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*Full Length Research Paper*

# Assessment of intervarietal differences in drought tolerance in chickpea using both nodule and plant traits as indicators

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**5 lines of Tunisian varieties of chickpea (*Cicer arietinum* L.) inoculated with *Mesorhizobium ciceri* UPMCa7 were monitored during the vegetative stage on sterilized sandy soil. 2 levels of soil moisture were compared (100 and 33% of field capacity). The work was aimed at assessing the relative tolerance of these lines to drought and then, to research relationships between the level of sensitivity of plant growth and N content to drought and nodule, leaf and root traits. Drought limited plant growth of Amdoun and Neyer and decreased N content of Chetoui, Amdoun and Neyer. The latter N shortage was associated with increase in nodule mortality and restriction of nodule growth. In view of their minimal decrease in plant biomass and N content, Beja and Kesseb were the most tolerant varieties. Inter- varietal differences for water stress effects on nodule, root and leaf traits were limited to (i) change in root to shoot ratio (ii) loss of chlorophylls and (iii) nodule mortality. Each of these traits was considered as an indicator of stress tolerance. These indicators predict that the most tolerant variety was Beja based on higher increase in root to shoot ratio and Beja and Kesseb based on lower nodule mortality. When choice of the varieties should depend on the likelihood of water stress during the culture, Amdoun which presented the higher biomass per plant and the higher nitrogen content in control condition and ranked similarly to all other varieties in stress condition might represent a reasonable trade- off between high growth and stress tolerance when the probability of water stress to occur during the culture period is low.**

**Key words**: *Cicer arietinum*, drought stress, nodule characteristics, tolerance.

## INTRODUCTION

*Cicer arietinum* L. (chickpea) is an important food legume crop of Mediterranean populations. In Tunisia, it shares the first rank with faba bean and it is a winter-spring crop grown in semi-arid regions. Generally, legumes are highly sensitive to water deficit stress (Mahieu et al., 2009). Drought conditions may limit production of le-gumes by affecting nodule functioning (Ashraf and Iram, 2005; Clement et al., 2008). The effect can be due to (i) restriction of carbohydrate transport from leaves to nodule (Singh and Singh, 2006) (ii) reduced in shoot N demand reflected by the Rubisco depletion affected negatively malate dehydrogenase and glutamate-oxalate transaminase nodule's activities (Aranjuelo et al., 2009) (iii) less water which hinders the transport of N-products away from the nodules (Ramos et al., 2003) (iv) direct effects on nodule gas permeability (Ramos et al., 2003) and/or (v) the alteration of nodule metabolic activity (Clement et al., 2008).

Water deficit can also induce premature senescence of nodules (Puppo et al., 2005). During this process, many changes occur in nodules, for example the external colour of the N-fixing tissues of the nodule changes from red (due to functional leghaemoglobin) to green (indicat-ing alteration of this protein) (Swaraj and Bishnoi, 1996), decrease in leghaemoglobin content (Garg and Manchanda, 2008), decrease in nodule membrane inte-grity (Mhadhbi et al., 2009), degradation of bacteroids (Herder et al., 2008), increase of proteinase activities in nodules (Groten et al., 2006) and loss of N-fixation acti-

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vity regardless of physiological and biochemical mechanisms of $N_2$ fixation inhibition by water deficit stress, there is evidence that legume species have significant genetic variation in their ability to fix $N_2$ under drought conditions (Ashraf and Iram, 2005; Charlson et al., 2009). However, there is no available information on the variability of drought tolerance in chickpea cultivated in symbiosis condition. The aim of this work was to establish easy use of indicators of chickpea tolerance to drought based on simple traits of plants and nodules. 5 chickpea varieties were compared. Their relative tolerance was assessed from plant biomass and N content and a variety of traits of nodules, roots and leaves were used as indicators.

