low temperatures increase membrane permeability measured by electrolyte leakage, although the nature and change in biochemical and functional properties of the membranes are still not entirely clear (Yuchan *et al.*, 2014). The response of the cell membrane to low temperatures has not been already characterized in *H. petiolaris*.

Photosynthesis is the main physiological process studied under low temperature conditions in many species, including *Arabidopsis* (Uemura *et al.*, 1995), alfalfa (Peoples *et al.*, 1978), rice (Zhi-Hong *et al.*, 2005), corn (Fryer *et al.*, 1995), wheat (Groom and Baker, 1992), and barley (Greer *et al.*, 1991).

In sunflower, low temperatures influence chlorophyll content and fluorescence (photochemical efficiency of photosystem II). These traits have been used to assess tolerance to low temperatures, demonstrating that significant differences exist in plants exposed to low temperatures with lower chlorophyll content and fluorescence values in plants subjected to cold treatment compared to control plants (Allinne *et al.*, 2009).

The carbohydrate content is a well known parameter used in cold response studies. Accumulation of metabolites in the cytoplasm and low molecular weight solutes such as carbohydrates is another important metabolic change observed in plants after exposure to low temperature (Guy, 1990). Cold stress increases carbohydrate concentration in plant tissues (Antikainen and Pihakaski, 1994; Shu *et al.*, 2009) often associated with an increased tolerance to low temperatures (Perras and Sarhan, 1984; Guy *et al.*, 1992).

There is limited information on this subject in sunflower compared to other economically important crops such as rice (Baruah *et al.*, 2009), maize (Bhosale *et al.*, 2007), wheat (Baga *et al.*, 2007), canola (Asghari *et al.*, 2008), and sorghum (Knoll *et al.*, 2008). This fact and the importance of sunflower crop in Argentina raised the need for this study as a first step to find characters of low temperature tolerance in wild *Helianthus* materials and their eventual transfer to sunflower. Sunflower and *H. petiolaris* have low sexual compatibility as a result of several chromosomal rearrangements that are responsible for the partial reproductive isolation of both species (Rieseberg *et al.*, 1995). Despite these barriers, interspecific hybrids with intermediate morphology are often found in the geographical areas in which the two species coexist (Ureta *et al.*, 2008). In a previous work we reported hybridization events between *H. petiolaris* and sunflower recorded in several populations of central Argentina (Gutierrez *et al.*, 2010a).

The goal of this study was to characterize by morphological and physiological traits the response to cold from early grown plants of *H. petiolaris* populations with different life cycle and geographical distribution, acclimated and non-acclimated to low temperatures, to identify accessions with cold tolerance traits for future studies that can contribute to sunflower breeding.

## Materials and methods

### Plant material and cold stress experiment

Seeds from mature plants of eight *Helianthus petiolaris* populations were collected, which have germinated and flowered early in spring, in the second half

of August and reached senescence in late January, assuming that natural selection for cold resistance had been operating. A population, Victorica, with a normal life cycle, germinating in late September was also included (Table 1) striving for comparison. The seeds were germinated on wet trays after stratification for seven days at 4 °C. Then seedlings were placed in trays with growing substrate. Plants were grown in the greenhouse at 26 °C day/20 °C night with a photoperiod of 18 hours, up to the 4-leaf stage.

**Table 1:** *H. petiolaris* populations with early and normal life cycle expressed in months.

Population	Symbol	Province	Life cycle
Rivera	RIV	Buenos Aires	August – January
Carhue	CAR	Buenos Aires	August – January
Quenuma	QUE	Buenos Aires	August – January
Padre Buodo	PAB	La Pampa	August – January
Catriló	CAT	La Pampa	August – January
Colonia Barón	COB	La Pampa	August – January
Ataliva Roca	ATR	La Pampa	August – January
Alpachiri	ALP	La Pampa	August – January
Victorica	VIC	La Pampa	September – March

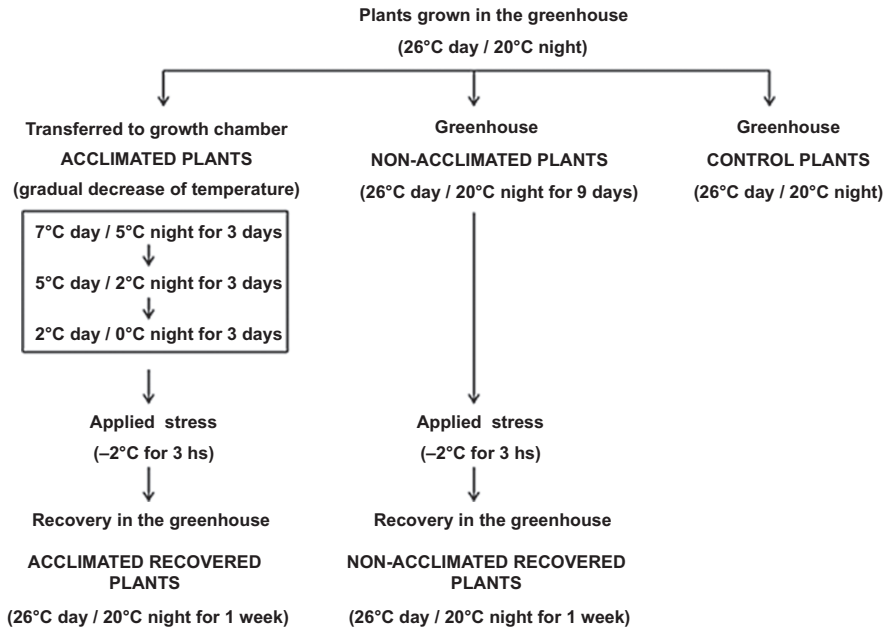
Experiments were performed in five groups of *H. petiolaris* plants: acclimated plants under cold stress, non-acclimated plants under cold stress, acclimated plants under cold stress followed by 7-day recovery, non-acclimated plants under cold stress followed by 7-day recovery, and control plants (Figure 1). For the nine accessions, five plants of each group were assessed.

