Effect of drought on growth, physiological and biochemical processes of chickpea-rhizobia symbiosis

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ABSTRACT

The effects of drought on growth, several physiological and biochemical processes in six winter varieties (Zhour, Rizki, Douyet, V46, V34 and P37) of chickpea (Cicer arietinum L.) and two rhizobial strains (MC07 and MC10) were studied. The experiment was conducted under greenhouse conditions. Seedlings were grown under three regimes moistening and inoculated separately: 100 % of field capacity (control), 80% of field capacity (optimal irrigation) and 40% of field capacity (water deficit). The results showed that the hydric deficit had significantly perturbed the dry biomass, proline activity, total chlorophyll and nitrogen contents. Moreover, this constraint negatively affected the water deficit saturation (WDS), the membrane permeability and the stomatal conductance of leaves. Under drought, the varieties Zhour and Rizki showed a better water efficiency that was translated by high level in proline accumulation, membrane stability, total chlorophyll and nitrogen contents. These parameters were maintained at the adequate levels with the rhizobial strain MC07 which showed a tolerance in the drought condition. On the contrary, the symbiotic combination least powerful according to the studied parameters is formed by the variety P37-MC10.

Key words: Chickpea, Drought, Nitrogen contents, Proline activity, Tolerance, WDS.

INTRODUCTION

Chickpea (Cicer arietinum L.) is among first leguminous plants with seeds domesticated by the man since antiquity and is cultivated in the large area in the arid and semi-arid zones: Americas, Mediterranean basin, East Africa, the Middle East, Asia and Australia (Jayashree et al.2005) where approximately 90% of world’s chickpea is grown under rainfed conditions (Kumar and Abbio 2001). The chickpea is cultivated from farming practices rainfed conditions, is traditionally sowed in spring. This type of agriculture accounts for more than 60% agricultural areas in Morocco. It provides much of the food consumed by Moroccan people. But, sowing in spring exposes the culture to terminal drought (Kashiwagi et al.2013). The winter chickpea could have an important place in the culture systems of the Moroccan semi-arid zones. Indeed, it was observed that the winter chickpea has a yield potential higher than that of the spring chickpea (Pala and Mazid 1992).

The drought is the most important cause which limits the agricultural production and decreases the efficiency of dry lands. To resist the drought, the plant recourse to changes has morphologic, physiologic and biochemical properties (Xiong et al.2006). These changes include water efficiency, osmotic adjustment, photosynthetic activity and accumulation of proline (Dhanda et al. 2004; Kalefetoglu and Ekmekçi 2009; Farissi et al. 2011). The drought conditions, reduces yield and produced biomass of the chickpea (Leport et al. 2006) and decreased the relative water content (Chandrasekar et al. 2000). The drought delays the development of the growth as well of harvest, outputs as by decreased the leaf and root potential of water (Grzesiak et al. 1997). The leaf chlorophyll contents are an important indicator in determining the photosynthesis rate and the Dry weight (DW) (Ghosh et al. 2004). In general, the drought changes the chlorophyll contents (Faroq et al. 2009). The accumulation of osmolytes allows the maintenance of the membrane structural integrity (Conroy et al. 1988) and the osmotic adjustment is a factor of resistance in the drought. The proline is one of the osmolytes, which quickly answers than other amino acids in the plant in case of deficit hydric. In the chickpea roots, rhizobia form nodules by the secretion of lipochito oligosaccharidic Nod factors (NFs). This organ reduces dinitrogen in NH₄⁺ who is assimilated by the plant host (Masson-Boivin et al. 2009), which supplies the carbon and the energy sources in bacteroids in the form of succinate and malate (Lodwig and Poole 2003). Several studies showed that the hydric deficit affects negatively the symbiosis of legumes - rhizobia by accelerating the nodules senescence, decreasing their number (Ashraf and Irám 2005), their leghemoglobin contents and reduce the nitrogenase activity (Figueiredo et al. 2008). Our study searches to, move forward...
the sowing of the chickpea in winter by drought-resistant varieties for increase yield.

**MATERIALS AND METHODS**

**Treatments and plant culture:** The experiments of this study were carried out under greenhouse conditions with an approximate temperature of 29/18°C (day/night), 50-80% of relative humidity and 16 hour (h) photoperiod (22 Klux) at the Faculty of Sciences and Techniques, Marrakesh.