## MATERIAL AND METHODS

### Growth conditions and experimental procedures

The experiment was conducted in a greenhouse of the Biotechnological Center at Borj Cedria (35 km south-east of Tunis), during June 2005. Sterilized seeds of 5 local chickpea (*C. arietinum* L.) lines named Beja, Neyer, Amdoun, Kesseb and Chetoui were germinated in plastic Petri dishes. 4-day-old seedlings were individually transplanted in sterilized plastic pots (16.5 cm diameter and 15.5 cm height) filled with loam sandy soil (3.1 kg). Prior to transplantation, soil was abundantly washed with distilled water to remove nutrients content and it was heat sterilized in metal buckets at 380°C for 4 h. After transplantation, the seedlings were inoculated with rhizobial suspension (strain *Mesorhizobium ciceri*, UPMCa7). They were irrigated with nutrient solution (Vadez et al., 1996) at field capacity for 21 days. The average day and night temperatures were 35 ± 5°C and 20 ± 2°C, respectively. The day length was 14 h and the relative humidity was 55 ± 5% by day and 80 ± 5% at night. Functional nodules were established during this period. After 21 days, plants were divided into 2 lots of 6 plants each, one irrigated with tap water at 100% field capacity(control plants) and the second one at only 33% field capacity (stressed plants)

The water volume necessary to reach 100% field capacity was determined by measuring soil water content after cession of drainage. Regular wetting (every 2 days) enabled to restore the moisture of soil at its nominal value.

### Measurements

Plants were harvested after 14 days of treatment (that is, 39 days after germination). The midday leaf water potential of the 3 younger leaves of the main stem was determined using a pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA, USA) according to Scholander et al. (1965). Harvested plants were divided into leaves, stems, roots and nodules and the fresh weight (FW) of plant parts was determined. Leaves were counted and their surface area was measured using a portable area meter (LI-3000A). Electrolyte leakage of leaves was measured on the third youngest (fully expanded) leaf as described by Dionisio-Sese and Tobita (1998). Relative water content (RWC) was calculated in the same leaves using the as RWC (%) = 100 FW - DW / (TW - DW) (Schonfeld et al., 1988). Fresh weight (FW) was determined within 2 h after harvest. Turgid weight (TW) was obtain-ed after soaking leaves in distilled water in test tubes for 12 h at room temperature (about 20°C) under laboratory room ceiling light. After soaking, leaves were quickly and carefully blotted dry with tissue paper in preparation for determination turgid weight. Dry weight was measured after oven drying samples at 60°C for 48 h. Chlorophyll content of leaves was determined using the method of Bruinsma (1963). Fully expanded and mature leaves were randomly selected for this purpose. Nodules were removed, counted and photographed with digital camera. Leghaemoglobin content was measured according to Becana et al. (1986). Shoots, roots and nodules dry weight was determined after oven drying for 72 h at 80°C. Total nitrogen was determined by Kjeldahl method. Statistical analysis was performed using a computer program (Statistica$^{TM}$ software). Data were analysed by 2 way ANOVA (treatment and chickpea lines as factors, Table 1). Least significant difference (LSD) post hoc tests were used for mean comparison within varieties when ANOVA indicated significant effect of the considered factor. Significance differences between the 2 treatments in each variety was examined accordingto student's t- test at p = 0.05. Data are represented by means of 6 replicates ± SEM.

## RESULTS

### Plant growth

No significant variability appeared among varieties for whole plant biomass, but significant effect of treatment was observed for whole plant biomass (Table 1). In control condition, the plant biomass (dry weight, DW) differed slightly among varieties, with the higher level for Amdoun and Neyer (Figure 1). In these 2 varieties growth was significantly affected by water stress (p = 0.05). However, the absolute values of the plant biomass under stress did not significantly differ between the five varieties. The root biomass of control plants did not differed between varieties (Table 1) . It was augmented by the water stress only in Chetoui (p = 0.05, Figure 2). Values in stressed plants of all varieties were statistically similar. However, the root to shoot ratio and its response to water stress signify-cantly differed between varieties (Table 1), it was signify-cantly augmented in water stressed Beja, but not the other varieties (p = 0.05, Figure 2).

### Nodules

The N content of the whole plants was significantly diminished by water stress in Chetoui, amdoun and Neyer (p = 0.05, Figure 1) varieties, but this response did not discriminate between varieties (Table 1). The water stress significantly increased nodule defects or mortality (Figure 4), especially in Chetoui and Amdoun. In these varieties, the proportion of empty nodules with dark coloration reached 50 - 60% at the harvest, in contrast to the 3 other varieties in which it was only 10 to 26% (Table 2). In both control and stress condition, Kesseb presented less nodules per plant than the other varieties. No effect of the water stress could be detected for this parameter (p = 0.05, Figure 3). On the contrary, the biomass of non-empty nodules per plant presented the same pattern as observed for N content (Figure 3), with a clear decrease upon water stress and no significant inter-varietal difference (Table 1). Only leghaemoglobin concentration in non-empty nodules depended on both varieties (highest values in Chetoui and Neyer) and treatments, with a large decrease in Amdoun and Neyer upon water stress (p =