## Morphological and physiological traits

To evaluate the phenotypic response to low temperature, the following growth traits were studied for each treatment: plant height, leaf width and leaf length. To evaluate the physiological response to low temperature the following traits were studied for each treatment: electrolyte leakage, chlorophyll content, and glucose content.

## Electrolyte leakage

Leaf discs (2 cm diameter) from young fully expanded leaves were rinsed three times with demineralised water and placed in tubes with 10 mL of demineralised



**Figure 1:** Flow chart of five treatments applied to the studied accessions.

water. Electrolyte leakage (EL) was measured with a conductimeter (Twin Con B-173, Horiba, Japan) after 24 h of floating at room temperature. Then, tubes were boiled for 90 min (100°C) to kill the leaf tissue and release the total electrolytes (TE). Results were expressed as relative electrolyte content (REL) calculated as  $(EL/TE) \times 100$  (%).

## Chlorophyll content

The relative chlorophyll content measurements were performed with a Minolta SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan) on healthy leaves. Three SPAD meter readings were taken on interveinal areas of each leaf, and the average reading was recorded to represent the SPAD value of a particular leaf.

## Glucose content

The glucose content was determined on fully developed young leaves. Dry tissue was weighed and 50 mg were homogenized in a mortar with 3 ml of 0.1 M

sodium acetate buffer pH 4.8. For determination of initial glucose a 500  $\mu$ l aliquot of homogenate was analyzed by the enzymatic method of glucose oxidase – peroxidase using the Lab Wiener kit. The remaining homogenate was incubated in a thermostatic bath at 100 °C for 20 min. Then, 140  $\mu$ l amyloglucosidase (10 mg/ml) was added to a 1.4 ml aliquot and incubated at 55 °C for one hour. A dot blot assay with Lugol reagent was done to verify the complete enzymatic hydrolysis of the treated material. At the end of the incubation time the reaction was stopped by subjecting the sample to 100 °C for one minute. Samples were kept on ice until the determination of total glucose. The samples were centrifuged at 14.000 rpm for 10 min; finally the total glucose content of the supernatant was determined by measuring the glucose released by the glucose oxidase – peroxidase enzymatic method. The absorbance of the reaction mixture at 505 nm against a blank reagent and its concentration was determined in comparison with a standard glucose of 1 g/l solution provided by the test kit.

## Statistical analysis

ANOVA and Principal Component analysis were performed on the data set. Plant height, leaf width, leaf length, electrolyte leakage, chlorophyll content and glucose content were considered as dependent variables across populations and treatments. Means were compared by the Tukey test ( $P \leq 0.01$ ). All the analyses were done with InfoStat (Di Rienzo *et al.*, 2011).

## Results

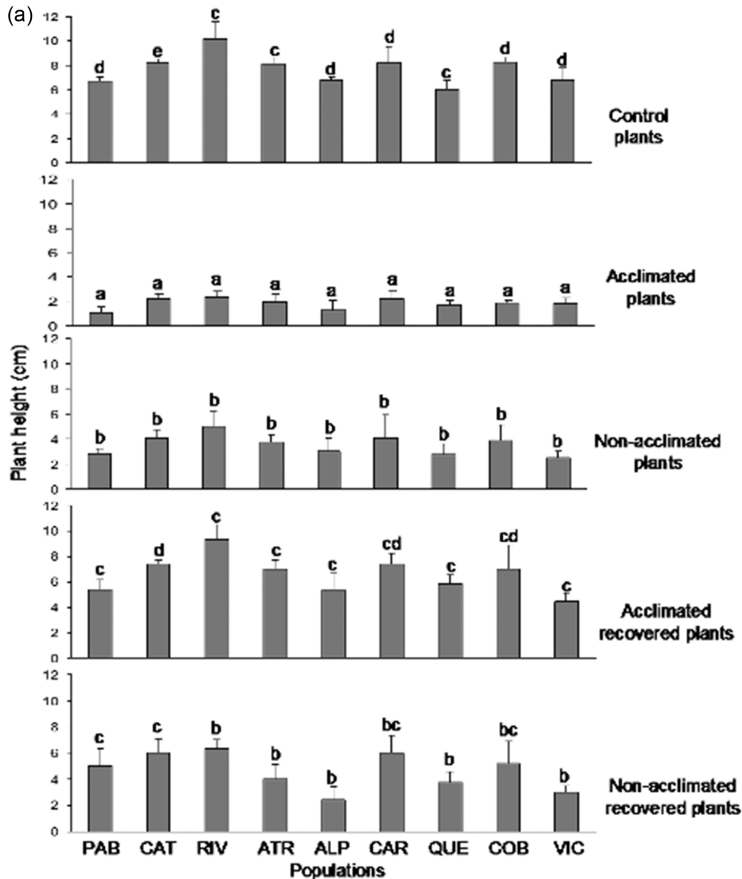
### Morphological traits

Among growth traits, plant height showed the greatest differences between treatments, with leaf width the less affected trait.

