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Plant water relations and photosynthetic activity in three Tunisian chickpea (Cicer arietinum L.) genotypes subjected to drought

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Abstract: Chickpea (Cicer arietinum L.) is an important food crop grown under rainfed conditions in Mediterranean regions in which drought is a major limiting factor for production. In these areas little attention is given to legumes, and efforts to identify drought-tolerant genotypes are primarily focused on major cereal crops. In the current study a greenhouse experiment was conducted to assess the effects of drought stress on plant growth, photosynthesis, and water relations in 3 Tunisian chickpea genotypes (Cicer arietinum L.). Drought was applied, and soil humidity was maintained at 30% of field capacity (stressed plants) or 100% of field capacity (control plants) for 3 weeks. A close relationship between plant growth, and photosynthetic activity, leaf water status was observed. In comparison to Chetoui and Kesseb, Amdoun exhibited the greatest plant growth and photosynthetic activity, the lowest drought intensity index, and important osmotic adjustment under drought stress. Water use efficiency clearly differentiated the studied genotypes.

Key words: Drought, Cicer arietinum, photosynthesis, water potential, osmotic adjustment, water use efficiency

Introduction

Chickpea (Cicer arietinum L.) is one of the world’s most important, but lesser-studied, leguminous food crops, grown on nearly 10 million hectares across the Americas, the Mediterranean basin, East Africa, the Middle East, Asia, and Australia (Jayashree et al. 2005). While in the developed world it represents a valuable crop for export, in the developing world it provides a protein-rich supplement to cereal-based diets. Chickpea seed contains 13%-33% protein, 40%-55% carbohydrate, and 4%-10% oil (Stallknecht et al. 1995).

Chickpea is generally grown without irrigation, planted in the post-rainy season, and survives until to harvest despite progressively increasing drought. Increased exposure to terminal drought following spring sowing decreases season length and delays flowering, and reduces dry matter production, water use efficiency (Brown et al. 1989), plant height, and seed yield (Singh et al. 1997). Although chickpea is considered a drought-sensitive species, the selection and breeding of genotypes and/or cultivars that can grow under water-deficit conditions constitute a more effective method of minimizing the repercussions of exposure to drought.

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Adaptation research has confirmed the importance of drought avoidance in chickpea. Although chickpea has a number of characteristics consistent with dehydration postponement and tolerance, such as deep rooting (Saxena et al. 1994), high-level soil water extraction (Zhang et al. 2000), and osmotic adjustment (Leport et al. 1999), its primary adaptive strategy to drought stress appears to escape through early phenology (Berger et al. 2006).

Changes in the water balance and the amount of water available in soil can be crucial for crop yield (Fuhrer 2003). On the other hand, physiological characteristics of plants are correlated with the water potential (Hsiao 1973). Low water potential due to reduced water availability negatively affects plant growth (Ohashi et al. 2000), photosynthesis (Ogen and Öquist 1985), plant cell enlargement (Nonami et al. 1997), and hormone balance (Munns and Gramer 1996). Other physiological effects of drought on plants include reduced cyclin-dependent kinase activity, which results in slower cell division, and inhibition of growth under water deficit conditions (Schuppler et al. 1998).

The intensity of water deficit is commonly evaluated by the leaf water potential ($\Psi_w$). Values of –0.9 and –2.7 MPa represent moderate and severe drought for coffee, respectively (Da Matta et al. 1997). Stomatal closure is among the first responses to water stress and is assumed to be the main cause of impaired photosynthesis induced by drought, as stomatal closure limits CO$_2$ availability to the mesophyll (Chaves 1991). In view of this, a decrease in net photosynthesis under drought depends more on the availability of CO$_2$ in the chloroplast than on the leaf water potential (Sharkey 1990). Osmotic adjustment is known to be an important physiological mechanism of water retention and cell turgor maintenance (Morgan 1984). The accumulation of solutes, such as amino acids, organic acids, ions, and soluble sugars, is associated with active osmotic adjustment during drought stress (Guicherd et al. 1997).