**Bacterial material:** The inoculum consists of three strains of rhizobia: MC07 and MC10 were provided by the laboratory: Unit of Plant Biotechnology and Symbiosis Agronomy Research (INRA)-Marrakesh. Originating from semi-arid regions (south of Morocco), were selected for their symbiotic performance and their drought tolerance (8 % PEG600).

**Plant material:** Six seeds kabuli type of winter chickpea (Cicer arietinum L.) varieties (Rizki (Rz), Zhour (Zh) and Douyet (Dt)) were provided by the National Institute of Agronomic Research (INRA)-Settat and three were supplied by INRA-Meknes: P37, V34 and V46. Seeds surface was disinfected by immersion in ethanol 95% during 30 seconds and 5 min in 5% sodium hypochlorit and rinsed several times by sterile deionized water. After, the seeds are transferred to plastic pots measuring 20 cm tall and 16 cm diameter. Each pot contained 2000 g of sterile sand and peat with the proportion 5:1 respectively and the number of seedlings adjusted to 6 per pot. Seven days after, the seedlings were inoculated with 10 mL of each strain of rhizobia Containing approximately 10^8 CFU/mL (CFU = Colony-forming unit). Plants were watered three times a week with the distilled water and the nitrogen free nutrient solution during the trial period (Bargaz et al. 2013). After one week, plants randomly selected were subjected to maximal irrigation (100%).

**Biomass measurements:** Fresh weight (FW) of the shoots and roots was directly weighed. The dry weight (DW) determined after samples passed 48h under 70°C and weighed. To standardize the data, the results were expressed as the relative reduction of yield in comparison to the control plant using the formula (Ghoulam et al. 2002):

$$\text{Relative reduction} \% = \left(\frac{1}{n} \times \text{(Stressed/ Control)}\right) \times 100$$

**Hydric parameters:** Foliar samples (0.1 g) took at the levels of the young leaves and weighed (Fresh weight: FW), then deposited in Petri dish by keeping them in distilled water for 4 h during 24 hours, the samples were weighed (turgid weight: TW). Finally, they were dried during 24 hours in 80°C and then weighed (Dry weight: DW).

**Water deficit saturation (WDS):** Plant tissues are generally in situations of hydric deficit and WDS can explain this situation. Farissi et al. (2013) defined as follows:

$$\text{WDS} \% = \frac{TW/FW}{(TW-DW)} \times 100$$

**Membrane permeability (Electrolyte leakage):** It was assessed as described by Ghoulam et al. (2002). The disturbance of the membrane permeability by the hydric stress was considered by the trans-membrane flow of the electrolytes leaving the cells. The foliar samples were rinsed three times with deionized water to eliminate the ions surface then placed in closed vials with 25 mL of deionized water. After incubated during 24h in a rotary shaker (100 t/min) at 25°C, we determined the electrical conductivity of the solution (Lt) using a conductivity meter (Hannah Instruments HI8820N). Samples were then autoclaved at 120°C during 20 min after equilibration at 25°C, the last electrical conductivity (Lo) was measured. The electrolyte leakage was defined as follows:

$$\text{Electrolyte leakage} \% = \frac{(L_t/L_o)}{L_o} \times 100$$

**Stomatal conductance:** It was based on the measure of CO2 entering rate, or water vapor exiting through the leaf stomata and expressed in mmol H2O/m².s on leaves using a porometer (Leaf porometer, model SC1, DECAGON DEVICES, version 2012) under a temperature of 25°C and a relative humidity of 55 ± 5%.

