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Early tests on tolerant kabuli chickpea (Cicer arietinum L.) genotypes selection for drought stress

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In order to determine early selection parameters for drought stress tolerance, an experiment was carried out, in situ, in pots under controlled climatic conditions. Drought stress tolerance of eight “kabuli” chickpea type accessions (Béja1, Amdoun 1, Nayer, Kasseb, Bochra, FLIP96-114C, FLIP88-42C and ILC3279) was evaluated with four amounts of irrigation: 100, 75, 50 and 25% of the water reserve easily usable (WREU). The assessment of the drought stress intensity on the chickpea genotypes was based on four parameters namely: the relative water content, the foliar index, the chlorophylls (a and b) contents and the chlorophyll fluorescence parameters. The first three parameters require destructive vegetable material techniques and various handling which can bring about many errors. On the other hand, the chlorophyll fluorescence parameters have the advantage of being non-destructive, direct reading, reliable and rapid. The results analysis showed that the drought stress has negatively affected all the studied parameters. The chickpea genotypes had a broad genotypic variability toward the drought stress and various physiological and chlorophyll fluorescence answers. The identification of the drought stress tolerant genotypes appears complicated and uncertain. The drought tolerance index showed that genotypes: ILC3279, Béja1 and Nayer are the most tolerant; whereas FLIP96-114C, FLIP88-42C and Kasseb are the most sensitive.

Key words: Chickpea (Cicer arietinum L), early tests, selection, drought tolerance.

INTRODUCTION

Through the world, chickpea (Cicer arietinum L.) is the second important seed legume (Shepherd, 2007). In the Mediterranean basin, where this species is traditionally conducted as a spring culture (Singh, 1997), high temperatures, in particular those of the beginning of summer expose winter and spring chickpea cultures to the thermal and drought stresses. These abiotic stresses are caused by the terminal drought and are worsened by the precipitations scarcity and the exhaustion of the water stock in the ground (Shepherd and Turner, 2007). The spring sowing allows this species to avoid the winter freezing and cold and the proliferation of certain diseases as the anthracnose (Ascochyta rabiei) at the youthful plant stage (Walker, 1996). However, the exposure of this culture to the final drought shortens its farming cycle and delays its flowering. It reduces the dries matter production (Hughes et al., 1987), the water use efficiency (Brown et al., 1989), the plant height and the grain yield (Singh et al., 1997).

In Tunisia, chickpea culture is generally localized at the sub humid zones, and occupies the third place in terms of the sowing area after faba bean and pea (DGPA, 2008) with a surface of nearly 10 000 ha. Although, winter chickpea is more productive (Ben et al., 1999), spring chickpea is more cultivated and accounts 60% of the sowing surfaces in this species. In our regions, two types of drought stress affect the chickpea culture. One is

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Abbreviations: DAS, Days after sowing; FM, fresh matter; WREU, water reserve easily usable; RWC, relative water continent; IF, index foliar; Chl(a), chlorophyll a content; Chl(b), chlorophyll b content; Chl(a)/Chl(b), plant photosynthetic system effectiveness; Chl(a) + Chl(b), total chlorophyll a content; F0, initial fluorescence; Fv, variable fluorescence; Fv/Fm, PS II maximum quantum yield; Fv/F0, PSII photochemical effectiveness or PSII maximum primary photochemical yield; DSTI, drought stress tolerance index.
intermittent and caused by the rupture of precipitations and the other is final and occurs during flowering and filling seeds phases. The most serious damage generated by the drought stress appears on chickpea cultivated in the semi-arid zones. The grain yield is very slight and never exceeds 700 kg ha\(^{-1}\) (Aouani et al., 2001). The national production does not meet the internal needs and annual imports were nearly 19,000 t (Skrypetz, 2004).

To increase production and improve the productivity of the chickpea culture, it would be useful to act on two levels. The first one would be based on the extension of this species culture to the semi-arid zones which cover two thirds of the Tunisian territory (MEAT, 2001). In addition, in Morocco, the chickpea culture has gradually extended from semi-arid to the arid regions where the edapho-climatic conditions such as salinity, pH and temperatures have harmful effects on the establishment of this culture (Mâatallah et al., 2002). The second alternative would be to select drought stress tolerant varieties. Early tests like relative water content, foliar index, chlorophylls (a and b) content and chlorophyll fluorescence can be adopted to evaluate the drought stress intensity and screen the tolerant genotypes.