In Tunisia chickpea is grown in the post-rainy season without irrigation and has a very low yield. A better understanding of the physiological basis of changes in water stress resistance could be used to select varieties and genotypes with better productivity. The aim of the present study was to evaluate the tolerance of 3 Tunisian chickpea genotypes to drought, to identify a suitable index for further screening, and to select chickpea genotypes tolerant to drought stress.

Materials and methods

Plant material and treatment

Three Tunisian chickpea (Cicer arietinum L.) genotypes were used: Amdoun, Kesseb, and Chetoui. Five seeds were planted in 1-kg pots filled with a potting soil mix (pH: 6.0; N: 225 mg kg$^{-1}$; P: 245 mg kg$^{-1}$; K: 275 mg kg$^{-1}$; EC: 0.7 mS cm$^{-1}$). Plantlets were watered daily with tap water. On the 21st day after sowing (DAS) the seedlings were thinned to 1 healthy plant per pot. Treatments were administered from 21 to 42 DAS. All pots were watered to initial weight with tap water every other day to maintain 30% FC (drought stressed plants) and 100% FC (control plants).

The experiment was conducted in a glasshouse (at the Graduate School of Life and Environmental Sciences, Tsukuba University, Tsukuba, Japan) under natural light, with a 14-h photoperiod and a temperature of 25 °C/17 °C (day/night). Relative humidity was about 75%. Ten plants per treatment and per genotype were maintained.

Biomass production and water relation

For growth quantification samples of fresh material were weighed 42 DAS, and then dried at 70 °C for 72 h to estimate dry weight biomass and to calculate the DII (drought intensity index). Plant dry weight of all stressed (DWs) and control (DWc) genotypes was used to calculate the DII (Fischer and Maurer, 1978) as follows:

$$DII = 1 - \frac{DWs}{DWc}$$

DII is a measure of the severity of drought based on plant growth.

Water use efficiency for plant growth (WUE DW) was calculated as the ratio of total plant biomass (g DW) to leaf water content (mL g$^{-1}$ DW). Water use efficiency for photosynthetic activity (WUE PH) was calculated as the ratio of net photosynthesis (mol CO$_2$ m$^{-2}$ S$^{-1}$) to leaf water content (mL g$^{-1}$ DW).

Leaf relative water content (RWC) was determined according to the methods of Barrs and Weatherley.
(1962), based on the following equation: 

\[ \text{RWC} = \frac{(\text{FW} - \text{DW})}{(\text{SW} - \text{DW})} \times 100, \]

where FW is leaf fresh weight, DW is dry weight of leaves after drying at 85 °C for 3 days, and SW is the turgid weight of leaves after soaking in water for 4 h at room temperature (approximately 20 °C). Half of the third (from the top) fully expanded leaf was used.

The leaf water potential (11 \( \psi_w \)) was measured in the 3 upper fully expanded leaves 2 h after the beginning of the light period using a pressure chamber (model C52-SF, Wescor, Inc.) (Scholander et al. 1965).

For osmotic potential (OP) measurement the second half of the third (from the top) fully expanded leaf was collected, and then stored in an Eppendorf tube at –20 °C until analysis. The frozen samples were allowed to thaw for 20 min at room temperature. After thawing the samples, Eppendorf tubes containing the samples were perforated using a heated pin-needle to extract cell sap. Each Eppendorf tube was then encased in a second intact tube and centrifuged at 14,000 \( \times g \) for 15 min. The osmolality of the collected sap was evaluated using a dew point microvolt meter (model HR-33T, Wescor, Inc.). The osmolality of unknown solutions was estimated by calibrating the microvolt using a NaCl solution as a known osmotic potential. Osmotic adjustment was calculated as the difference in osmotic potential at full turgor between control (11 \( \psi_{11\text{turg}} \)) and stressed plants (11 \( \psi_{11\text{st}} \)) (Blum 1989).

