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RESPONSE OF FRIJOLILLO *RHYNCHOSIA MINIMA* (L) DC. TO SUPPLEMENTARY PHOSPHORUS WITH THREE SOIL MOISTURE CONDITIONS: II. SOLUTE ACCUMULATION

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□ The objective of this study was to evaluate the effects of induced drought conditions and phosphorous (P) application on osmotic adjustment as reflected in the accumulation of organic solutes in the leaves of frijolillo. The experiment took place under greenhouse conditions without climate control. Plastic containers were used measuring 20 cm high × 15 cm in diameter. In each container, five plants were evaluated from emergence to vegetative growth phase. Three soil moisture regimes were evaluated (25%, 50%, and 100% of field capacity) combined with four concentrations of phosphorous (0, 50, 100 and 150 mg kg⁻¹ of soil). A completely randomized block design with a factorial arrangement of 3 × 4 with four replications was used. The cellular osmotic adjustment as a response to drought stress in frijolillo was associated with the accumulation of sugars, amino acids and proline in that higher concentrations than the control were measured with moisture at 25%. Concentrations of chlorophyll and carotene increased as soil moisture levels decreased.

Keywords: *Rhynchosia minima*, drought stress, phosphorous, solute accumulation

INTRODUCTION

The study of non-domesticated legumes tolerant to conditions of abiotic stress is important for the improvement of crops for both human and animal consumption. Therefore, the study of physiological and biochemical changes is of great importance in the understanding the complex reactions of plants to salinity and drought (Tatar and Gevrek, 2008).

Under drought conditions the soil solution maintains a low osmotic potential, the plant also demonstrates a low intercellular osmotic potential to avoid a movement of water from plant cells to the soil producing an

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“osmotic dehydration.” The osmotic adjustment in almost all types of plants involves the synthesis and accumulation of organic solutes that reduces the osmotic potential of the cell to a level that provides a high potential for turgidity for plant development. In general the solutes which accumulate during osmotic adjustment under drought conditions are soluble sugars, amino acids, proline, glycinebetaine, potassium and alcohols derived from carbohydrates and certain organic acids (Abdalla and El-Khoshiban, 2007; Amede and Schubert, 2003; Esfandiari et al., 2008). Experimental evidence demonstrates that the addition of phosphorous, in soils with low levels of this element, helps to diminish the adverse effects of drought in some crops (Garg et al., 2004; Jones et al., 2005). Although other authors have found that this effect depends on the species under study (Abdel-Fattah et al., 2002).

Frijolillo (*Rhynchosia minima*) is a legume of indeterminate growth, annual or biennial, as determined by environmental conditions, endemic to the coastal plain of Nayarit, Mexico. This native legume grows from October to the beginning of the rainy season in June. Our interest in studying this species lies in its potential for use as a forage legume in that it is readily consumed by cattle in its wild state and is available at times of drought and under saline conditions. Because of this, the objective of this study was to evaluate the effect of drought and the application of phosphorous (P) on osmotic adjustment as reflected in the accumulation of organic solutes in the leaves of frijolillo.

MATERIALS AND METHODS

This experiment took place under greenhouse conditions. Seed of frijolillo was scarified for thirty minutes in a sulfuric acid solution. Solute concentration of frijolillo was evaluated with phosphorous application under three levels of soil moisture.

A mix of organic soil combined with a piroclastic material known locally as jal or pumice. The water retention capacity of this mix was determined to be 50% of the dry weight of the soil mixture. Plastic containers measuring 20 cm deep by 15 cm in diameter were filled with this mix. Five plants were allowed to develop in each container. Three moisture levels were utilized including the maximum capacity for water retention.

- 1) 100%. Maximum water-retention capacity. At first water was added to the soil mixture to the point of saturation and afterwards the containers were watered every third day.
- 2) 50%. One half of the maximum of soil water-retention capacity. Containers were weighed daily and only enough water was added to maintain soil moisture at 50% of maximum.

- 3) 25% of the maximum of soil water-retention capacity. Containers were weighted daily and only enough water was added to maintain soil moisture at 25% of maximum.

At the beginning of the experiment each container was fertilized with 200 mg nitrogen (N), 150 mg potassium (K) and 5 mg of Kelatex Multi® (a commercial mixture of micronutrients; Cosmocel, Monterrey, Mexico). Four concentrations of phosphorous were used to determine their effect on solute concentrations and physiological variables in plants under drought conditions. The concentrations of phosphorus were 0, 50, 100, and 150 mg P kg⁻¹ of soil.

