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Proline accumulation in pigweed plants (*Amaranthus dubius* Mart, and *Amaranthus cruentus* L.) growing under water stress conditions.[1]

Acumulación de prolina en plantas de pira (*Amaranthus dubius* Mart, y *Amaranthus cruentus* L.) creciendo en condiciones de estrés hídrico.

M. Ferrarotto S.[2]

Abstract

Many plants accumulate high levels of proline (Pro) in response to water stress, and this is noticeably evident in some mesophytic flowering plants. Generally, these levels are higher than those required to be used in protein synthesis. Even though Pro can act as an osmoprotectant, the increases in pool sizes of metabolites in stressed tissues do not necessarily connote adaptive significance. *Amaranthus dubius* is widely distributed as a weed in tropical regions, showing a high competitive capacity due to its high growth rate and efficient water extraction from the soil. However, *in situ* observations show that *A. dubius* wilts rapidly during laboratory manipulation or when submitted to short water stress periods. The selection of *Amaranthus cruentus* for this study was made on the basis that this species has been reported as drought tolerant. In this study, plants of *A. dubius* and *A. cruentus* were grown under water stress conditions (S) in order to determine if differences in the response in both species were related to Pro accumulation. Water hidric potential (ø) and relative water content (RWC) were determined. The Pro concentration on a fresh weight basis was determined colorimetrically. Dehydration was higher in *A. dubius* plants in S, and free Pro increased in both species.

Key words: *Amaranthus*, proline, hidric potential, relative water content.

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2 Universidad Central de Venezuela. Facultad de Agronomía. Av. El Limón. Maracay. Edo. Aragua. Aptdo. 4579. Tlf (fax) 0243-2464143.
e-mail: ferrarottom@agr.ucv.ve

Resumen

Muchas plantas acumulan niveles altos de prolina (Pro), en respuesta a condiciones de estrés hídrico, siendo muy marcada en algunas mesofitas con flores. Generalmente, estos niveles son mayores que los requeridos en la síntesis de proteínas. Aún cuando Pro puede actuar como un osmoprotector, incrementos en el pool de metabolitos en tejidos de plantas estresadas no necesariamente tiene una connotación de significación adaptativa. *Amaranthus dubius* comúnmente se distribuye en regiones tropicales, mostrando una alta capacidad competitiva debido a su alta tasa de crecimiento y su eficiencia en la extracción de agua del suelo. Sin embargo, observaciones *in situ* muestran que *A. dubius* se deseca rápidamente durante su manipulación en el laboratorio o cuando se somete a períodos cortos de estrés hídrico. La selección de *Amaranthus cruentus* para este estudio se realizó considerando que esta especie ha sido señalada como tolerante a la sequía. En este estudio, las plantas de *A. dubius* y *A. cruentus* se desarrollaron bajo condiciones de estrés hídrico (S) con el fin de determinar si las respuestas diferenciales de ambas especies a éstas condiciones se relacionan con la acumulación de Pro. Se determinó el potencial hídrico (ø) y el contenido relativo de agua (RWC). La concentración de Pro libre en base a peso fresco, se determinó colorimétricamente. En S, la deshidratación de *A. dubius* fue mayor, y el contenido de Pro en ambas especies se incrementó.

Palabras clave: *Amaranthus*, prolina, potencial hídrico, contenido relativo de agua.

Introduction

Lack of water has been a major selective force in plant evolution, and ability to cope with water deficits is an important factor in the natural distribution of plants and of crop distribution and productivity (6). Plant adaptations to dry environments can be expressed at four levels: phenological or developmental, morphological, physiological, and metabolic (7). Some studies report that the wild *Amaranthus dubius* Mart., has a fast biomass accumulation (12), high water extraction capacity (4) and high competitive capacity (10). For this reason it has been considered as a noxious weed in tropical and subtropical regions. Leaves of pigweed plants are used in human nourishment in Central America (19) and due to their nutritive value they are also used in pig feeding (16).

Among plant responses to water stress, proline (Pro) accumulation has been related with plant tolerance to hyperosmotic stress (9), and has been used as a metabolic indicator to stress tolerance (13).

In situ observations show that *A. dubius* plants present high susceptibility to water stress, They wilted fast, show a considerable growth reduction, low osmotic potential and present shortening of its life cycle under these conditions (5). The second species: *Amaranthus cruentus* L., is one of three species whose seeds are used in human feeding, being one of the main species cultivated in America (3). This species is well known by its tolerance to water stress and high photosynthetic efficiency in well illuminated conditions (15).

In this work we studied some aspects on hidric relations and Pro accumulation in *A. dubius* and *A. cruentus* growing under water stress conditions, in order to analyze if differential response of this species to water stress are related to Pro accumulation.