All the studied accessions showed significant differences in seedling height between acclimated and non-acclimated plants. For acclimated plants from all the accessions there were highly significant differences in plant height between recovered and non-recovered plants. Recovered plants from RIV, ATR, CAR, QUE, and COB did not differ from the controls. RIV had the greatest height values similar to the control. Non-acclimated plants had lower growth compared

to acclimated ones during the recovery period, since only two accessions differed from the non-recovered plants (Figure 2A).

Leaf width was less influenced by acclimation in most accessions, since only three out of nine accessions showed significant differences between acclimated and non-acclimated plants. After the recovery stage, six previously acclimated accessions had a significant growth when plants were compared to non-



**Figure 2:** Cold stress effect on growth traits: (A) Plant height (cm), (B) Leaf width (cm), and (C) Leaf length (cm) in control plants, acclimated plants, non-acclimated plants, acclimated recovered plants and non-acclimated recovered plants from nine *H. petiolaris* accessions. Each bar represents mean  $\pm$  SD ( $n = 5$ ) and different letters indicate significant differences in Tukey test ( $P \leq 0.01$ ).

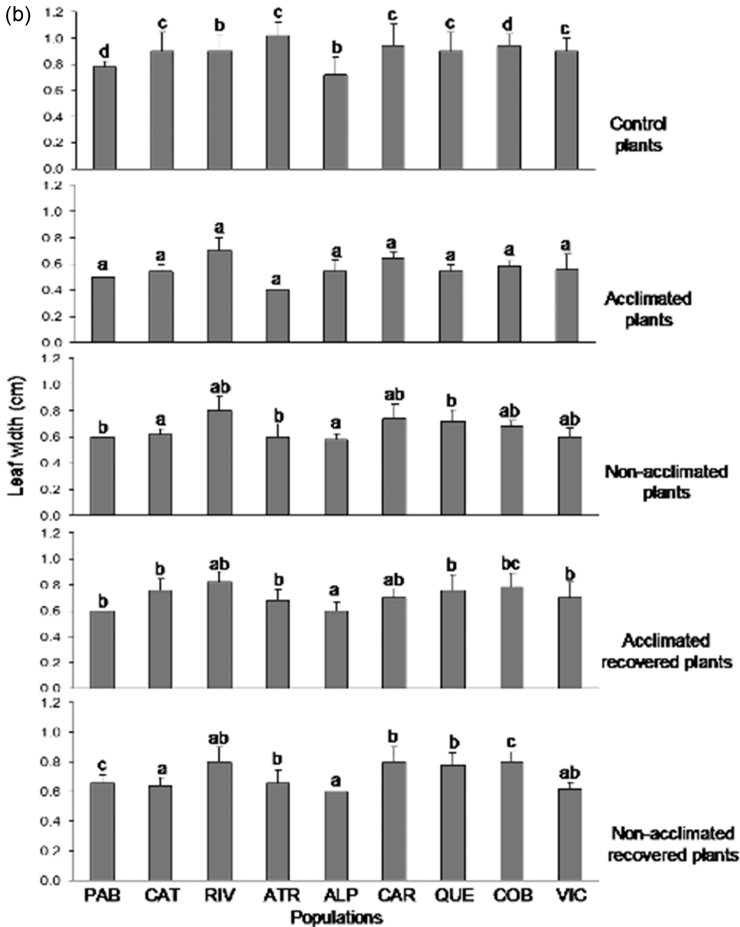


Figure 2: (continued)

acclimated ones. RIV values after recovery did not differ from their respective control (Figure 2B).

Six accessions showed significant differences in leaf length between acclimated and non-acclimated plants, whereas highly significant differences were found among acclimated recovered and non-recovered plants in all accessions. Acclimated plants of ATR, RIV, PAB, QUE, COB and CAT after recovery showed similar values to their respective controls, while no recovery was observed in non-acclimated plants, except for the RIV accession (Figure 2C).