**Gas exchange measurement**

Gas exchange measurements were made with an LI-6400 (LI-COR, Inc.) portable gas exchange system. Measurements were made on the 3 youngest fully expanded leaves. Photosynthesis was induced with saturating light (1000 \( \mu\text{mol m}^{-2} \text{s}^{-1} \)). This light was fitted to the standard 6-cm \(^2 \) clamp on the leaf chamber. Sample pCO\(_2\), flow rate, and temperature were kept constant at 362 mbar, 500 \( \mu\text{mol s}^{-1} \), and 25 °C, respectively.

**Statistical analysis**

The experimental design was 2 factorial, arranged in a completely randomized design with 10 replications. The first factor was genotype (Chetoui, Amdoun, and Kesseb), and the second factor was treatment (control: 100% FC; drought: 30% FC). Analysis of variance (ANOVA), using the AV1W MUSTAT program with orthogonal contrast and mean comparison procedures, was performed to detect differences between the treatments. Separation means were determined using the multiple range test with Fisher’s least significant difference (LSD) (P < 0.05) (Krouma et al. 2006).

**Results**

**Plant growth and photosynthesis**

When subjected to drought plant growth decreased significantly in all the tested genotypes. Even though it decreased, biomass production in Amdoun remained higher than that in Kesseb and Chetoui (Figure 1). In order to express this genotypic variability we calculated the DII (Table 1). The Amdoun genotype had the lowest value, as compared to Kesseb and Chetoui.

All plants cultivated under drought stress exhibited a significant decrease in photosynthetic activity, as compared to the controls. Water deficit significantly decreased net photosynthesis (Figure 2), stomatal conductance (Figure 3), and transpiration (Figure 4). As for plant growth, the same trend in variation was observed; Amdoun was the least...
affected by drought, followed by Kesseb and Chetoui. Net photosynthesis decreased by 33%, 48%, and 51%; stomatal conductance decreased by 28%, 64%, and 70%; and transpiration decreased by 27%, 54%, and 61%, respectively, in Amdoun, Kesseb, and Chetoui.

A strong relationship between biomass production and photosynthetic activity was observed under drought stress (Figure 5). Amdoun had the highest plant growth and highest photosynthetic activity, followed by Kesseb and Chetoui.

**Plant water relations**

The relative water content decreased in the leaves of plants subjected to water deficit. This decrease was not significant in Amdoun (~8%), which maintained more hydrated leaves as compared to Kesseb and Chetoui (~23%) (Table 1). The water potential measured in control and stressed leaves showed an obvious decrease when plants were subjected to drought (Table 1). Amdoun had the highest potentiality of 11ψw decrease (2.1-fold greater than the control plants.), as compared to Kesseb (1.6-fold) and Chetoui (1.5-fold). Nevertheless, the calculation of osmotic adjustment based on the osmotic potential at full turgor showed that Amdoun developed a higher potentiality of osmotic adjustment than did the Kesseb and Chetoui genotypes (Table 2). This parameter was 1.8- and 2.5-fold more important in Amdoun than in Kesseb and Chetoui, respectively.

In order to investigate the other parameters implied in the drought tolerance of chickpea, we

<table>
<thead>
<tr>
<th>Relative water content</th>
<th>Water potential</th>
<th>DII</th>
</tr>
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<tbody>
<tr>
<td>control</td>
<td>drought</td>
<td></td>
</tr>
<tr>
<td>Amdoun</td>
<td>0.90 ± 0.08</td>
<td>-0.65 ± 0.05</td>
</tr>
<tr>
<td>Kesseb</td>
<td>0.87 ± 0.08</td>
<td>-0.64 ± 0.06</td>
</tr>
<tr>
<td>Chetoui</td>
<td>0.88 ± 0.07</td>
<td>-0.69 ± 0.07</td>
</tr>
</tbody>
</table>