**Table 1.** Results of variance analysis (ANOVA). Five chickpea varieties and two treatments (control and water stress) were compared.

| p values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Whole plant root and nodules | | | | | | | | |
| **Variables** | **Whole plant biomass** | **Whole plant N content** | **Root system biomass** | **Root /shoot ratio** | **Nodule biomass per plant** | **Nodule number** | **proportion of empty nodules** | **legheamoglobin** | **Mean Specific N fixation** |
| Factor "variety" | 0.145 | 0.121 | 0.036 | $2\ 10^{-04}$ | 0.326 | 0,028 | $2\ 10^{-05}$ | 0.005 | 0.004 |
| Factor "treatment " | 0.027 | $9\ 10^{-06}$ | 0.789 | 0,034 | $5\ 10^{-08}$ | 0.138 | $6\ 10^{-10}$ | 0.036 | 0.006 |
| Interaction | 0.033 | 0.184 | 0.085 | 0,035 | 0.202 | 0.992 | $2\ 10^{-05}$ | 0.060 | 0.277 |

| Leaves | | | | | | |
|---|---|---|---|---|---|---|
| **Variables** | **Leaf water potential** | **leaf number** | **chlorophyll concentration** | **Leaf surfacic mass** | **Electrolyte leakage** | **Relative water content** |
| Factor "variety" | $3\ 10^{-07}$ | 0.415 | $8\ 10^{-06}$ | 0.000 | 0.008 | 0.093 |
| Factor "treatment " | $3\ 10^{-13}$ | 0.004 | $1\ 10^{-14}$ | 0.002 | 0.159 | 0.347 |
| Interaction | $2\ 10^{-07}$ | 0.940 | $2\ 10^{-07}$ | 0.704 | 0.244 | 0.702 |