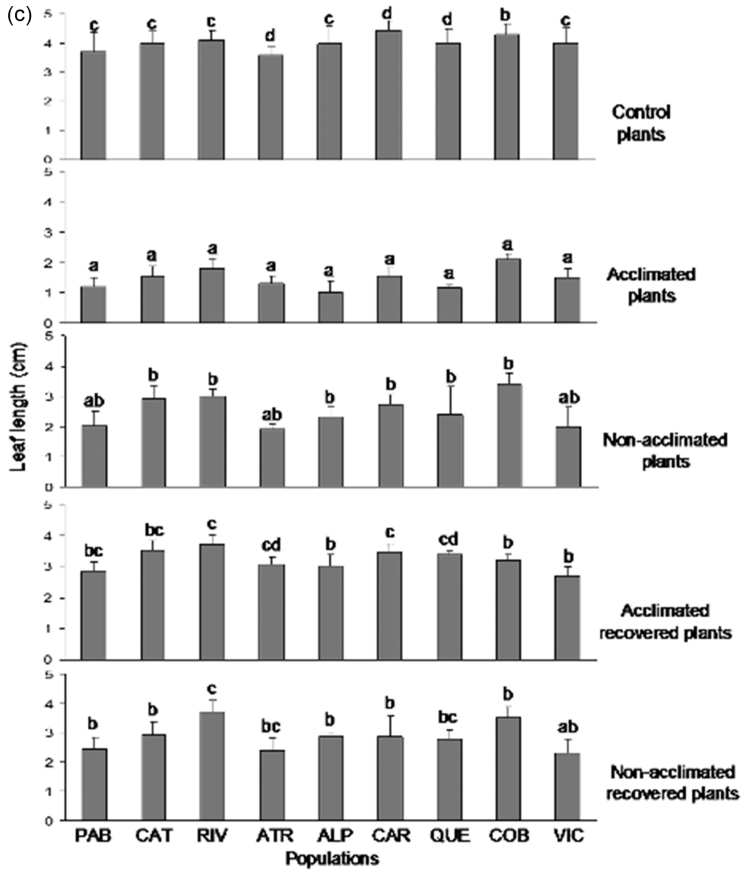


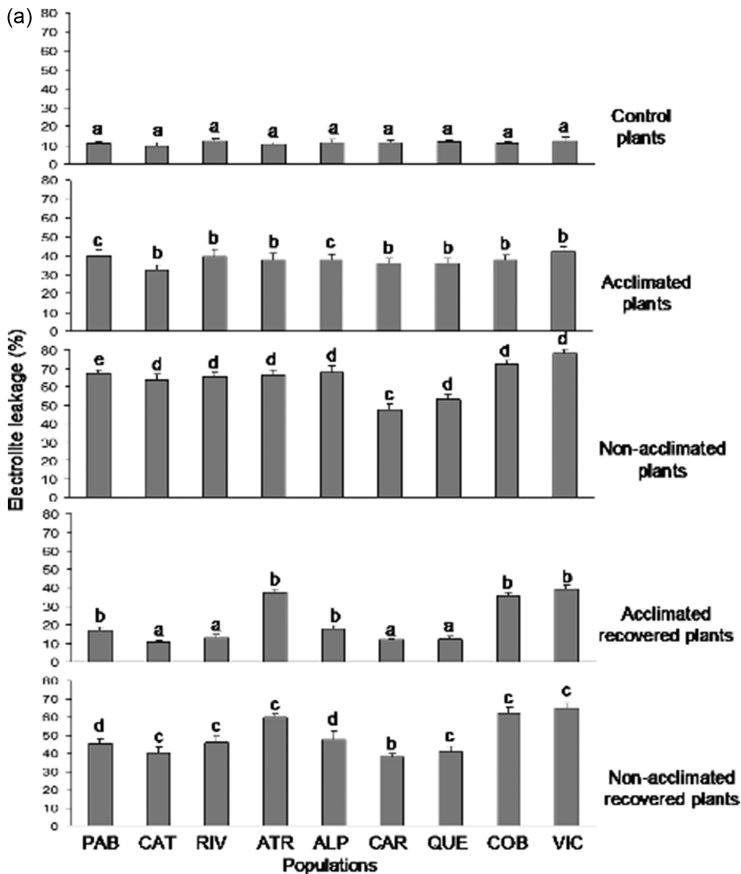
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## Physiological traits

Electrolyte leakage as a measure of membrane integrity greatly increased when the seedlings were subjected to low temperatures. As a whole, the lowest values corresponded to controls with a mean leakage of 11%, and highest values were for non-acclimated plants with a mean of 65%. Highly significant differences were found in acclimated vs non-acclimated plants, and acclimated recovered vs non-acclimated recovered plants from all the accessions. VIC showed the highest values for all treatments after the cold stress. This accession together with COB and ATR after recovery showed similar values to their respective acclimation treatment.

Acclimated CAT, CAR, QUE, and RIV accessions had the lowest values after recovery and did not differ among themselves and from their respective controls. Recovery values of the remaining acclimated accessions differed significantly from the controls (Figure 3A).

The lowest values of chlorophyll content corresponded to acclimated plants subjected to low temperature and the highest values were for controls. Highly significant differences were found among acclimated and non-acclimated plants



**Figure 3:** Cold stress effect on physiological traits: (A) Electrolyte leakage (%), (B) Chlorophyll content (SPAD values), and (C) Glucose content ( $\text{mg}\cdot\text{g}^{-1}$ ) in control plants, acclimated plants, non-acclimated plants, acclimated recovered plants and non-acclimated recovered plants from nine *H. petiolaris* accessions. Each bar represents mean  $\pm$  SD ( $n = 5$ ) and different letters indicate significant differences in Tukey test ( $P \leq 0.01$ ).