**Total chlorophyll content:** It was measured using the method described by Arnon (1949). It was extracted with acetone in a mortar, using a proportion of 200 mg of fresh leaf tissue and 5 mL of acetone (80%, v/v). Total Chlorophyll content was measured after centrifugation (10 min at 5000xg), the absorbance (OD) of the supernatant was measured at 663 and 645 nm. Chlorophyll concentration was calculated using the formula:

$$\text{Total Chlorophyll} = 8.02 \text{ OD}_{663} + 20.20 \text{ OD}_{645}$$

**Proline contents:** There were determined using the method described by Bates et al. (1973). Proline was extracted from leaf samples (100 mg FW) with 2 mL of 40% (methanol: water). The tubes were incubated in a water bath at 85°C for 30 min, then 1 mL of extract was mixed with 1 mL of a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3: 2:v/v) and 25 mg ninhydrin. After 1 h incubation at 100°C, the reaction was terminated in ice bath to stabilize the purple color of the extract 5 mL of toluene was added to each tube and the absorbance of top purple aqueous layer was measured at 528 nm in a spectrophotometer. The proline concentration was determined according to the standard curve obtained using reference proline solutions.

**Total nitrogen contents:** These were determined using the Kjeldahl method (Bargaz et al. 2012). A dry leaf sample (0.5 g) put in Buchi tubes with 1 g of catalyst mixture (potassium sulphate (100 g), copper sulfate (20 g) and
selenium powder (2.0 g) and concentrated sulfuric acid (10 mL). During Zh, the digestion of the organic matter was held at 400°C. After 5 min of distillation, the distillate was recuperated in H₃BO₃ solution at 1% and titrated by hydrochloric acid (N/100). The total nitrogen content was estimated and expressed in mg/plant.

**Statistical analysis:** It was performed using the SPSS 21.0 software (SPSS, Chicago, Illinois, USA, 2012). Two-way analysis of variance (ANOVA II) was performed using three replicates per treatment for all studied parameters. The means values and standard errors were calculated. For means comparison of the considered parameters, Tukey’s test was applied.

**RESULTS AND DISCUSSION**

**Effect of water on growth:** The hydric stress was significantly reduced (p<0.001, Table 1) the dry biomass of all the studied chickpea varieties compared to their controls deficit (Table 1). The irrigation by FC₁, showed a reduction of the shoot dry weight of the more intense to the V34, V46 and P37 varieties (40 to 74 %) than that of Rz, Zh and Dt (12 to 40%). This difference was much higher by irrigation FC₂; indeed this reduction for the V34, V46 and P37 varieties varies between 50 and 79 % while it is only from 17 to 55 % for the Rz, Zh and Dt varieties respectively. The minimal percent reduction is obtained with the symbiotic winter variety zhour-MC07 in FC₁ (12 %) and FC₂ (17 %) respectively. At the root system (Table 1), the irrigation by FC, showed a higher significant (p<0.001, Table 1) reduction in the V34, V46 and P37 (25 to 51 %) than that of Rz, Zh and Dt varieties (7.6 to 30.8 %). This reduction is much higher with the irrigation by FC₂. Indeed, this reduction varies between 35.5 to 60 % in the V34, V46 and P37 varieties while it is only 10.75 to 41 % for the Rz, Zh and Dt varieties. Under hydric stress FC₂, the lowest reduction is observed with the symbiotic zhour-MC07 (10.75 %) in the root and the strongest reduction are noted at the symbiotic P37-MC10 (79 %) in the shoot. We noted also, a strong correlation (r=0.942) between shoot and root dry under FC₁. These results show a better resistance of the Rz, Zh and Dt varieties by strain MC07 under hydric stress. So, Silva et al. (2007) showed that under drought, the vigour of the seedlings of sugarcane can be used for the identification of the tolerant varieties. The shoot dry weight under water stress reduced more to root dry weight. In parallel, the extension of roots in the ground for the search for water is a mechanism to avoid the drought (Benjamin and Nielsen 2006). Generally, symbiotic combination plants hosts and rhizobial strain more tolerant in the drought form between them symbioses for decreased the effect of the hydric stress (Ben Rhomdhan et al. 2009).