Mefti et al. (2001) indicated that the relative water continent (RWC), often considered as an excellent indicator of the water state plant, is directly related to the cells volume and can reflect the balance between leaves water provisioning and perspiration rate. Under drought conditions, RWC is strongly attenuated by the increase in the water deficit and the plant age (Moinuddin and Khanna-Chopra, 2004).

Watson (1947) defined the foliar index (FI) as being an adimensional variable, which translates the ratio of the total unilateral surface of the photosynthetic tissue by the ground unit area occupied by the considered plants. Hadri et al. (2005) indicated that the determination of this agronomic parameter is necessary in many agronomic research fields and especially in the quantification of the water-requirement cultures. The foliar index is influenced by the vegetable material (Sheldrake and Saxena, 1979), the culture growth stage (Gate, 1995) and climatic conditions and particularly precipitations (Nogueira et al., 1994).

During the seeds filling phase, high chlorophylls (a and b) content is required and contributes to the grain yield elaboration (Blum, 1988). Drought stress negatively affects the chlorophylls tissue content (Rong-hual, et al., 2006) and the plant photosynthetic system effectiveness (Chl(a)/Chl(b)) (Kathiresan and Kannan, 1985; Garg et al., 1998). Low chlorophylls (a and b) content indicates an inhibition of the photosynthetic apparatus and a reduction of the carbohydrates favorable for the magnification of seeds and the filling pods (Farquhar et al., 1989).

According to Percival and Sheriffs (2002), during screening of drought stress tolerant genotypes, on detached leaves in vitro culture, chlorophylls fluorescence fluorescence permits to evaluate the state of the foliar tissues integrity and the components of the photosynthetic apparatus, particularly, the thylacoidienne membrane. It provides a fast and precise technique to detect and measure the plants tolerance. In vivo, it allows early detection of the drought stress even before appearance of the tissues degradation physical signs (Meinander et al., 1996).

According to Meinander et al. (1996), in situ culture, quantum yield (Fv/F0) permits to measure the plant strength and can be used like early diagnostic test. Whereas the PSII photochemical effectiveness (Fv/F0) or PSII maximum primary photochemical yield is regarded as a good indicator of the PSII activity and its photochemical effectiveness for drought stress tolerance (Govindjee et al., 1981; Dekkaki et al., 2000). It enables us to evaluate the plant photosynthetic capacity (Meinander et al., 1996) and to characterize the osmotic sensitivity induced by sodium chloride or polyethylene glycol (Bounaqba, 1998).

The chlorophylls fluorescence parameters, such as fluorescence initial, maximum and variable, quantum yield and the PSII photochemical effectiveness are affected by the abiotic stresses. In response to drought, some of these parameters are reduced and photosynthetic apparatus processes are highlighted (Percival and Sheriffs, 2002). Initial fluorescence (F0) is slight in the absence of drought stress (Spalling et al., 1983), thermic stress (Havaux, 1992) and osmotic stress (Bounaqba, 1998) and increases in case of stresses. Maximum fluorescence (Fm), quantum yield (Fv/Fm) and the PSII photochemical effectiveness (Fv/F0) are high in the absence of stress and decrease whenever there is stress persists. Under optimal environmental conditions, quantum yield is about 0.8. In drought stress conditions, it shows a reduction (Eyletters and Bourrié, 1986; Percival, and Sheriffs, 2002). Bounaqba (1998) underlined that, at the sensitive plants, yellowing leaves, generated by abiotic stresses, represented an increasingly electrons transfer blocking intensity, structure deterioration and a reduction of the PSII primary active reactional centers.

MATERIALS AND METHODS

Experimental site

The trial was carried out in High Agronomic Institute of Chott Mariem, experimental field which is located in the eastern center of Tunisia which belongs to the semi arid Superior bioclimatic stage (altitude: 6 m above the sea; latitude: 35°52 North; longitude: 10°38' Est).

Edapho-climatic conditions of the experimental site

The ground, is silt-clay-sandy type (USDA, 1951), alkaline, with relatively low organic matter content and low salinity. It is characterized by a total porosity 52.45%, a field capacity 20.47%
Table 1. List of used kabuli chickpea genotypes.