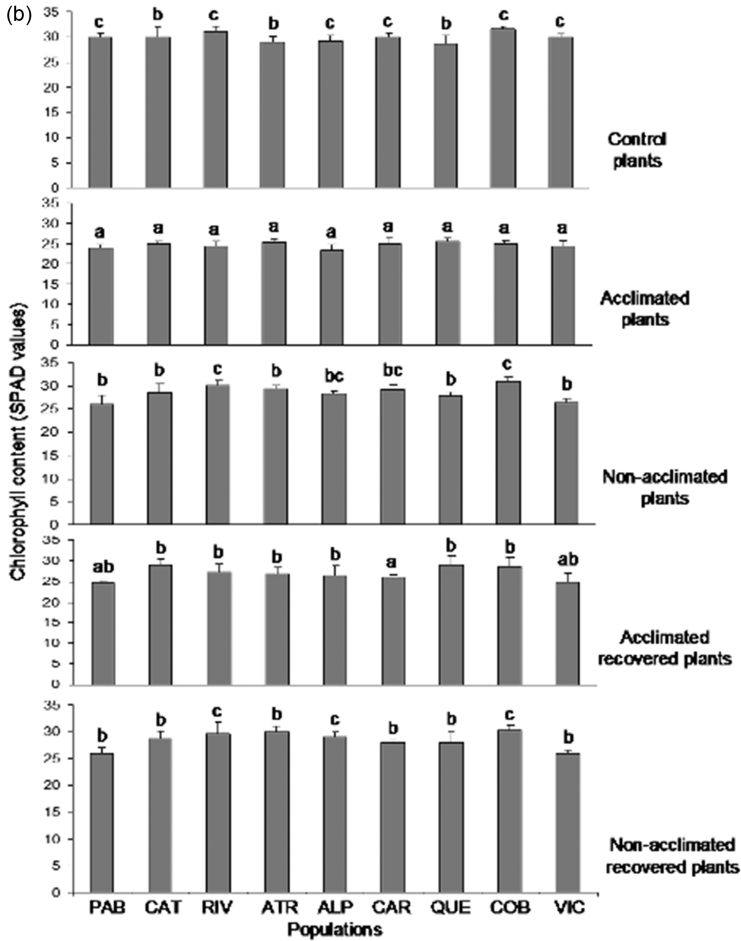


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from all accessions. Chlorophyll values of PAB, CAR, and VIC were the lowest among all accessions. ATR, QUE and CAT showed good recovery since their values were not significantly different from controls. No significant differences were found after recovery among non-acclimated plants from all the accessions (Figure 3B).

For glucose content, highly significant differences were found among acclimated and non-acclimated plants from PAB, CAT, RIV, CAR, QUE, and COB. The remaining accessions (ATR, ALP and VIC) did not differ significantly between

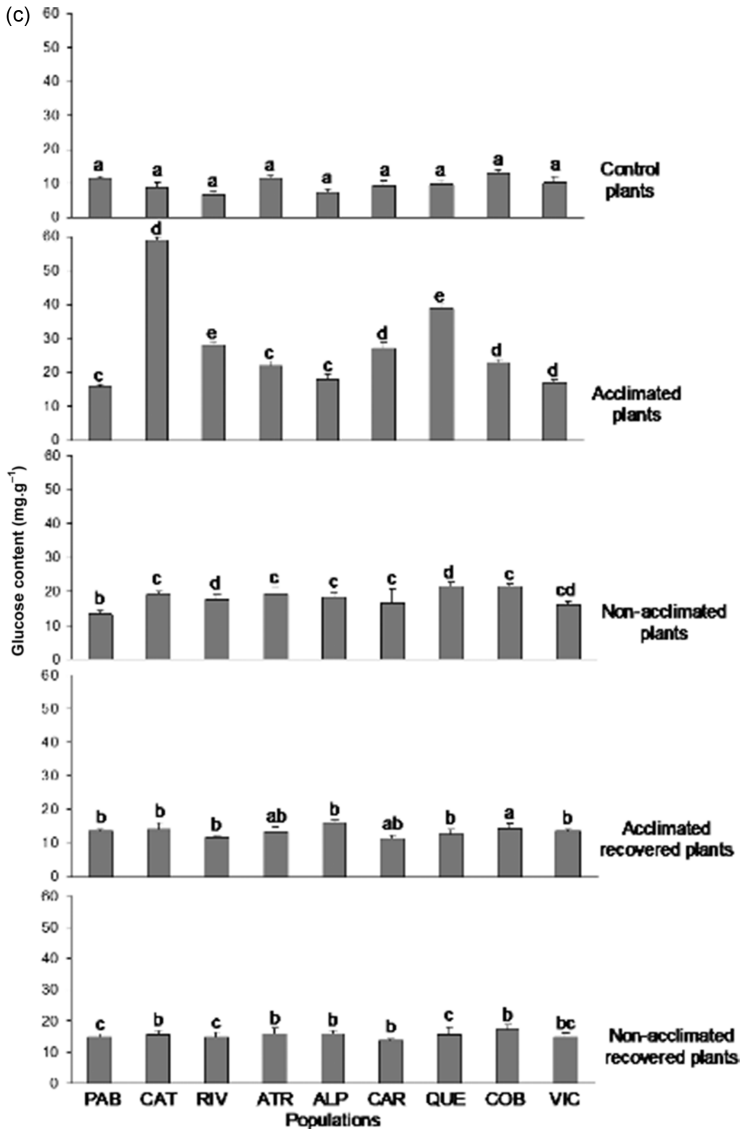


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both treatments. The lowest values of glucose content were for controls and the highest ones were for acclimated plants, with the extreme accessions CAT and QUE with means of  $59$  and  $39$   $\text{mg.g}^{-1}$  respectively; RIV with  $28$   $\text{mg.g}^{-1}$  and CAR with  $27$   $\text{mg.g}^{-1}$ . The remaining accessions showed lower values; below  $23$   $\text{mg.g}^{-1}$ .

Accessions CAR, RIV, and QUE showed the lowest glucose levels after the acclimated recovery period. ALP, PAB, COB and VIC showed the lowest recovery values after acclimatization. After recovery, acclimated plants showed lower values than non-acclimated plants for all the accessions, although for CAT, ATR, ALP, CAR and VIC significant differences were not found among both treatments (Figure 3C).