**Effect of drought on water deficit saturation:** The comparison between the varieties showed a significant difference (p<0.001, Table 2) between the combinations studied. The increase of water deficit saturation (WDS) was proportional to the drought and the high levels of this variable were noted at FC₁. Indeed, we observed an efficiency of the water with the combination Zhour-MC07 (24.13 %) under FC₁, following by Rizki-MC07 (26.8%) and a strong reduction was observed in P37-MC10 (45.8%). Moreover, we observed a strong correlation (r=0.862) between shoot dry and WDS under FC₂. In parallel, Huang et al.(2000) indicated that the water efficiency was an important element to determinate the resistant varieties under hydric deficit. The combination Zh-MC07 was able to maintain an adequate

**Table 1:** Effect of water deficit on: Shoot reduction weight, root reduction weight in six chickpea varieties (V46, V43, P37, ZH, RZ and DT) inoculated with rhizobial strains MC07 and MC10. Values are means of three replicates ± standard errors.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Shoot reduction (%)</th>
<th>Root reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symbiotic combination</strong></td>
<td><strong>Shoot</strong></td>
<td><strong>Root</strong></td>
</tr>
<tr>
<td>FC₁</td>
<td>FC₂</td>
<td>FC₁</td>
</tr>
<tr>
<td>V46-MC07</td>
<td>28±1.2</td>
<td>50±3</td>
</tr>
<tr>
<td>V46-MC10</td>
<td>28.7±1.5</td>
<td>65±3</td>
</tr>
<tr>
<td>V34-MC07</td>
<td>22±1</td>
<td>40±2.5</td>
</tr>
<tr>
<td>V34-MC10</td>
<td>23±1</td>
<td>55±2.5</td>
</tr>
<tr>
<td>P37-MC07</td>
<td>32±1</td>
<td>58.7±2.1</td>
</tr>
<tr>
<td>P37-MC10</td>
<td>32.3±1.6</td>
<td>73.7±1.2</td>
</tr>
<tr>
<td>Zh-MC07</td>
<td>10.1±0.4</td>
<td>12.1±0.8</td>
</tr>
<tr>
<td>Zh-MC10</td>
<td>11.1±0.5</td>
<td>22.1±0.9</td>
</tr>
<tr>
<td>Rz-MC07</td>
<td>11.3±0.6</td>
<td>20±1.3</td>
</tr>
<tr>
<td>Rz-MC10</td>
<td>11.2±0.7</td>
<td>32±1.3</td>
</tr>
<tr>
<td>Dt-MC07</td>
<td>12.6±0.5</td>
<td>25±1.9</td>
</tr>
<tr>
<td>Dt-MC10</td>
<td>13.2±0.7</td>
<td>40±1.9</td>
</tr>
</tbody>
</table>

**Statistical analysis:**

<table>
<thead>
<tr>
<th>Symbiosis</th>
<th>Water deficit</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>564.63***</td>
<td>364.13***</td>
<td>107.5***</td>
</tr>
<tr>
<td>1128.3***</td>
<td>6123.04***</td>
<td></td>
</tr>
<tr>
<td>210.2***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*:* Significance at 0.05 probability level; **: significance at 0.01 probability level; ***: significance at 0.001 probability level; NS: not significant at 0.05.
level in this parameter. Indeed, is probably related to a good osmotic adjustment capacity to conserve the functional and structural integrity of tissues (Farissi et al. 2013). On the basis of this criterion, the Rz and Zh chickpea was found the most efficient (Soltani et al. 1999).

**Effect on membrane permeability**: Significant increases of leaves electrolyte leakage in almost all varieties inoculated under hydric constraint ($p<0.001$, Table 2). Under irrigation treatment $FC_2$, the comparison between studied symbiotic combinations showed that the lowest percentages of electrolyte leakage were observed in combinations Zh-MC07 and Rz-MC07 (18 and 19% respectively). In contrast, the highest values have been observed in symbiotic MC10-V34, V46 and P37 varieties, particularly P37-MC10 (33.8%) and V46-MC10 (31.6%). Furthermore, a strong correlation ($r=0.942^{**}$) was noted between WDS and membrane permeability under $FC_2$. An increase in electrolyte leakage indicates that the membrane integrity is affected. Indeed, the rate of electrolyte leakage is a physiological index which reflects the degree of cellular membranes stability of plants in stress conditions (Farissi et al. 2013a).

**Effect on stomatal conductance**: Under $FC_2$, significantly reduced in all of the studied varieties ($p<0.001$, Table 2). We noted higher reductions between symbiotic combinations under irrigation treatments and their respective controls. This confirms the results found by Aranjuelo et al. (2011). Under $FC_2$ this parameter varied between 50 and 210 mmol m$^{-2}$s$^{-1}$ respectively in Zh-MC07 and P37-MC10. The positive effect of the WDS was noted by controlling the behavior of stomata under hydric stress, maintaining leaf surface and facilitating the absorption of water (Thameur et al. 2012). In addition, the tolerant varieties develop mechanisms for a better use of fixed CO2. These mechanisms are explained to differential day/night control (Rosales et al. 2012). Strong correlation ($r=0.908^{**}$) was noted between WDS and stomatal conductance under $FC_2$.

