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RESPONSE OF FRIJOLILLO RHYNCHOSIA MINIMA (L) DC. TO SUPPLEMENTARY PHOSPHORUS WITH THREE SOIL MOISTURE CONDITIONS: I. GROWTH AND DEVELOPMENT

A. Madueño-Molina,1 D. García-Paredes,1 J. Martínez-Hernández,2 R. Bugarín-Montoya,1 and J. I. Bojórquez-Serrano1

1Unidad Académica de Agricultura, Universidad Autónoma de Nayarit, Tepic, Mexico
2Colegio de Postgraduados, Instituto de Recursos Naturales, Montecillos, Mexico

This study was undertaken with the objective of evaluating the response of frijolillo, grown with three levels of soil moisture and supplemented with phosphorus (P). The experiment was carried out under greenhouse conditions without climate control. Plants were tested during the period from germination to vegetative growth stage. Three soil moisture levels (100, 50, and 25% of field capacity) combined with four levels of P (0, 50, 100, and 150 mg kg\(^{-1}\)) were tested in a factorial arrangement with four replications. This species showed tolerance to drought stress since its leaf area, dry mass of stems and root mass did not decrease with 50% of constant moisture. Furthermore, with the highest concentrations of phosphorous (100 and 150 mg kg\(^{-1}\)) there was more aerial mass accumulation. Finally, with maximum drought stress (25% moisture) growth of main stem decreased. However, root growth had a maximum length under the lowest moisture conditions.

Keywords: drought stress, P rates, growth rate, drought tolerance index, Rhynchosia

INTRODUCTION

In the tropical zone there is great diversity of wild legume plants that represent a potential for domestication and agricultural use as edible crops for human use or by grazing cattle. Frijolillo (Rhynchosia minima L.) is a wild legume distributed on the coastal plain in Nayarit, Mexico. In this area, it grows during the dry season, even in saline soils. Therefore, this species is used by cattle as an alternative forage during the dry season. Plants are subjected to different conditions of environmental stress and according with Chen et al. (2000) drought is one of the most serious affecting crop
production in the world. In general, all processes of growth and development in plants are affected by water deficit. Several studies mention that water stress produces biomass and leaf area diminution, because of reduced number and size of leaves and the inhibition of the expansion of growing foliage (Boutraa and Sanders, 2001; Saleem, 2003; Fazeli et al., 2006). Also, others authors have found that optimum levels of phosphorous (P) in soils may help in reducing the negative impact of drought conditions in crops (Al-Karaki et al., 1996; Garg et al., 2004; Jones et al., 2005; Singh and Singh, 2006). The objective of this research was to evaluate the effect of three levels of soil moisture and the application of phosphorous on the growth and development of frijolillo.

MATERIALS AND METHODS

A pot experiment, filled with a mix of pumice material and organic soil in a 50:50 proportion by volume, was conducted under greenhouse conditions without climate control. The water retention capacity in grams, was determined for this mix. Five plants were grown in each pot. Three soil moisture conditions, 100, 50 and 25% of field capacity were tested during six weeks. The water saturation was maintained by weighting the pots. Four concentrations of phosphorous (0, 50, 100 and 150 mg kg\(^{-1}\) soil) were used to observe the effect on the agronomic characteristics of frijolillo. In addition, every pot was fertilized with a mixed material of nitrogen (N), potassium (K), and micronutrients (200, 150, and 5 mg kg\(^{-1}\) soil, respectively).

The experiment had a factorial A × B arrangement, in a completely randomized design with four replications. Treatment A was three levels of moisture and treatment B was four concentrations of phosphorous. 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). The measurement of the response variables was undertaken every week during six weeks. The characteristics evaluated were defined as follows:

Length of main stem (LS): This was the average length from five plants measured every week and was used to evaluate the dynamic of plant growth.

Length of main root (LR): The average length of main root from five plants measured every week.