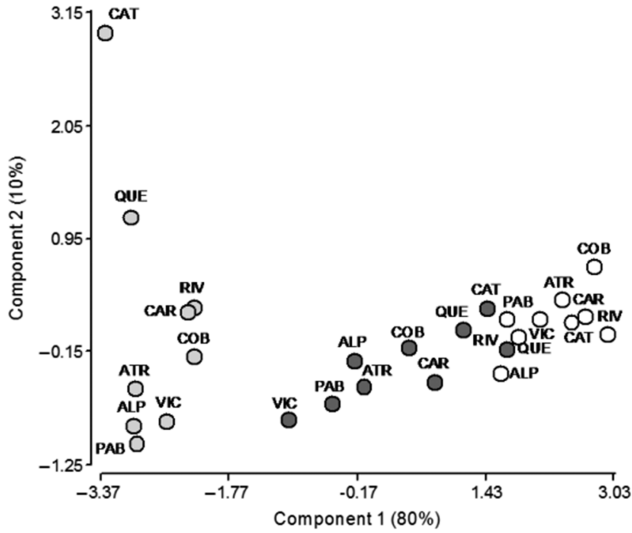
**Table 2:** Comparison of overall means of six traits: plant height (PH), leaf width (LW), leaf length (LL), and physiological traits: electrolyte leakage (EL), chlorophyll content (CC), glucose content (GC) in the nine *H. petiolaris* accessions with all treatments. Different letters indicate significant differences according to Tukey test ( $P < 0.01$ ).

Treatment	PH	LW	LL	EL	CC	GC
Control	7.7 d	0.9 c	4.0 d	11.3 a	29.9 d	9.9 a
Acclimation	1.8 a	0.6 a	1.5 a	37.7 c	24.7 a	27.6 c
Non-acclimation	3.5 b	0.7 b	2.5 b	64.7 e	28.5 c	18.1 b
Acclimation + recover	6.6 c	0.7 b	3.2 c	21.8 b	27.0 b	13.5 ab
Non-acclimation + recover	4.6 b	0.7 b	2.9 bc	49.4 d	28.4 c	15.3 ab

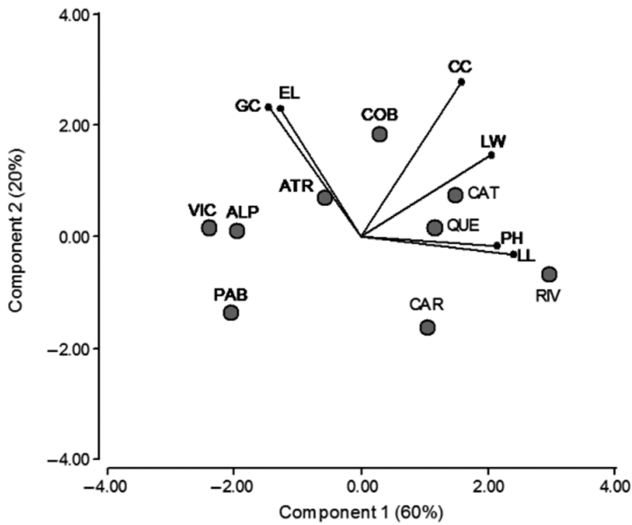
On the whole, significant differences between acclimated and non acclimated plants were found for all the studied traits. Table 2 shows the overall effect of acclimation, since mean values of acclimation + recover were the closest to control values. Recovered acclimated plants showed marked changes in mean values compared to those of recovered non-acclimated plants.

Principal Component Analysis (PCA) was based on three treatments (acclimated plants, acclimated + recovery, and control plants) and explained 90 % of the variation in the first two axes (Figure 4). In PC1 all the variables had similar loadings, whereas in PC2 the largest loading corresponded to the glucose content variable. Acclimated recovered plants were placed between acclimated plants and control plants mainly based on morphological and physiological traits. Some recovered plants were closer to the control plants, these corresponding to CAT, CAR, QUE and RIV accessions that showed the best behavior and recovery values after cold stress.

The best performance and recovery values to cold stress are shown in Figure 5. In PC1 the morphological variables accounted for the largest loadings, while in PC2 the largest loadings corresponded to glucose content and conductivity. Acclimated accessions CAT, CAR, QUE, and RIV had the lowest electrolyte leakage values after recovery and the highest values of chlorophyll content



**Figure 4:** Principal component analysis of morphological and physiological traits in nine *H. petiolaris* accessions. Color corresponds to acclimated without recovery (light grey), acclimated with recovery (dark grey), and control (white) treatments.



**Figure 5:** Principal component analysis (biplot) of growth traits: plant height (PH), leaf width (LW), leaf length (LL) and physiological traits: electrolyte leakage (EL), chlorophyll content (CC), glucose content (GC) in the nine *H. petiolaris* accessions with acclimated recovery treatment.

corresponded to QUE and CAT. Accessions CAR, RIV, and QUE showed the lowest glucose levels after recovery and RIV achieved the best morphological values.

## Discussion

This study of nine geographically diverse accessions of *Helianthus petiolaris* identified considerable natural variation for cold tolerance as well as for acclimation ability. Changes induced by cold stress affected all morphological and physiological traits. The phenotypic response to low temperatures was significant for the treatments after cold stress showing a slow but constant growth in acclimated plants. Non acclimated plants had a delay in growth after the recovery period compared to acclimated plants that significantly recovered, reaching similar control values in some cases. Overall, the accession with best morphological recovery was RIV and the worst was VIC, the population that did not germinate early. A recent study (Okami *et al.*, 2015) on the response of morphological traits in seedlings of three rice lines recovered after drought stress showed that there was a rapid phenotypic growth during the recovery phase and that there were genotypic differences among lines. Amongst morphological traits, leaf width was the least plastic trait. Early experiments in our lab comparing *H. petiolaris* and sunflower HA89 seedlings growing at low temperature showed non-comparable results on survival and plant height. Differences in seed size, germination energy, and plant architecture between plants of both species accounted for those results, likely blurring differences due to physiological basis. For this reason, we did not include sunflower materials in the present experiments (Gutierrez *et al.*, 2010b; Hernandez *et al.*, 2015).