<table>
<thead>
<tr>
<th>No</th>
<th>Genotype</th>
<th>Pedigree</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beja1</td>
<td>INRAT 93-1</td>
<td>Tunisian</td>
</tr>
<tr>
<td>2</td>
<td>Amdoun1</td>
<td>Be-se-81-48</td>
<td>Tunisian</td>
</tr>
<tr>
<td>3</td>
<td>Nayer</td>
<td>FLIP 84 - 92 C</td>
<td>Tunisian</td>
</tr>
<tr>
<td>4</td>
<td>Kasseb</td>
<td>FLIP 84 - 460 C</td>
<td>Tunisian</td>
</tr>
<tr>
<td>5</td>
<td>Bochra</td>
<td>INRAT 87 ou FLIP 84 - 79 C</td>
<td>Tunisian</td>
</tr>
<tr>
<td>6</td>
<td>FLIP69114C</td>
<td>X93 TH 74/FLIP87-51CFLIP91-125C</td>
<td>ICARDA/ICRISAT</td>
</tr>
<tr>
<td>7</td>
<td>FLIP88-42C</td>
<td>X85 TH 230/ILC 3395 x FLIP 83-13C</td>
<td>ICARDA/ICRISAT</td>
</tr>
<tr>
<td>8</td>
<td>Chetoui</td>
<td>ILC3279</td>
<td>ICARDA/ICRISAT</td>
</tr>
</tbody>
</table>

and permanent wilting point 8.19%. This zone is characterized by a drought which covers five months (May to September). Averages annual rainfall and evaporation are respectively 370 and 1320 mm/an. The minimal and maximum temperatures have the respective mean values 14.3 and 23°C. The relative hygrometry and wind speed are respectively 70% and 2.3 m.s⁻¹. During the trial, temperature and relative hygrometry variations are followed by a thermohygrographe beforehand calibrated.

Experimental design and sowing

The trial is carried out, with eight "kabuli "chickpea genotypes and four amounts of irrigation: 100, 75, 50 and 25% of reserve easily usable (WREU), according to a randomized block experimental design with three replications. Sowing is carried out, in situ, April 16, 2008, with four delayed weeks compared to normal spring sowing date (Malhotra and Johansen, 1996), in pots (24 cm of diameter and 24 cm height) filled with arable land and laid out under a hemispherical greenhouse covered with polyethylene of 180 μ thickness and aired the two sides.

Vegetable material

The vegetable material is composed of eight "kabuli " chickpea genotypes of which are commercial varieties registered by the INRAT in the Tunisian catalogue of obtaining vegetable (Béja1, Amdoun1, Nayer, Kasseb, Bochra and Chetoui (ILC3279)) and two improved lines, FLIP96-114C and FLIP88-42C, pleasantly provided by the ICARDA in the framework of "Legume International Testing Program (LITP)" Alep; Syria (Table 1).

Irrigation

The irrigation water, coming from Nebhana dam, is characterized by an electric conductivity, measured at 25°C, evaluated at 1.09 ms/cm². It contains a dry residue of 0.70 g/l including 0.25 g/L sodium chlorides. The WREU, evaluated at 464 ml, is calculated according to the principle cited by Soltner (1981);

\[ RFU = \frac{1}{2} \left[ \left( \frac{Cc - pF}{Chl(a)/Chl(b)} \right) \right] * D_{ap} * V \]

With Cc: Field capacity; pF: Permanent wilting point; D_{ap}: Apparent density; V: ground pot volume. The culture potential evapotranspiration (ETc) was determined by the relation:

\[ ETc = Kc*ETO \]

The reference evapotranspiration (ETO) was calculated starting from the formula of Blanney-Criddel (Doorenbos and Pruitt, 1977). The farming coefficient (Kc) and the durations of the physiological phases of adopted chickpeas are those used by FAO (Allen et al., 1998).

Studied parameters

The studied parameters are:

1) The relative water contents (RWC, %) are determined by Barrs and Weatherley method (1962).
2) The foliar index (Ff) is measured at the end of the vegetative growth phase, namely 45 days after sowing (DAS). Samples of each chickpea seedling have been taken according to genotype, water treatment and block. At the laboratory, leaves composed of leaflets and rachis, were cut down. The leaf area was determined, in cm², using apparatus “AREA METER” (Model LI - 3100, S.R. NO LAM 653 LI GOR THE USA). The foliar index corresponds to the ratio of the leaf area by the surface occupied by seedling which corresponds to the pot section (Heller et al., 1993).
3) The chlorophylls a and b content: Chlorophylls a and b are extracted according to the method cited by Bounaqba (1998). Chlorophyll a (Chl(a)) and Chlorophyll b (Chl(b)) contents expressed in mg.g⁻¹ of FM, are calculated according to the principle dosage proposed by Arnon (1949).
4) The total chlorophylls content represents the sum: Chl(a) + Chl(b).
5) The effectiveness photosynthetic system translates the ratio: Chl(a)/Chl(b).
6) The chlorophyll fluorescence is measured using a portable fluorometer (Fluorescence Induction Monitor; FM 1500) which reports the following parameters automatically:

a) Initial fluorescence (F0) is the minimal value of the fluorescence when all the electrons acceptors of the photosystem II (PSII) are completely oxidized. It originates in chlorophylls which form the collecting antennas of the PSII.