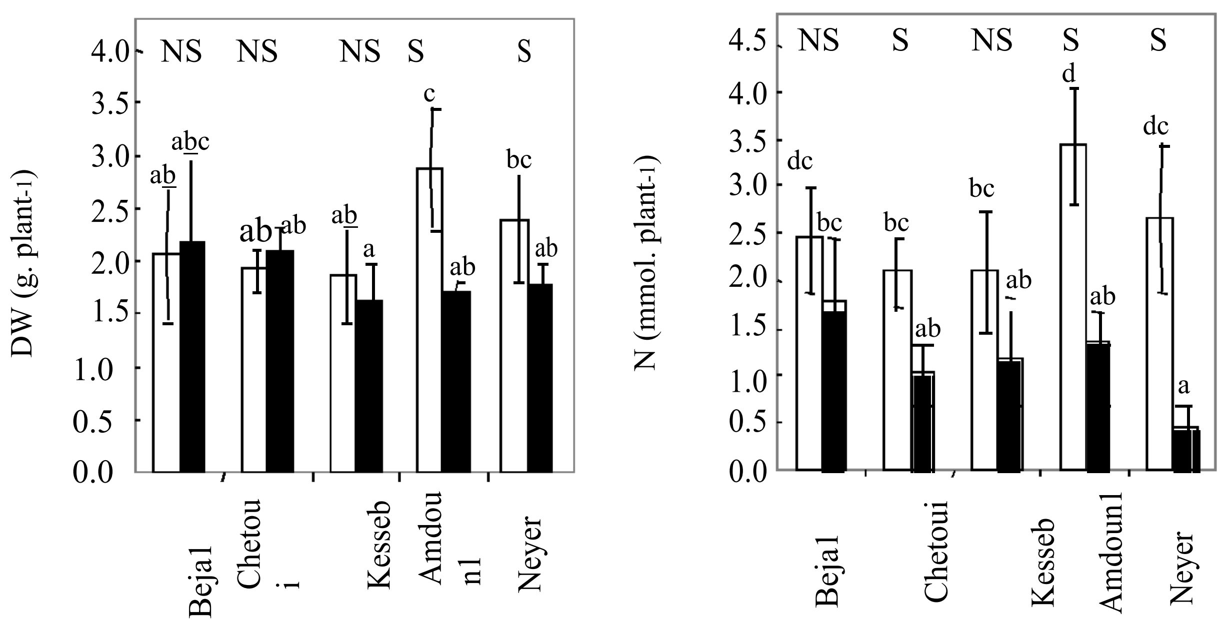


**Figure 1.** Effect of water stress on growth and nitrogen fixation. Growth was estimated as whole plant dry weight (DW) at the final harvest. Nitrogen fixation was estimated as N content of whole plants grown in the absence of N in the culture solution. Means ± SE (n = 6). Differences among chickpea lines were analyzed using ANOVA. Different letters indicate significant difference ($p$ = 0.05). S, NS: significant, respectively non significant mean difference within each variety, according to Student t test at $p$ = 0.05. Open bars: control. Full bars: water stress.

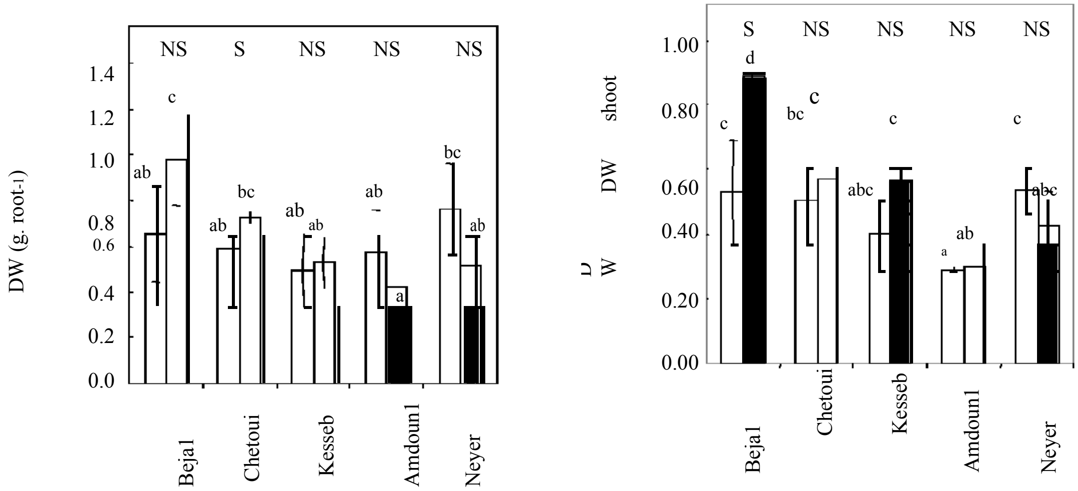


**Figure 2.** Effect of water stress on root growth. Root growth was estimated as root system dry weight (DW) at the final harvest (left panel). The right panel shows the root to shoot DW ratio. Five chickpea lines are compared. Means ± SE (n = 6). Differences among chickpea lines were analyzed using ANOVA. Different letters indicate significant difference (*p* = 0.05). S, NS: significant, respectively non significant mean difference within each variety, according to Student t test at *p* = 0.05. Open bars: control. Full bars: water stress.

**Table 2.** Nodule characteristics of five chickpea varieties and effect of water stress. The plants were cultivated for for 14 days either in control condition or at 33% field capacity. Values are means ± standard errors (n = 6). For each parameter different letters indicate significant differences ($p < 0.05$).

| | | Varieties | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Treatments** | **Beja** | **Chetoui** | **Kesseb** | **Amdoun** | **Neyer** |
| Individual nodule | control | 6.6± 3.6abc | 6.1 ± 2.3abc | 7.3 ± 0.8bc | 5.1 ± 0.5abc | 7.8 ± 2.2c |
| biomass (mg DW) (1) | water stress | 4.5 ± 1.4ab | 4.2 ± 0.1ab | 7.1 ± 2.2bc | 3.4 ± 0.1a | 4.1 ± 1.3ab |
| Specific N fixation | control | 15.5± 2.8dc | 11.7 ± 3.2bc | 14.1 ± 2.3c | 18.7 ± 0.8d | 16.3 ± 0.6dc |
| (mmol N. $g^{-1}$ nodule DW) (1) | water stress | 15.8± 5.9dc | 9.3 ± 3.0ab | 11.9± 4.5bc | 14.1 ± 2.2c | 7.2 ± 4.6a |
| Empty nodule number | control | 0 | 0 | 0 | 0 | 0 |
| (% of total nodules) | water stress | 10 ± 6 a | 60 ± 10 b | 12 ± 13 a | 51 ± 13 b | 26 ± 4 a |
| Leghaemoglobin | control | 3.9± 1.9abc | 8.7 ± 3.1d | 4.3 ± 2.6bc | 5.1 ± 1.4bc | 6.3 ± 1.8dc |
| (mg $g^{-1}$ nodule FW) (1) | water stress | 5.2 ± 1.4bc | 6.4 ± 3.0dc | 5.0 ± 3.2bc | 1.4 ± 0.7a | 3.2 ± 1.2ab |