The SPAD-502 provides a quick nondestructive measurement of chlorophyll content in leaves. It is an effective tool for estimating total leaf pigment concentration since there is a significant positive relationship between leaf chlorophyll and SPAD values (Hawkins *et al.*, 2009; Yinghui *et al.*, 2013). Low temperatures inhibit the accumulation of chlorophyll in actively growing leaves (Allinne *et al.*, 2009). The chlorophyll content was lower in stressed plants than in control plants maintained in the greenhouse. Given that plants were subjected to gradual temperature drop for nine days reaching  $-2^{\circ}\text{C}$  by the stress time, it could be considered similar to the response found by Liu *et al.* (2013) on oats in which chlorophyll content decreased with time, significantly decreased at freezing temperatures after seven days. Mantoan Benetti *et al.* (2015) evaluated the tolerance of *Annona emarginata* plants to water stress followed by a recovery period and no damage was found, demonstrating high photoprotective ability.

Our results after the recovery time are similar, since the values in chlorophyll content increased in acclimated ATR, QUE and CAT accessions reaching similar values to the controls.

The lower chlorophyll content of *H. petiolaris* plants exposed to low temperatures could reflect the process of photoprotection, because of the modification in pigment composition to enhance the ability to dissipate light energy excess as heat through the xanthophyll cycle (Demmig - Adams and Adams, 1996; Haldimann, 1998). The photoprotection process leads to a decrease in chlorophyll content and an increase of xanthin content, as demonstrated in maize (Leipner *et al.*, 1999) and tomato (Venema *et al.*, 1999) under low temperature.

The glucose and similar solutes contribute to the decrease of osmotic potential which leads to cytoplasmic freezing point reduction preventing cell dehydration and freezing (Yelenosky and Guy, 1989). The metabolism of soluble carbohydrates influences plant tissue sensitivity at low temperatures (Shao *et al.*, 2008), being excellent substances for osmotic adjust in higher plants (Uemura and Steponkus, 2003; Shao *et al.*, 2008). A recent study in tomato where the amount of soluble carbohydrates was measured at optimal, intermediate, and low temperature, showed that in cold tolerant plants low and intermediate temperatures increased soluble carbohydrates in leaves compared with cold-sensitive ones (Ntatsi *et al.*, 2014). In this study it was observed an increased glucose content in all accessions previously acclimated to cold, with a marked effect in CAT and QUE. This increase in leaf carbohydrate content would indicate activation of metabolic processes occurring during acclimation to prevent significant damage at the cellular level as in *Ricinus communis*, where carbohydrates mobilized from the roots before a heat stress (Ribeiro *et al.*, 2014). In pea, low temperature increases the carbohydrate content reflecting the antioxidant properties of fructose and their potential protective role in plants exposed to cold stress (Bogdanovic *et al.*, 2008). In *Vitis vinifera*, accumulation of soluble sugars in the branches protects the plant from damage caused by freezing (Han-yu *et al.*, 2014). Similarly, we found a strong correlation between the total soluble sugar content and low temperatures. Therefore, the increased sugar levels represent an important defense against cold stress in *H. petiolaris*. The glucose content after a week of recovery decreased significantly in acclimated CAT, QUE, CAR and RIV accessions, showing some tolerance to cold stress which allows a restart development and vegetative growth when temperature is optimal (Venema *et al.*, 1999). Furthermore, cold stress affects the properties of the plasma membrane causing changes and structural damage. The plasma membrane plays a central role in the activation of primary responses to chilling and in maintenance of cellular homeostasis and it is likely the



primary site of cold injury (Yuchan *et al.*, 2014). The main effect of temperature is fluidity of membrane lipids, which determine the chemical and physical properties of cell membranes and are the most important for membrane functionality under conditions of low temperature (Lyons, 1973). Changes in the membrane lipid composition of many plant species have been reported in response to low temperatures and represent great differences in cold sensitivity (Whitaker, 1993; Badea and Basu, 2009; Zhang *et al.*, 2013). These changes result in increased permeability because they affect cell membrane integrity and cell compartment under stress conditions. The degree of cell membrane injury induced by cold stress can be reflected by the electrolyte leakage. Relative conductance is a good indicator to indirectly evaluate plant responsiveness to cold stress. The extent of cell membrane damage is intensified when low temperature tension extends in time (Liu *et al.*, 2013). In our experiment the same was observed when non-acclimated plants were subjected to abiotic stress without hardening. As in Campos *et al.* (2003) the recovered plants in CAT, CAR, RIV and QUE accessions returned to normal conductivity evidencing a low impact on cell membrane permeability. This study demonstrated that *H. petiolaris* acclimated plants had less electrolyte leak compared to non-acclimated plants in cold stress; this shows a positive effect of the acclimatization stage, as in chickpea (Kazemi *et al.*, 2014) where acclimated plants showed greater tolerance to cold stress than non-acclimated ones, indicating the strong influence of the hardening process.

Table 2 highlights that acclimation is a very important feature for the plant tolerance level. Acclimation enables plants to modify structures and functions as a response to stress in order to reduce damage and improve fitness (Pollet *et al.*, 2011) and it is genetically determined (Janska *et al.*, 2010) so different accessions can exhibit different acclimation ability. In this study acclimation favored cold tolerance and recovery in all the accessions, although VIC was the less efficient in recovery after cold stress.