Table 1. Relative water content (RWC, %), Water potential (MPa) and drought intensity index (DII) in 3 chickpea genotypes subjected to drought.
calculated the water use efficiency for plant growth (WUE DW) and photosynthetic activity (WUE PH) (Table 2). The first parameter is defined as the ratio of total plant biomass (g DW) to leaf water content (mL g\(^{-1}\) DW) and the second as the ratio of net photosynthesis (mol CO\(_2\) m\(^{-2}\) S\(^{-1}\)) to leaf water content (mL g\(^{-1}\) DW). These ratios are considered more reliable estimates of water use efficiency under drought because they take into account the water content together with plant growth or photosynthesis. The obtained results show that all the tested genotypes exhibited decreased water use efficiency for plant growth and photosynthetic activity under drought conditions. Genotypic differences were observed and Amdoun had the highest values of these 2 parameters when subjected to drought; this decrease was significantly lower in Amdoun (–27% and –24% of the control) than in Kesseb (–46% and –47% of the control), and Chetoui (–47% and –48% of the control) for WUE DW and WUE PH, respectively.

The correlation between plant growth and osmotic adjustment (Figure 6a), and between photosynthesis and osmotic adjustment (Figure 6b) indicates a strong relationship between these parameters. Amdoun is usually characterized by its performance, as compared to Kesseb and, in particular, to Chetoui. Our results show that there was a strong relationship between stomatal conductance and water status under drought stress (R\(^2\) = 0.99); in the control plants this correlation was less pronounced (R\(^2\) = 0.25).

**Discussion**

Exposure to drought stress inhibited plant growth in chickpea. Genotypic differences were observed and Amdoun was less affected as compared to Kesseb and

Table 2. Osmotic adjustment at full turgor (OA, MPa), water use efficiency for plant growth [WUE DW, g DW (mL g DW\(^{-1}\))^\(-1\)] and water use efficiency for photosynthesis [WUE Ph, μmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) (mL g DW\(^{-1}\))^\(-1\)] in 3 chickpea genotypes subjected to drought.

<table>
<thead>
<tr>
<th></th>
<th>WUE DW</th>
<th>WUE Ph</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>drought</td>
<td>control</td>
</tr>
<tr>
<td>Amdoun</td>
<td>0.87 ± 0.07</td>
<td>0.63 ± 0.05</td>
<td>3.24 ± 0.34</td>
</tr>
<tr>
<td>Kesseb</td>
<td>0.79 ± 0.07</td>
<td>0.43 ± 0.04</td>
<td>3.34 ± 0.27</td>
</tr>
<tr>
<td>Chetoui</td>
<td>0.71 ± 0.06</td>
<td>0.38 ± 0.04</td>
<td>2.69 ± 0.21</td>
</tr>
</tbody>
</table>
Chetoui. In fact, this genotype produced 1.4-fold more biomass than Kesseb and Chetoui. The calculation of the DII supports these intraspecific differences, and confirms the tolerance of Amdoun and the obvious sensitivity of Chetoui. A common adverse effect of water stress on crop plants is reduced fresh and dry biomass production (Farooq et al. 2009). Mohammadian et al. (2005) reported that the leaf area index, leaf dry weight, shoot dry weight, and root dry weight decreased under drought stress, as compared to non-stress conditions. Osmotic regulation can enable the maintenance of cell turgor for survival or for assisting plant growth under severe drought conditions in pearl millet (Shao et al. 2008). The observed reduction in plant height was associated with a decline in cell enlargement and increased leaf senescence in A. esculentus under water stress (Bhatt and Srinivasa Rao 2005). Brown et al. (1989) and Singh et al. (1997) reported that spring sowing (terminal drought) decreases dry matter production, plant height, and seed yield. Rosales-Serna et al. (2004) reported similar results in common bean subjected to drought and observed that overall yield reductions due to drought treatments were greater in the drought-susceptible cultivars than in the tolerant cultivars. Other researchers reported that drought-resistant cultivars might be more efficient at photoassimilate production and translocation to the seeds (Samper and Adams 1985), indicative of an obvious relationship between photosynthesis and yield.