(1) Empty nodules excluded

0.05, Table 2).

The mean specific N fixation provides a crude measure of the nodule efficiency. It was estimated by rationing the whole plant N content to the (non-empty) nodule mass (DW) per plant. Although decrease was observed upon stress treatment in Amdoun and Neyer varieties (p=0.05, Tab. 2), the interaction between treatment and variety factors was not significant (Tab. 1).

### Leaves

Water stress lowered the leaf number, but this effect was only significant in Chetoui (Table 3). In both control and stress conditions, the 5 varieties presented statistically similar leaf mean thickness (estimated as the leaf surfacic mass, that is, leaf fresh weight rationed to leaf surface area). The latter parameter was augmented by water stress, but this behavior was significant only in Kesseb and Amdoun varieties (p = 0.05, Table 3). Leaf tissue hydration (RWC) did not present significant difference between varieties nor between treatments and no interaction between these factors could be observed for this parameter (Table 1). In contrast to RWC, the leaf water potential (Table 1) was strongly dependent on variety, Kesseb presenting a higher value (-0.42 Mpa) compared to the other ones in control condition (ca. -1.15 MPa). It was lowered in water stressed plants, to similar values in all varieties. Decrease in concentration of total chlorophylls in leaf tissues upon water stress occurred for values in Chetoui and Neyer) and treatments, with a large all varieties and the magnitude of this effect differed significantly between them (p = 0.05, Table 3). In stressed plants of Chetoui, Amdoun and Neyer the chlorophyll content per g leaf FW was only 10 to 20% that of control plants, but this value was maintained at ca. 30 - 50% in Beja and Kesseb. Finally, the electrolyte leakage from