Materials and methods

Seeds of *A. dubius* were collected from areas near to the Plant Physiology and Tropical Weeds and Crop Metabolism Laboratory of the Agronomy Faculty, Maracay, Venezuela, were this study was conducted. Seeds of *A. cruentus* were obtained from the Botanical Garden of Bonn, Germany. Plants of both species were grown from seed in a greenhouse with metallic net walls under the following conditions: temperature minimal 22ºC, max. 25ºC; relative humidity minimal 42%, max. 96%; solar radiation 251,23 $cal.cm^{-2}.día^{-1}$. A group of 10-15 seed were sown in 6 Kg plastic bags with sterilized soil (4% clay/ 34% slime/ 62% sand) with pH=7.9 and 0.77% organic matter. At 12 days after emergence, plants were selected and thinned to one per bag conforming one lot of 120 individuals. Plants were daily watered to field capacity until 25 days after planting (d.a.p.), at this moment, water was withheld in half of bags establishing 4 treatments with 30 plants in each one. To: *A. dubius* irrigated (AdW); T1: *A. dubius* non irrigated (AdS); T2: *A. cruentus* irrigated (AcW); T3: *A. cruentus* non irrigated (AcS). The ø and RWC were determined every 5 days, twice a day (at 06:00 and 13:00 hours). For ø the first full expanded leaf nearest to the apex was placed in a pressure chamber. RWC was calculated obtaining 10 discs of 1 cm diameter from the second full expanded leaf nearest to the apex. RWC was calculated as wact/w x 100, were wact = fresh weight-dry weight and w = saturated weight-dry weight. Saturated weight was registered after rehydrating discs during 14 days with 10 ml of distillated water and dry weight was obtained after drying discs in an oven at 70 ºC for 48 hours. Initial (20 d.a.p.) and final (60 d.a.p.) free Pro content were determined using a colorimetric analysis (1) in samples from plants growing in irrigated (W) and non irrigated conditions (S). Soil water content was calculated every 5 days by the gravimetric method weighing 50 g samples from 3 different strata of each bag in an oven at 110 ºC during 48 hours. The humidity retention curve was made through the pressure pan method at 0.01, 0.1, 0.2, 0.3 and 0.5 MPa at the Soil Physic Laboratory of the same Faculty.

Analysis of non parametric variables with Kruskal-Wallis test was made with a multiple comparison in order to detect significant differences or no differences between irrigation and species at 5% level of significance.

Results and discussion

RWC in excised discs of *A. dubius* was 85-100% and 79-98% for *A. cruentus* when determined at 06:00, showing not considerable variation between species when growing under well irrigated conditions. In plants growing under water stress RWC showed a progressive diminution starting at 35 d.a.p. At 50 d.a.p. RWC was 43% when ø was -5.53 MPa and soil water content 3.4% in *A. dubius*. At the same sampling time *A. cruentus* RWC was 65% when ø was -2.17 MPa and soil moisture was 4.8%. When soil presented a water content between 20-29% (-0.1 MPa) in dry weigh basis, and ø was -0.5 MPa, Pro content of both species was low. However, when soil water content dropped to 3-4% (less than -0.5 MPa) and ø was low (-3 MPa), free Pro content increased in both species (Table 1). When RWC values where compared between species, in non irrigated conditions, the results indicated that the dehydration of *A. dubius* tissue was higher than *A. cruentus*. Metabolic differences that appear among genotypes, may reflect differences in water status reached, rather than differences in metabolism at a given water status (8). In measurements made at 13:00 hours no significant differences in ø and RWC in the studied species were found.

A very marked increase (10 to 100 fold) of free Pro content occurs in leaf tissue of some mesophytic as a response to water stress (8). Foliar free Pro accumulation constitutes an indicator of drought sensitivity, of species (11). In this work plants exposed to a lower ø were able to accumulate higher levels of free Pro, *A. dubius* seems to be more sensitive to desiccation than *A. cruentus*. The increased of Pro levels above the required levels to be used in protein synthesis are considered by some authors as a part of an adaptative strategy to tolerate hidric stress (9). Furthermore, in this study, the increase on the levels of Pro was not very marked to be considered as responsible for an osmoregulation. Difference in Pro content between plants of the same species under W and S was observed, this indicated that under water stress conditions, the amount of free foliar Pro content increases in comparison with plants from W. At 20 d.a.p free Pro content was higher in *A. cruentus* than in *A. dubius* (0.66 and 0.38 mmol . g pf $^{-1}$) (Table 1). The relevant fact is that 40 days later, both species showed an increase of the free Pro content. Probably the higher initial Pro content in *A. cruentus* is more related with genotype differences of the species or could have been a response to high temperatures which this species was higher than in its site of origin (17).