A completely randomized experimental design with a factorial A × B arrangement, with four replications was employed; where factor A corresponds to the three levels of soil moisture, and factor B corresponds to the four concentration of phosphorus (0, 50, 100, and 150 mg P kg⁻¹ of soil mixture).

Six weeks after emergence of sprouts a sampling to determine total soluble sugars, total soluble amino acids, proline, and photosynthetic pigments was undertaken. The analysis of total soluble sugars was determined using the technique proposed by Buysse and Merckx (1993). The analysis of free amino acids was determined according to the process described by Pin and Takahasii (1966). Free proline concentrations were analyzed by following a protocol suggested by Magné and Larther (1992). And finally the photosynthetic pigments were quantified according to the technique described by Lichtenthaler (1987).

The data collected was evaluated with an analysis of variance and mean comparison (Tukey's $P \leq 0.05$) using SAS version 6.12 software (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Using an analysis of variance, highly significant statistical differences were detected amongst moisture levels and phosphorous concentrations and their interactions as manifested in soluble sugars, proline concentrations in leaf tissue, and in photosynthetic pigments (Tables 1 and 2).

Table 1 shows the independent effects of moisture and phosphorus over the solute concentration in leaves of frijolillo. The concentration of soluble sugars in leaf tissue was greater at lower levels of moisture and oscillated between 2705 to 3232 $\mu\text{g g}^{-1}$ of fresh weight in the gradient from 100 to 25% moisture with an average of 2990 $\mu\text{g g}^{-1}$. In regards to the phosphorous factor, a positive correlation was observed, with an increment in concentration of sugars from 2712 to 3224 $\mu\text{g g}^{-1}$ of fresh weight in the gradient from 0 to 150 mg P kg⁻¹ of soil.

TABLE 1 Effect of moisture and phosphorus on solute accumulation in leaves of frijolillo

Factor	Sugar	AA	Proline	Chl-a	Chl-b	Carotene
----- $\mu\text{g g}^{-1}$ -----						
Moisture						
100 (%)	2705 c [§]	433 c	24 c	1399 c	600 c	288 b
50	3033 b	511 b	31 b	1492 b	639 b	367 a
25	3232 a	710 a	41 a	1551 a	677 a	370 a
Phosphorus						
0 (mg kg ⁻¹)	2712 d	502 d	28 c	1538 a	667 a	343 a
50	2957 c	529 c	33 b	1456 b	612 b	344 a
100	3068 b	569 b	23 c	1462 b	653 a	342 a
150	3224 a	608 a	44 a	1467 b	623 b	338 a

Chl-a = chlorophyll-a; Chl-b = chlorophyll-b.

[§]Means in columns with the same letter are statistically similar (Tukey's, $\alpha = 0.05$).

The concentration of amino acids in the leaf tissues increased in accordance with soil phosphorous concentration. The amino acids concentration ranged from 502 to 608 $\mu\text{g g}^{-1}$ fresh weight on a gradient from 0 to 150 mg P kg of soil. The highest concentration of amino acids was detected under low moisture conditions (433 to 710 $\mu\text{g g}^{-1}$ fresh weight, on a gradient from 100 to 25%).

The concentration of free proline in leaf tissues oscillated between 24 to 41 $\mu\text{g g}^{-1}$ of fresh weight on a gradient from 100 to 25% moisture with the medium being 32 $\mu\text{g g}^{-1}$ of fresh weight. The accumulation of proline

TABLE 2 Effect of the interaction between moisture and phosphorus on solute accumulation in leaves of frijolillo