Development of optimal leaf area is important to photosynthesis and dry matter yield. As in the present study (not shown), drought stress primarily reduced leaf growth and in turn leaf area in many plant species (Wullschleger et al. 2005; Farooq et al. 2009). In the present study significant decreases in net photosynthesis, stomatal conductance, and transpiration were associated with a significant decrease in plant growth. A strong relationship between biomass production and net photosynthesis under water deficit ($R^2 = 0.96$) was observed (Figure 5). The tolerant genotype Amdoun had the highest biomass production, photosynthetic activity, transpiration, and stomatal conductance. The last parameter seems to be the main factor in photosynthesis and limited transpiration under drought conditions. According to Baker (1993), there

![Figure 6. Relationship between biomass and osmotic adjustment (OA) and photosynthesis and osmotic adjustment (OA) in 3 Tunisian chickpea genotypes subjected to drought stress. Vertical and horizontal bars represent ± standard errors of means of 10 replicates.](image)

![Figure 7. Relationship between stomatal conductance and water potential in 3 Tunisian chickpea genotypes subjected or not to drought stress. Vertical and horizontal bars represent ± standard errors of means of 10 replicates.](image)
is a direct relationship between reduced intercellular CO₂ concentrations due to stomatal closure and decreases in CO₂ assimilation. Although drought impaired photosynthesis, it did not damage the photosynthetic apparatus (Zanella et al. 2004). The reduction in photosynthesis in *Myracrodruon urundeuva* under drought conditions occurred primarily because of stomatal closure, rather than damage to PSII (Zanella et al. 2004). In coffee plants under drought conditions partial maintenance of the quantum yield of PSII was observed despite photosynthesis suppression. In this case some processes could have contributed to the maintenance of electron flow, such as the Mehler reaction and photorespiration (Lima et al. 2002). In olive trees the photosynthetic apparatus was resistant to both weak and moderate drought, and stomatal closure was the main factor that limited photosynthesis. In the model plant for a drought-resistant legume (*Medicago truncatula* 'Jemalong') leaf relative water content and exchange gas parameters were significantly affected only under severe drought conditions (soil water content was below 25%) (Nunes et al. 2008).

The observed water status and relations in plants showed that drought decreased the relative water content, water potential, and osmotic adjustment. The utilization of leaf RWC as an indicator of plant water status is usual (Lawlor and Cornic 2002). In several legume cultivars of *Phaseolus vulgaris* (Costa Franca et al. 2000), *Vigna glabra* (Scotti Campos et al. 1999), and *Lupinus albus* (Pinheiro et al. 2001) the close relationship between RWC and predawn water potential observed during progressive water deficit supports its utilization as an indicator of plant water status. In the present study Amdoun maintained better leaf hydration than did Kesseb and Chetoui.

The osmotic adjustment was 1.8- and 2.5-fold greater in Amdoun than in Kesseb and Chetoui, respectively. In fact, the ability to decrease the water potential and osmotic adjustment allowed this genotype (Amdoun) to maintain better leaf hydration and photosynthetic activity. Figure 6 illustrates this explanation and the positive correlation between biomass production and osmotic adjustment (6a), and that between photosynthesis and osmotic adjustment (Figure 6b). Similar studies have reported a significant correlation between growth and osmotic adjustment in pea plants subjected to drought stress (Sanchez et al. 2004). These authors suggest that the reduction in plant and leaf growth rates is an early phenomenon that occurs before decreases in the leaf water potential can be detected. Blum (1989) observed that plants with high osmoregulatory capacity exhibit more growth than plants with low osmoregulatory capacity. Our results also show that leaf water status interacts with stomatal conductance under drought stress. Figure 7 indicates that there is a good correlation between these 2 parameters, with R² reaching 0.99. In the control plants this correlation was less pronounced (R² = 0.25).

Taken as a whole, the present results show that plant growth, photosynthetic activity, and osmotic adjustment were interdependent. We suggest that the tolerance or sensitivity of chickpea to drought is related to its capability to maintain good leaf water status. This parameter is highly correlated with stomatal conductance, which controls photosynthesis and transpiration and, in turn, plant growth. The tolerance of the Amdoun genotype seems to have been the result of the equilibrium between many physiological parameters (water use efficiency, stomatal conductance, photosynthesis, and transpiration), ensuring plant growth under drought stress.

References


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