In order to have a global interpretation of the dynamic of levels of free Pro in vegetal tissue, its relevant to consider a group of elements as two phases in the dynamic of Pro accumu-

Table 1. Free foliar Pro content (µmol.g.pf^{-1}) in *A. dubius* and *A.cruentus* growing under irrigated (W) and non irrigated (S) conditions[1].

Species	Conditions	µmol. g pf $^{-1}$ 20 d.a.p	% Soil Humidity 20 d.a.p	Hídric Pot. (MPa)	µmol. g pf $^{-1}$ 50 d.a.p	% Soil Humidity 50 d.a.p
A. dubius	W	0.38 ± 0.08	20.07 ± 6.71	-0.5	0.41 ± 0.09	25.19 ± 30-00
A.dubius	S	-	-	-2.98	5.51 ± 4.25	3.41 ± 0.26
A.cruentus	W	0.66 ± 0.13	20.10 ± 1.58	-0.53	0.42 ± 0.11	29.55 ± 10.67
A.cruentus	S	-	-	-2.83	3.91 ± 1.37	4.85 ± 0.87

[1]Each value represent the average of free Pro determination from nine absorbance measurements.

lation: the time in which the final free Pro level is reached, and the time that takes this level to increase or diminish during the recuperation period with irrigation. In this sense, plants that accumulate a higher Pro level in a shorter period of time seems to be able to recuperate faster than plants that accumulate Pro in a larger period of time (14).

Species more tolerant to water stress, would increase their free Pro content up to higher levels slower than less tolerant or more sensitive species. It is unlikely that ø itself is the effect for proline accumulation, because slowly stressed plants may have low Pro concentration at ø values sufficient to elicit considerable Pro accumulation in plants of the same genotype stressed rapidly (18).

The results reported by (5), studying *A. dubius* plants show that Pro accumulation under water stress conditions in this species, occurs in a shorter period of time that under irrigation. This fact support the idea that in *A. dubius*, Pro accumulation seems to be more related with the recuperate capacity than with its tolerance to water stress conditions, being this species, under this criteria, less tolerant to desiccation.

Considering that the values of free Pro content and ø are similar with the values reported for some sorghum cultivars, we can think that increases in free Pro levels in *A. dubius* probably gives to this species more probabilities to recuperate from dehydration with less damage at cellular level (2).

In order to interpret the plant response to water stress through Pro accumulation it is important to consider not only the amount of amino acid accumulated, but other elements related with the morphological expression of the stressing factor as a result of its influence in the plant metabolism. In this work free Pro content in both species increased under water stress conditions, this fact can be consider as a sign of susceptibility to water stress. However, the observance of a significant reduction in size and biomass accumulation in *A. dubius* plants under water stress conditions in comparison with *A. cruentus* (5) is an element that let conclude that in this species free Pro accumulation seems to be an indicator of less tolerance to water stress condition.

Considering water as a factor, it makes differences in the amount of free Pro levels in each species separately (Table 2). The hidric condition of the soil affected water content of plants, reflected in the decreased of ø and RWC in non irrigated conditions. For this reason both parameters can be considered as good indicators of the hidric status of plants. Table 2 shows that differences were due to irrigation, more than due to the factor species.

Table 3 shows differences in free Pro levels comparing both species. No statistically differences were found considering species as a factor. So, analyzing the factor free Pro content, the studied species are not statistically different. Comparing free Pro content at 60 d.a.p in both species, we did not find differences; both species were able to increase its free Pro content in response to water stress conditions.

Pigweed plants under non irrigated conditions showed an increase

Table 2. Differences in free Pro levels in *A. dubius* and *A. cruentus* plants growing under irrigated (W) and non irrigated (S) conditions at 50 d.a.p. Differences according to Kruskal and Wallis (5% of probability) due to the factor irrigation.

Variable	Irrigation	*A. dubius*	*A. cruentus*
Pro	W	0.4138 b	0.4198 b
	S	5.5119 a	3.9074 a

Table 3. Differences in free Pro levels in *A. dubius* and *A. cruentus* plants growing under irrigated (W) and non irrigated (S) conditions at 50 d.a.p. Differences according to Kruskal and Wallis (5% of probability) due to the factor species.

Variable	Species	W	S
Pro	1	0.4138 a	5.5129a
	2	0.4198 a	3.9074a

Differences according to Kruskal and Wallis (5% of probability) due to the species factor.

in the level of Pro respect to the irrigated plants of each species (Table 1). At this point, any firm conclusions about the adaptive role of Pro can be established. Differences in Pro accumulation between the two species seems to be related to genotypic differences in control of plant water status during stress and recuperation after the stress period.

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