Leaf area (LA): This characteristic was calculated using the following equation:

\[ \text{LA} = (L \times A)(0.6667) \]

Where LA is the leaf area in cm\(^2\), L is maximum length, A is maximum width, and 0.6667 is a constant value used as a correction factor.
Leaf weight (LW), stem weight (SW), and dry root weight (RW). The average weight, in grams, was calculated from five plants in each experimental unit, which were oven dried at 65°C until a constant weight was reached. Dry matter of stem (DMS): This was calculated by LW + SW. Stem-leaf ratio (SLR): This was calculated by LW/SW. Shoot-root ratio (S-R): This was calculated by RW/DMS. Salinity tolerance index (STI): A salinity index for stem and root was calculated according to the equation proposed by Maiti et al. (1996):

\[
\text{STI} = \frac{\text{Dry weight of shoot under stress}}{\text{Dry weight of shoot in control}}.
\]

**RESULTS**

The analysis of variance, for agronomic characteristics of frijolillo, showed statistical differences between P concentrations and levels of moisture as well as for their interaction, as can be demonstrated by the mean differences (Tables 1 and 2).

Main effect of moisture level and phosphorous concentration. Stem growth was directly related to soil moisture conditions. This variable had a similar effect with the lowest rate of phosphorous (0 and 50 mg kg\(^{-1}\) soil). Greatest growth was obtained with 100 mg kg\(^{-1}\) soil of phosphorous (Table 1).

Leaf area increased with the high levels of soil moisture (100 and 50%) and was inhibited with low moisture levels (25%). The effect of phosphorous on this variable, shows a direct response and was statistically different. Dry matter of stems (DMS) was higher with high percentage of moisture. The production of DMS was the same with 0, 50, and 100 mg kg\(^{-1}\) soil of phosphorous. The highest production of DMS was obtained with 150 mg kg\(^{-1}\) soil of phosphorous (Table 1). Root dry matter production (DMR) was higher with the levels of 100 and 50% of moisture in soils.

**TABLE 1** Morphological characteristics of frijolillo in response to moisture and phosphorous

<table>
<thead>
<tr>
<th>Factor</th>
<th>Length of stem (cm)</th>
<th>Leaf area (cm(^2))</th>
<th>DMS (g)(^{\dagger})</th>
<th>DMR (g)(^{\dagger})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>66.8 a(^{$})</td>
<td>258.6 a</td>
<td>11.1 a</td>
<td>8.6 a</td>
</tr>
<tr>
<td>50</td>
<td>53.6 b</td>
<td>240.9 a</td>
<td>11.3 a</td>
<td>9.1 a</td>
</tr>
<tr>
<td>25</td>
<td>25.7 c</td>
<td>74.8 b</td>
<td>6.5 b</td>
<td>5.8 b</td>
</tr>
<tr>
<td>Phosphorous (mg kg(^{-1}) soil)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>45.3 c</td>
<td>163.8 c</td>
<td>9.3 b</td>
<td>8.6 a</td>
</tr>
<tr>
<td>50</td>
<td>43.1 c</td>
<td>156.7 d</td>
<td>9.2 b</td>
<td>7.5 b</td>
</tr>
<tr>
<td>100</td>
<td>57.0 a</td>
<td>188.0 b</td>
<td>9.6 b</td>
<td>7.6 b</td>
</tr>
<tr>
<td>150</td>
<td>49.4 b</td>
<td>250.6 a</td>
<td>10.6 a</td>
<td>7.7 b</td>
</tr>
</tbody>
</table>

\(^{\dagger}\)Dry matter of stem; \(^{\dagger}\)dry matter of root.

\(^{\$}\)Means in columns with the same letter are statistically similar (Tukey’s P ≤ 0.05).
Drought moisture conditions significantly affected the production of dry matter of stems and roots. Yield of DMS ranged from 11.1 to 6.5 g for 100% and 25% of moisture, which reflects a reduction of 41.6%. Yield of DMR ranged from 8.6 to 5.8 in the same levels of moisture, a decrease of 32.6%. Dry matter in both tissues, with soil moisture of 100 and 50%, were statistically similar and greater than that of 25% (Table 1). For stems the best response was with 150 mg kg\(^{-1}\) soil of phosphorous. In roots, phosphorous had a negative influence, decreasing dry matter with the increase of phosphorous.

The length of stem was greater with 100% moisture plus 150 mg kg\(^{-1}\) soil of phosphorous, however, with 50% moisture the optimum concentration of phosphorous was 100 mg kg\(^{-1}\) soil (Table 2). The highest leaf area was obtained with 100% and 50% moisture combined with 150 mg kg\(^{-1}\) soil of phosphorous. The treatment with least leaf area was that with the lowest levels (25% moisture and 0 mg kg\(^{-1}\) soil of phosphorous). The lowest production of dry matter of stem and root was for treatments with major drought stress (Table 2).