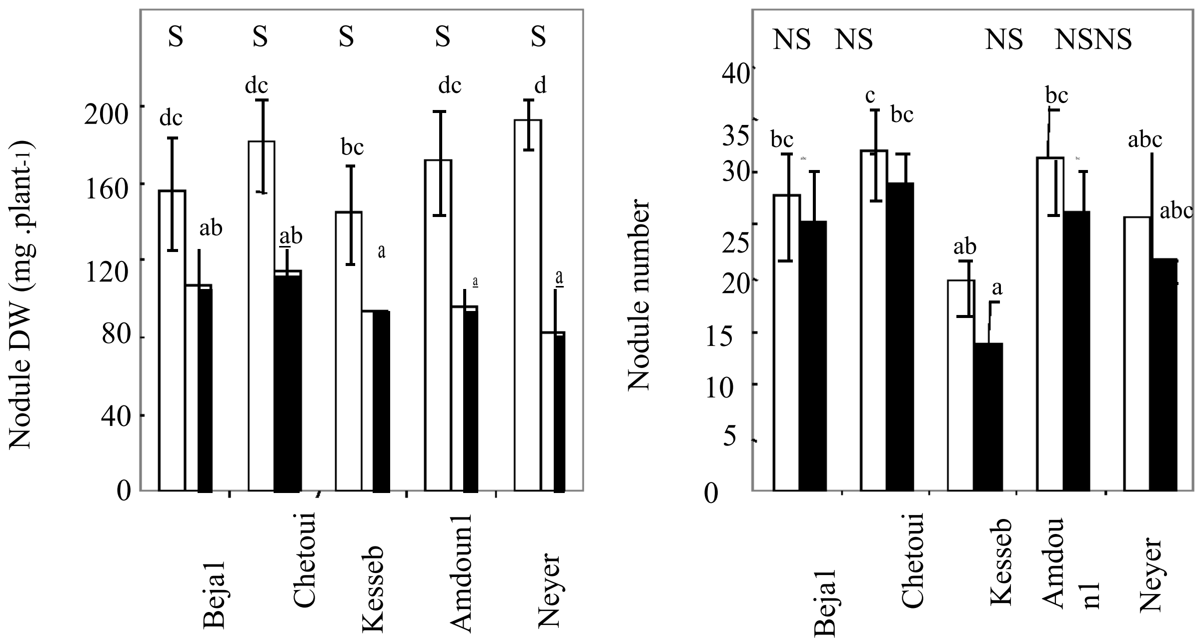


**Figure 3.** Effect of water stress on nodule development. Empty nodules were excluded. Left panel: total biomass of nodules. Right panel: number of nodules per plant. Five chickpea lines are compared. Means ± SE (n = 6). Differences among chickpea lines were analyzed using ANOVA. Different letters indicate significant difference ($p = 0.05$). S, NS: significant, respectively non significant mean difference within each variety, according to Student t test at $p = 0.05$. Open bars: control. Full bars: water stress.

**Table 3.** Leaf characteristics of five chickpea varieties and effect of water stress. The plants were cultivated for 14 days either in control condition or at 33% field capacity. Means ± standard errors ($n = 6$). For each parameter different letters indicate significant differences ($p < 0.05$).

| | | Varieties | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Treatments** | **Beja** | **Chetoui** | **Kesseb** | **Amdou** | **Neyer** |
| Leaf number | control | 50.3 ± 15c | 41.0 ± 1.1abc | 46.7 ± 17bc | 46.7 ± 15bc | 43.3 ± 8abc |
| (plant$^{-1}$) | water stress | 41.0 ± 13abc | 25,0 ± 2,3a | 31.0 ± 14ab | 37.7± 12abc | 30.3 ± 14ab |
| Total chlorophylls | control | 680± 170c | 1483 ± 95f | 1080 ± 192e | 910 ± 70d | 838 ± 218cd |
| (µg.g$^{-1}$ leaf FW) | water stress | 447 ± 82b | 176 ± 44a | 504 ± 40b | 212 ± 9a | 141 ± 22a |
| Leaf surfacic mass | control | (41 ± 8) 10$^{-4}$abc | (59 ± 21) 10$^{-4}$ed | (32 ± 1) 10$^{-4}$ab | (34 ± 5) 10$^{-4}$abc | (29 ± 14) 10$^{-4}$a |
| (g.cm$^{-2}$) | water stress | (51 ± 10) 10$^{-4}$cd | (76 ± 9) 10$^{-4}$e | (38 ± 4) 10$^{-4}$abc | (47 ± 5) 10$^{-4}$bcd | (51 ± 21) 10$^{-4}$cd |
| Electrolyte | control | 40± 12b | 20 ± 12a | 28 ± 8ab | 20 ± 9a | 24 ± 6a |
| leakage (%) | water stress | 39± 11b | 20 ± 2a | 30 ± 12ab | 38± 12b | 26 ± 7ab |
| Relative water | control | 85.7± 5,0ab | 80.6 ± 8,4ab | 74.3 ± 5.9a | 76.2± 7,3ab | 84.0 ± 5,0ab |
| content (RWC, %) | water stress | 87.4 ± 12,4ab | 83.3 ± 15,1ab | 75.2 ± 11.7a | 74.0 ± 8,7a | 90.1 ± 4,0b |
| Leaf water | control | -1.15 ± 0.00b | -1.13 ± 0.13b | -0.42 ± 0.21a | -1.17 ± 0.03b | -1.15 ± 0.06b |
| potential (%) | water stress | -1.55 ± 0.00cd | -1.67 ± 0.13d | -1.53 ± 0.07cd | -1.43± 0.07c | -1.43 ± 0.03c |

leaf tissues differed significantly between the varieties (Table 1) that of Beja being twice that of Chetoui and 1.6 fold that of Neyer. However, significant effect of water stress could be detected only in Amdoun (p = 0.05, Table 3).

## DISCUSSION

The results show that water deficit significantly limited plant growth (Amdoun and Neyer) and N fixation (Chetoui, Amdoun and Neyer). Inter-varietal variability was observed for plant biomass in control condition, but not under water stress. Probably, this constraint generated some limiting factor for plant growth. Considering the change in whole plant biomass due to water stress leads to distinguish 2 sets of varieties, namely Beja, Chetoui and Kesseb in which there was no significant response and Neyer and Amdoun in which growth was inhibited.