Moisture (%)	Phosphorus (mg kg ⁻¹)	Sugar	AA	Proline	Chl-a	Chl-b	Carotene
----- $\mu\text{g g}^{-1}$ -----							
100	0	2331 g [§]	404 f	19 f	1427 ef	629 cde	281 b
50	0	2799 de	417 f	26 ef	1579 ab	657 bc	378 a
25	0	3005 c	685 bc	39 bcd	1610 a	714 a	374 a
100	50	2751 ef	418 f	25 ef	1390 fg	577 g	300 b
50	50	2896 d	428 f	28 def	1467 e	607 efg	367 a
25	50	3224 b	740 a	46 ab	1512 cd	654 bcd	365 a
100	100	2689 f	478 e	19 f	1403 fg	609 ef	300 b
50	100	3224 b	644 c	25 ef	1455 e	668 b	358 a
25	100	3292 b	699 ab	26 ef	1528 c	681 b	371 a
100	150	3049 c	433 f	35 cde	1378 g	586 fg	275 b
50	150	3216 b	557 d	45 abc	1469 de	624 de	370 a
25	150	3409 a	718 ab	52 a	1553 bc	660 bc	369 a
CV%		1.32	3.19	14.80	1.20	2.05	3.29

Chl-a = chlorophyll-a; Chl-b = chlorophyll-b.

[§]Means in columns with the same letter are statistically similar (Tukey's, $\alpha = 0.05$).

in the leaves was not consistent with the concentration of P. Although the higher concentrations of proline were measured with P at 150 mg kg⁻¹.

Chlorophyll decreased as soil concentration of P increased from 0 to 50 mg P kg⁻¹. At all levels of soil moisture the highest concentrations of chlorophyll-a were obtained at the lowest levels of P combined with highest levels of drought stress. Likewise, the concentration of chlorophyll-b was greatest at the lowest concentration of P combined with the highest level of drought stress.

Carotene concentrations were greater at lower soil moisture levels (25 and 50%) compared to control. However, the correlation with P concentration was insignificant at all moisture levels.

Table 2 shows the combined effect of moisture and phosphorus over solute concentrations in leaves of frijolillo. The highest concentration of sugar was obtained with 25% of moisture combined with P at 150 mg kg⁻¹, and the lowest concentration with 100% of moisture without phosphorus. Amino acids concentration was higher when soil moisture was low (25%). The application of phosphorus at 100 mg kg⁻¹, combined with 50% of moisture, increased the AA concentration. The content of proline had a significantly increased with phosphorus at 150 mg kg⁻¹, with all percentage of moisture. The content of chlorophyll-a and chlorophyll-b had a negative correlation with the content of phosphorus in soil. The lowest percentage of moisture reached a major content of chlorophyll.

In general, the amount of phosphorus had no significant effect on the content of carotene. However, the concentration of carotene was significantly higher with 25 and 50% moisture condition.

The concentrations of sugars, amino acids, and free proline across the whole gradient of moisture levels were similar at the end of the experiment. For this reason, irrigation was suspended to all treatments in order to evaluate the response of frijolillo to this stressor. The concentration of sugars increased on average from 3060 to 6207 $\mu\text{g g}^{-1}$ of dry weight which is equivalent to an increase of 203% in seven days. The treatment with 25% moisture responded most rapidly (Figure 1).

The concentration of amino acids increased on average from 604 to 3364 $\mu\text{g g}^{-1}$ of fresh weight which reflects an increase of 557% in seven days. Plants maintained at 25% soil moisture responded most rapidly although they stabilized after five days (Figure 2). Plants under the 50 and 100% moisture regimes continued to accumulate amino acids up to seven days at which time they died.

The concentration of proline increased on the average 23 to 1150 $\mu\text{g g}^{-1}$ of fresh weight, which reflects an increase of 5000% in seven days with similar responses across the soil moisture gradient (Figure 3). However, the plants at 100 and 50% soil moisture died after seven days. By contrast, the plants under the 25% moisture regime maintained turgidity during this same period.