**Stem**

Length of stem increased from 5.3 to 66.8 cm, from the start of observations to 35 days. A linear trend was observed with all levels of moisture (Figure 1), however, the slope to this response decreased with reference to the availability of water. At the end of this study (35 days) the length of stem was 66.8 cm. with 100% moisture and 25.7 cm for 50% moisture, which represents a reduction of 62%.

With 50 mg kg\(^{-1}\) soil of phosphorous, there was no significant effect on growth of stem, as compared with control, for all moisture conditions.
However, with 100 mg kg$^{-1}$ of soil phosphorus, the effect was positive for all moisture treatments. Finally, with 150 mg kg$^{-1}$ soil of phosphorous only, a positive effect was demonstrated with the treatment without moisture deficit (100% moisture) and negative effect with 50% and 25% moisture (Figure 2).
Root

Length of root increased from 10 to 51 cm, during the period of the experiment. The response to the levels of moisture had a linear trend (Figure 3). Final length of root was 38, 46 and 51 cm, respectively with 100%, 50% and 25% moisture.

Leaf Area

Leaf area at 35 days of the study was 238.6, 240.9 and 74.8 cm$^2$, for 100%, 50% and 25% soil moisture, with an average of 184.8 cm$^2$ and a coefficient of variance of 2.03%. The levels of 100% and 50% of moisture were similar and higher than 25%, which correlated with a decrease of 68.7% (Figure 4). Leaf area had a quadratic trend in response to phosphorous concentration in soil, with moisture at 50% and 100%, increasing from 215 to 305 cm$^2$ (Figure 5). However, with 25% soil moisture, the response was linear with an increase from 60.3 to 82.5 cm$^2$.

Leaf-Stem Ratio and Root-Stem Ratio

The leaf/stem ratio was affected by water stress, decreasing from 2.19 in control to 1.74 and 1.81 with 50 and 25% moisture, respectively (Table 3). The root/stem ratio increased from 0.78 in control to 0.81 and 0.89 for 50 and 25% moisture.
TABLE 3 Root/stem ratio, leaf-stem ratio and tolerance indexes for stem, root and biomass

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soil moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Root/stem ratio</td>
<td>0.89</td>
</tr>
<tr>
<td>Leaf/stem ratio</td>
<td>1.81</td>
</tr>
<tr>
<td>Tolerance index for stems</td>
<td>0.58</td>
</tr>
<tr>
<td>Tolerance index for roots</td>
<td>0.67</td>
</tr>
<tr>
<td>Tolerance index for biomass</td>
<td>0.62</td>
</tr>
</tbody>
</table>

FIGURE 4 Leaf development of frijolillo under three soil moisture (H) conditions.

FIGURE 5 Leaf area of frijolillo as influenced by phosphorous and three levels of water (H).
TABLE 4  Correlation between moisture and traits of frijolillo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Moisture</th>
<th>LS</th>
<th>LA</th>
<th>DMS</th>
<th>DMR</th>
<th>TIS</th>
<th>TIR</th>
<th>TIB</th>
<th>RSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>0.923</td>
<td>0.748</td>
<td>0.946</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td></td>
<td>0.942</td>
<td>0.999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMS</td>
<td>0.740</td>
<td></td>
<td></td>
<td>0.999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMR</td>
<td>0.669</td>
<td></td>
<td></td>
<td></td>
<td>0.995</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIS</td>
<td>0.741</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td>0.995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIR</td>
<td>0.668</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.995</td>
<td>0.998</td>
<td>0.995</td>
</tr>
<tr>
<td>TIB</td>
<td>0.712</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.998</td>
<td>0.998</td>
</tr>
<tr>
<td>RSR</td>
<td>0.907</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.941</td>
</tr>
<tr>
<td>LSR</td>
<td>0.888</td>
<td>0.643</td>
<td>0.359</td>
<td>0.347</td>
<td>0.251</td>
<td>0.348</td>
<td>0.251</td>
<td>0.309</td>
<td>0.612</td>
</tr>
</tbody>
</table>

LS = length of stem; LA = leaf area; DMS = dry matter of stem; DMR = dry matter of root; TIS = tolerance index for stem; TIR = Tolerance index for roots, TIB = tolerance index for biomass; RSR = root/stem ratio; LSR = leaf-stem ratio.