A better recovery performance following acclimation period was observed in four out of nine accessions (CAR, CAT, RIV, and QUE); this is summarized in Figures 4 and 5. In Figure 4 the acclimated recovered plants were placed between acclimated plants without recovery and control plants but CAT, CAR, QUE, and RIV recovered plants were closer to the control plants that showed the best behavior and recovery values after cold stress. Figure 5 shows the nine *H. petiolaris* accessions with acclimated recovery treatment. Principal Component 1 separates accessions with larger size from those with higher glucose content and electrolyte leakage following the recovery period. This figure shows that accessions CAT, QUE, CAR, and RIV have the highest morphological values, lowest glucose content and lowest electrolyte leakage after cold stress in acclimated plants.

## Conclusions

The tolerance to low temperatures in plants of nine *H. petiolaris* accessions was evaluated, eight having an early germination in the field and one with normal life cycle. The four populations (CAR, CAT, RIV and QUE) with better cold stress performance were geographically close in the eastern part of the studied region, while the least cold tolerant accession corresponded to a population in the western edge (VIC); this is a more continental site with lower mean temperature. Results point to a natural adaptation related to the geographical site. The genetic variability within each population and cold temperature response makes of temperature an interesting parameter that influences local genetic structure through natural selection. Since low temperatures a main determinant of growth and given the observed differences in cold tolerance between accessions, low temperatures seem an important selective pressure for survival in early stages following germination in *Helianthus petiolaris*. This is evidenced by the better performance of plants from populations that germinated earlier in the wild. All accessions increased their cold tolerance considerably when plants were previously exposed to an acclimation process. In spite of this, physiological differences in cold tolerance during early growth stages demonstrated between accessions, suggest the existence of heritable components. Therefore this study regards the invasive species *H. petiolaris* as a genetic resource to contribute to sunflower breeding in the search of cold tolerant cultivars.

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## **Tolerancia al estrés por frío durante las etapas tempranas de crecimiento de poblaciones naturalizadas de *Helianthus petiolaris***

### **Resumen**

En los últimos 10 años, la superficie de cultivo de girasol en Argentina se ha desplazado hacia el suroeste como resultado de la expansión del cultivo de soja. Siembras tempranas requieren una mayor tolerancia a las bajas temperaturas con el fin de optimizar el uso de los recursos de agua por el cultivo, especialmente en la etapa de plántula, cuando el estrés por frío limita el crecimiento y la productividad. *Helianthus petiolaris* es un recurso genético silvestre que ha contribuido a la producción de cultivares modernos de girasol. El objetivo de este estudio fue evaluar la tolerancia a las bajas temperaturas en plantas de *H. petiolaris*. Rasgos morfológicos (altura de la planta, ancho de la hoja y longitud de la hoja) y rasgos fisiológicos (fuga de electrolitos, contenido de clorofila, y contenido de glucosa) se compararon en ocho accesiones con germinación temprana en el campo y una accesión con ciclo de vida normal. Plantas aclimatadas y no aclimatadas fueron sometidas a estrés por frío seguido de un período de recuperación. Los rasgos fisiológicos mostraron diferencias en la tolerancia al frío durante las primeras etapas de crecimiento y para la capacidad de aclimatación entre accesiones, lo que sugiere la existencia de componentes hereditarios. Cuatro poblaciones fueron las más tolerantes al frío entre las accesiones estudiadas, lo que sugiere una adaptación natural relacionada con el lugar geográfico. Los resultados apuntan a que *H. petiolaris* es útil como recurso genético para contribuir a la mejora de girasol en la búsqueda de cultivares tolerantes al frío.

## **La tolérance du stress par froid dans les premières phases précoces de croissance de populations naturalisées de *Helianthus petiolaris***

### **Résumé**

Dans les 10 dernières années, la surface de culture de tournesol dans l'Argentine s'est déplacée vers le sud-ouest comme résultat de l'expansion de la culture de soja. Des semences précoces requièrent une plus grande tolérance aux basses températures afin d'optimiser l'utilisation des ressources de l'eau par la culture, spécialement dans les premiers états de la plante, quand le stress par froid limite le croissance et la productivité. *Helianthus petiolaris* est un recours génétique sauvage qui a contribué dans la production de tu cultivaras modernes d'un tournesol. L'objectif de cette étude a été d'évaluer la tolérance aux basses températures aux plantes de *H. petiolaris*. Des traits morphologiques (hauteur de

la plante, largeur de la feuille et la longueur de la feuille) et des traits physiologiques (s'enfuit des électrolytes, un contenu de chlorophylle, et contenu de glucose) ont été comparés dans huit consentements à une germination précoce dans le champ et un consentement à un cycle de vie normale. Des plantes acclimatées et non acclimatées ont été soumises à un stress par froid suivi d'une période de récupération. Les d'traits physiologiques ont montré des différences dans la tolérance au froid durant les premières étapes de croissance et pour la capacité d'acclimatation entre des consentements ce qui suggère l'existence de composants héréditaires. Quatre populations ont été les plus tolérantes au froid entre les consentements étudiées ce qui suggère une adaptation naturelle relative au lieu géographique. Les résultats pointent qui *H. petiolaris* est utile comme recours génétique pour contribuer aux améliorations de tournesol dans la recherche de cultiveras tolérants au froid.