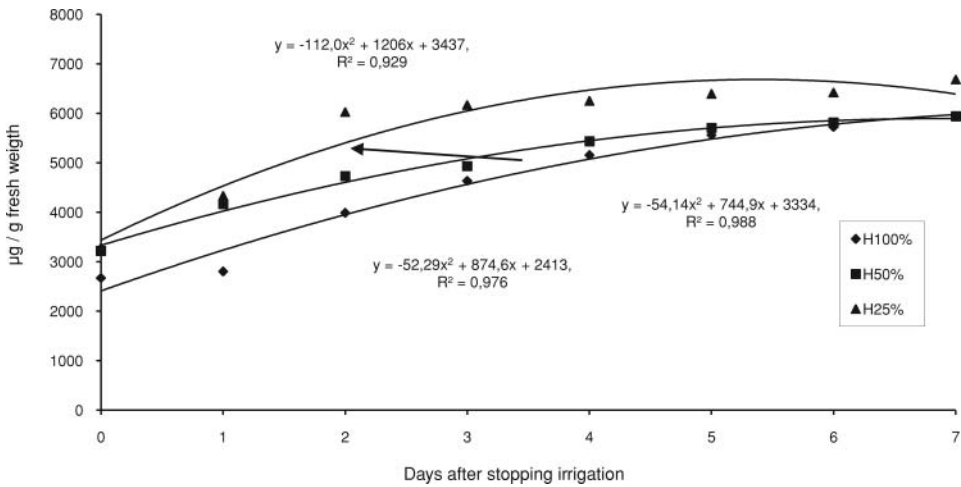


FIGURE 1 Content of sugar in leaves of frijolillo after stopping irrigation in plants under three soil moisture conditions.

DISCUSSION

Amongst abiotic factors that most affect plant production, water stress, caused by either drought or salinity, ranks as one of the most important (Najafi et al., 2006). One response of plants to water stress is the accumulation of certain compounds. In this study, the concentration of sugars increased in direct proportion to P concentrations. Also, plants subjected to drought stress concentrated more sugars in their leaves. Fazeli et al. (2006) found that in certain varieties of sesame, the concentration of reduced sugars increased at all levels of soil moisture when compared with control. This sugar accumulation may play an important role in the osmotic adjustment of plants

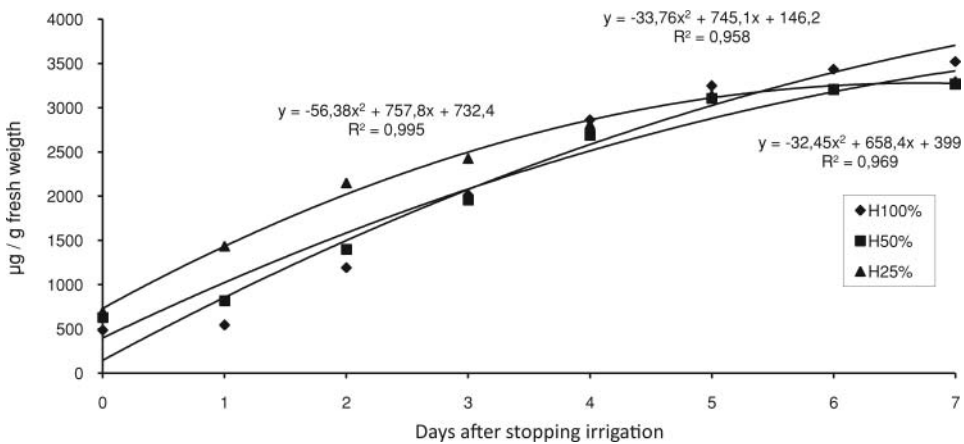


FIGURE 2 Content of amino acids in leaves of frijolillo after stopping irrigation in plants under three soil moisture conditions.

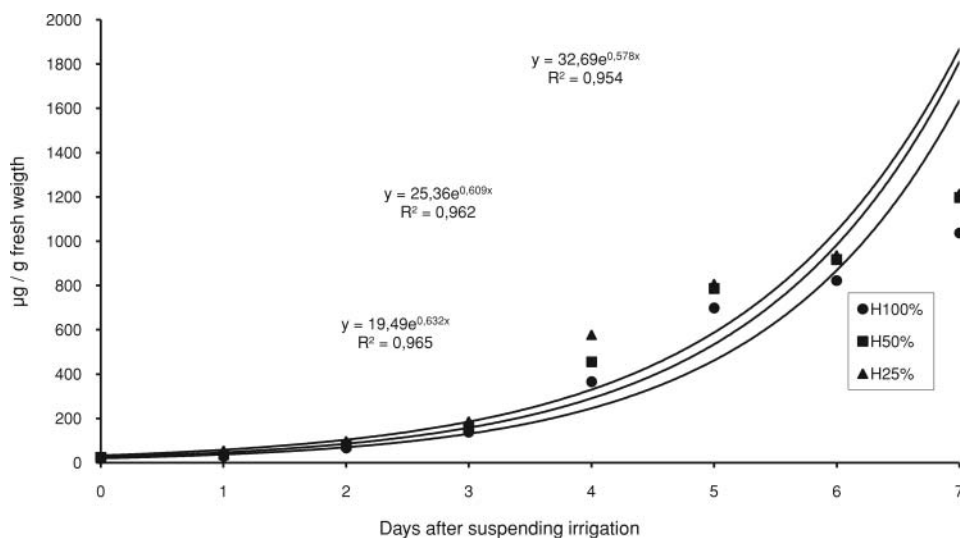


FIGURE 3 Proline content in leaves of frijolillo after suspending irrigation in plants under three soil moisture conditions.

under stress as has been reported by Miazek et al. (2001) and Khavari-Nejad et al. (2008).