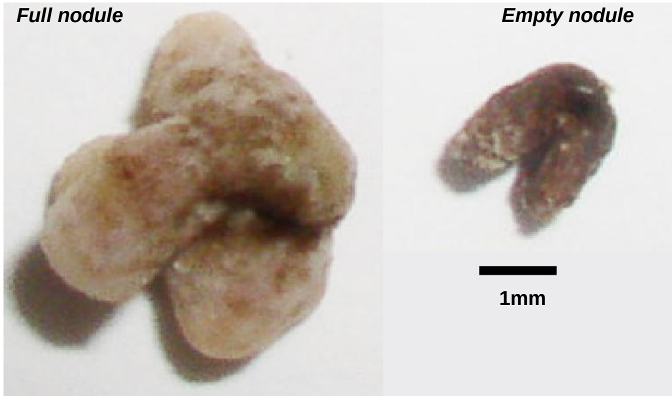


**Figure 4.** Nodule morphology. Left: full nodule; right: empty nodule. These examples are for Chetoui. The same morphology was observed for the other studied varieties.

However, since the latter plants were the taller in control condition, their biomass under stress was reduced to the same value as that of the other varieties. Differences between varieties appeared for plant development under stress, evidenced as an increase in root to shoot ratio specifically in Beja. Such drought (Shao et al., 2008) and to mineral deficiency, for instance N shortage (Hessini et al., 2009). Drought significantly limited the N content of the whole plants of Cheoui, Amdoun and Neyer, suggesing that N provision for plant growth was limited in these varieties. Since nodule number was poorly dependent on water stress, the putative N shortage of stressed plants would result from nodule functioning rather than nodule initiation. This hypothesis is likely because drought strongly decreased total nodule biomass. This phenomenon could result from adverse effect of drought on individual nodule growth, and from nodule abortion, as indicated by the significant effect of stress on the proportion of empty, dark nodules. Nodule blackening and/or emptying are indicators of degeneration (Gross et al., 2002). The black colour is due presumably to the accumulation of a dark-staining material within the cortex cells (Ramos et al., 2003). It has been reported that leghaemoglobin content declined in dehydrated nodules subjected to severe drought (Figueiredo et al., 2008). In our investigation, this parameter decreased in Amdoun and Neyer, the only varieties which showed a decrease in the nitrogen fixation per unit mass of non-empty nodule. The reduction of nodule leghaemoglobin content can be attributed to early nodules degeneration related probably to the production of $O_2^-$ radicals (Mhadhbi et al., 2009). However, reports on the relationship between symbiotic nitrogen fixation and nodule leghaemoglobin content are controversial (Irigoyen et al., 1992; Gonzalez et al., 2001).

According to Ashraf and Iram (2005) drought do not seem to influence the colonization of roots by rhizobia but it suppresses the growth of nodules. The high sensitivity of chickpea nodule development as compared to other plant parts suggests that water deficit specifically affected nodule development. Inhibition of nodule development in stressed plants has been suggested to be due to restriction of carbohydrate transport from leaves to nodule (Singh and Singh, 2006). Leaf water relations were not dramatically affected by water stress. Their water potential was lowered, and their thickness was augmented, 2 classical mechanisms for avoidance of tissue desiccation. Indeed, their relative water content was maintained. The cell membrane integrity was preserved as indicated by the insensitivity of electrolyte leakage to water stress. However, leaf chlorophyll content was significantly lower in stressed plants. These observations suggest that limitation of the whole plant photosynthetic capacity rather than hydro unbalance might have limited assimilate provision to nodules.

In conclusion, this work permitted to purpose several indices to predict relative tolerance to drought of chickpea varieties. In view of the minimal decrease in plant biomass and N content, Beja and Kesseb were the most tolerant varieties. Beja was predicted as the most tolerant variety when the increase in root to shoot ratio was used as an indicator of tolerance. Minimal chlorophyll loss and minimal nodule mortality predicted Beja and Kesseb as the most tolerant varieties. However, these 2 varieties do not present the higher performance in the absence of stress. Amdoun which presented the higher biomass per plant in control condition and ranked similarly to all other varieties in stress condition might represent a reasonable trade-off between high growth and stress tolerance when the probability of water stress to occur during the culture period is low. Of course, the agronomic value of these predictions should be evaluated from seed yield rather than vegetative growth, in realistic agricultural condition.

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