**Effect on total chlorophyll contents**: The results, showed that total chlorophyll contents in all of the studied symbiotic combinations were significantly reduced by hydric stress ($p<0.001$, Table 2). The highest reductions are noted to combinations MC10 and V34, V46 and P37 varieties, in particular P37-MC10 (0.55 mg.g FW$^{-1}$). The moderate reductions are observed at the combination MC07 – Zhour, Rizki (10 and 9 mg.g FW$^{-1}$ respectively). Tolerant chickpea genotypes maintained relatively higher chlorophyll content (Mafakheri et al. 2010). Moreover, the increasing drought induces the reduction of the chlorophyll contents by increasing chlorophyllase activity (Jamil et al. 2007) and the instability of the chloroplast structures. Negative correlation was noted in $FC_2$ between chlorophyll content (Guo et al. 2008) and WDS ($r=-0.83^{**}$).

**Effect on proline contents**: The results indicated that the increased drought induced a significant increase ($p<0.001$, Table 2) in proline accumulation in all symbiotic combinations studied. At $FC_2$, a higher accumulation of proline was noted by symbiotic combination Zh-MC07 (964 mg.g FW$^{-1}$). In contrast, proline accumulation was lowest in combinations P37, V46 and V34 with rhizobia strain MC10 were 797, 803 and 806 mg.g FW$^{-1}$ respectively. Under stress conditions, plants have evolved complex mechanisms include osmotic adjustment by accumulation of compatible solutes such as proline (Routley 1966). The osmotic adjustment involves an active accumulation as proline in the plant in answer to the reduction of water (Morgan 1984). As a consequence, the reduction in the osmotic potential in the cell attracts the water into the cell and leads to maintaining its turgor (Turner et al. 2007). The osmotic adjustment was also reported in the chickpea in water deficit (Leport et al. 1999; Gunes et al. 2008).

**Effect on nitrogen contents**: This was significant reduced ($p<0.001$, Table 2) in all associations under drought stress, Zhour and Rizki varieties showed important shoot nitrogen contents (7.4 and 5.5 mg/plant respectively) when inoculated by MC07 strain. The lowest nitrogen content was noted with symbiotic P37-MC10 (0.55mg/plant). This decline is due to nitrogen metabolism decrease (Antolin et al. 1995). A strong interaction ($p<0.001$, Table 2) between symbiosis and the drought was observed for this parameter.

Table 2. Results of two-way analysis of variance (ANOVA II) of drought and symbiotic combination effects and their interactions for the considered parameters.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Symbiotic combination</th>
<th>Water deficit</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dF</td>
<td>F</td>
<td>dF</td>
</tr>
<tr>
<td>Water deficit saturation</td>
<td>5</td>
<td>27.95***</td>
<td>1</td>
</tr>
<tr>
<td>Electrolyte leakage</td>
<td>5</td>
<td>133.37***</td>
<td>1</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>5</td>
<td>66.13***</td>
<td>1</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>5</td>
<td>183.05***</td>
<td>1</td>
</tr>
<tr>
<td>Proline accumulation</td>
<td>5</td>
<td>65.16***</td>
<td>1</td>
</tr>
<tr>
<td>Nitrogen contents</td>
<td>5</td>
<td>132.46***</td>
<td>1</td>
</tr>
</tbody>
</table>

*: Significance at 0.05 probability level; **: significance at 0.01 probability level; ***: significance at 0.001 probability level; NS: not significant at 0.05.
CONCLUSION

In general, the exposure plant to hydric stress causes many reactions as the fresh weight decrease, the reduction in the chlorophyll contents and the disturbance of the stomatal activity and the accumulation of the proline. All the studied parameters showed that the symbiotic combination Rz, Zh and Dt varieties-MC07 was more resistant in the hydric stress.

REFERENCES