Tolerance Indices

The tolerance index values for stem, root and biomass, with 25% moisture were always lower than 1; however, with 50% and 100% moisture were equal or greater than one (Table 3).

In this study most of the traits of frijolillo were affected by moisture conditions and showed a high significant correlation between them (Table 4).

DISCUSSION

It is known that plants achieve better growth and development under optimal soil moisture conditions in as much as other growth factors not are limiting. However, when drought stress exists most of the morphogenetic characteristics are negatively affected. In this experiment, a decrease in length of stem, leaf area and dry matter of stem as well as dry matter of roots was observed under drought stress.

Length of stem decreased 62% with the treatment of greatest drought stress (25% of field capacity), as compared to the others levels of moisture. Nevertheless, even under these conditions, plants did not demonstrate leaf wilting, although they did demonstrate a slower yet constant growth rate. This reduction in size could be an adaptive strategy that allows the plants to maintain cell turgidity and to maintain active metabolic processes in order to continue growth and development. These results are in agreement with those presented by Boutraa and Sanders (2001) who mentioned that the characteristic most affected by drought stress, in common bean, was length of stem. In the same way, Qadir et al. (1999) and Saleem (2003) found that drought stress decreased height of plants in wheat varieties.

Root growth response to moisture level was not the same than that of stem growth, since root growth was greater when drought stress was more severe.
These findings coincide with Hsiao (1973), and Hsiao and Xu (2000), who mentioned that besides fixing the plants to soil, root growth helps plants to search for water and nutrients and one of the adaptive processes of drought resistant plants is that they develop a deep root system. However, these results contrast with those of Gupta et al. (1985) in safflower, since they found that drought stress caused less growth in roots than in stems.

Leaf area at the end of the experiment (35 days) was greater for treatments with 100 and 50% moisture. The treatment with 25% moisture had a reduction of 68.7% as compared with control. Several authors have found that reduction of leaf area as a consequence of drought stress may be considered an adaptive response of plants in order to decrease respiration rate and this way extend the period of water availability in the root zone (Hsiao and Xu, 2000; Paz et al., 2003).

Dry matter production of stems and roots was affected by water deficiency. Dry matter of stems decreased 41.6% with the treatment of highest moisture stress, while dry matter of roots decreased 32.6% under the same conditions. In both tissues, those treatments with 100 and 50% of moisture were statistically similar, and better than that of 25% moisture. These findings are in agreement with those reported by Ali et al. (2000) in sunflower; Paz et al. (2003) in Barberia and Fazeli et al. (2006) in sesame.

Phosphorous concentration in the soil solution had a significant influence in dry matter accumulation, which might be the result of greater availability of phosphorous for plant metabolism, since an increase in photosynthetic activity has been associated with a corresponding increase in phosphorous content in leaves (Foyer and Spencer, 1986). However, plants under drought stress had less dry matter accumulation than those under no stress, which could be a signal of a reduction in phosphorous consumption occasioned by stress and might be in detrimental to growth (Al-Karaki et al., 1996).

The leaf/stem ratio always was over 1.0, and this proves that in spite of the negative effects of drought stress, showed in leaf area reduction, this species produces a fair amount of leaves.

The root-stem ratio was greater with the lowest percentage of moisture, which means that plants prioritize root growth over stem growth to pursue soil moisture and this way minimize the effects of drought stress.

Tolerance indexes are indicators of expected yield, for crops under drought stress conditions as compared with a normal environmental condition. In this study, the treatment with 25% moisture had a dry matter production of 58% compared with the control. However, when soil water was at 50%, the expected yield was greater than control, which shows the adaptive capacity of this species to growth in low moisture environments (Table 3). Similar results were mentioned by Amhad et al. (2003) in wheat varieties.
In this experiment soil moisture showed a significant positive correlation with length of stem and with leaf area (Table 4). Furthermore, these three characteristics showed significant correlation with all the other variables, except for the leaf-stem ratio. Sultana et al. (2002) mentioned that the greatest yields of wheat were correlated with higher biomass production.

The response of frijolillo under drought stress conditions, proves that this species has tolerance to this abiotic condition. This pattern also was observed in other crops by other authors (Iqbal et al., 1999; Rahman et al., 2002; Gunes et al., 2006; Salehi et al., 2008).

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**REFERENCES**