Parsons (1979) mentions that plants which cope well with drought stress have a well-developed root system with deep vertical growth, with the capacity to increase the ratio roots:stem, the ability to reduce leaf size, possess stomata at low density and of reduced size, have greater enzymatic activity and produce greater quantities of amino acids, sugars, proline, and abscisic acid. In a similar way, Ahmed et al. (1987) observed that the transpiration rate, leaf area, and the content of nitrogen and sugars decreased upon lowering soil moisture from 90 to 30% of field capacity. However, the concentration of chlorophyll and carotenes as well as the number of stomata was not affected.

The photosynthetic capacity of plants is related principally to total foliar area and the photosynthetic activity in the leaves. The closing of the stomata reduces water loss, but it also reduces photosynthetic activity as per Begg and Turner (1976), which results in the reduction in absorption of carbon dioxide (CO₂). These authors concluded that if photosynthesis is indeed reduced by the closing of the stomata, prolonged and intense water stress can result in a decline in the activity of chloroplasts and enzymes and other non stomatal effects on photosynthesis.

Some studies with various species have reported a decrease in total photosynthesis owing to drought stress (Castañeda-Saucedo et al., 2006) with beans, (Najafi et al., 2006) in peas. However, in this study, the concentration of chlorophyll-a (Chla), chlorophyll-b (Chlb) and carotenes in leaf tissues increased when soil moisture declined from 100% to 50 and 25%. This response allows us to posit that in this species, photosynthetic activity increased

as a result of drought stress. Similar results were reported by Mohammadian *et al.* (2003) who found that in sugar beets, chlorophyll concentration was higher in drought plants. Likewise the concentration of P on the soil solution was negatively correlated with the accumulation of osmoregulators, which is consistent with the findings of several authors who found that the accumulation of high levels of proline is associated with stress, but not necessarily with increases in the concentration of P (Hanson *et al.*, 1977; Al-Karaki *et al.*, 1996).

Regarding osmotic adjustment, it is accepted that in all types of plants, the process involves the synthesis and accumulation of organic solutes which tend to reduce the osmotic potential of cells to a level which maintains a high level of turgidity in order to maintain growth rate. However, as indicated by Wyn Jones (1981), not all organic solutes have a positive effect, in that certain tissues when subjected to stress synthesize amines which are eventually toxic. The organic compounds of low molecular weight that are usually produced in response to a low soil water potential, are sugars, organic acids and amino acids. This correlates with the results of this study, which allows us to see that as the availability of water increased rapidly, the levels of soluble sugars, amino acids and proline reached higher levels than the control.

Schubert *et al.* (1995) reported that increases in the concentration of sugars and amino acids in stems, roots and root nodules in alfalfa were in response to drought. In a similar vein, Good and Zaplachinski (1994) found higher levels of amino acids with induced water stress in radish. The role of proline as an osmoregulator has been disputed at times, though the importance of free proline accumulation as an indicator of an adaptive strategy and as a mechanism for increased survival of plants during periods of drought stress, has been reported by several authors (Abdel-Nasser and Abdel-Aal, 2002; Al-Bahrany, 2002; Khavari-Nejad *et al.*, 2008; Tatar and Gevrek, 2008).

The response of frijolillo to the cessation of irrigation allowed us to observe that response was related to the moisture level to which they were subjected during the experiment. We observed that all plants in the control group (100% moisture) died after six days. The half of those plants maintained at 50% moisture survived this same period, while those plants maintained at 25% moisture showed no signs of wilting and maintained turgidity. This response demonstrates that the plant incorporates mechanisms which allow it to adjust or adapt its internal functions to drought stress. But the process is gradual, not spontaneous, so that the populations of frijolillo that germinate naturally during October and November are able to thrive during the dry season.

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