b) Maximum fluorescence (Fm) is the maximum value of the fluorescence, with the same light intensity. It is obtained when all the first electrons quinones accepteuses are completely reduced.

c) Variable fluorescence (Fv = Fm - F0) translate the difference between Fm and F0.

d) The PS II maximum quantum yield translated by the ratio (Fv/Fm), indicates the effectiveness use of light for photochemical conversion.

e) The PSII photochemical effectiveness (Fv/F0) or PSII maximum primary photochemical yield.

f) The drought stress tolerance index (DSTI) as defined by Fischer et al., (1983), is calculated according to the relation;
**RESULTS AND DISCUSSION**

**Relative water contents**

The relative water contents (RWC) variance analysis showed that there were very highly significant differences (P < 0.001) among the irrigations amounts, the genotypes and their interaction (Genotype X Amount irrigation) with 4% coefficient variation (Table 2). These results indicate that the administrated amounts of irrigation have engendered differences on the plant relative water contents, genotypic variability between chickpea accessions and these genotypes responded differently toward the amounts of irrigation.

The relative water contents, proportional to the amounts of irrigation, vary from 62.4 to 74.3% (Figure 1). The mean comparison, according to the amounts of irrigation, indicated that there are three homogeneous groups which translate a RWC decreasing gradient. The drought stress causes a significant reduction of the RWC in the plants tissues. Sure enough, with the amounts of irrigation 100 and 75% of the WREU, the RWC is the highest and the most similar; whereas with the amount 25% of the WREU, it is the slightest (Figure 1). Similar results were obtained by Mefti et al. (2001) and Moinuddin and Khanna (2004). These authors announced that the drought stress caused a significant reduction of the RWC. Basu et al. (2004) noticed that at the filling pods stage, the RWC of a rainfed culture. The last group, characterized by high RWC, is composed of genotypes: FLIP88-42C, FLIP96-114C, Amdoun1 and Kasseb. The second group contains: Nayer, Bochra and ILC3279 which have slight RWC (Table 3).

The RWC varies simultaneously according to the genotypes of chickpea and the amounts of irrigation from 50 to 81.1%. The mean comparison showed that there are nine homogeneous groups which are interfered. The first group, characterized by enhanced RWC, is composed of genotypes: Béja1, Amdoun1, Kasseb, FLIP88-42C, and ILC3279 with the amount 100% of the WREU; of Béja1, Kasseb, Bochra, and ILC3279 with the amount 75% of the WREU; of Nayer and FLIP96-114C, Amdoun1 and Kasseb. The second group contains: Nayer, Bochra and ILC3279 which have slight RWC (Table 3).

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Figure 1. Relative water content of the chickpea genotypes (*cicer arietinum* L.) according to the amounts of irrigation (AI) (bars accompanied by the same letter are not significantly different; Student, Newman and Keuls test at 5% level).

Table 3. Average values comparisons of the relative water content, the foliar index, the chlorophylls content, the photosynthetic effectiveness system and of the chlorophyll fluorescence parameters chickpea genotypes.

<table>
<thead>
<tr>
<th>Amounts of irrigation</th>
<th>RWC (%)</th>
<th>FI (%)</th>
<th>Chlorophylls content (mg/gMF)</th>
<th>Ratio : Chl(a)/Chl(b)</th>
<th>Parameters of chlorophyllian fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chl(a) + Chl(b)</td>
<td>Chl(a)</td>
<td>Chl(b)</td>
</tr>
<tr>
<td>Béja I</td>
<td>72.6a</td>
<td>0.120a</td>
<td>5.95d</td>
<td>3.46c</td>
<td>2.49d</td>
</tr>
<tr>
<td>Amdoun I</td>
<td>71a</td>
<td>0.122a</td>
<td>6.09c</td>
<td>3.54d</td>
<td>2.55c</td>
</tr>
<tr>
<td>Nayer</td>
<td>67.1b</td>
<td>0.152a</td>
<td>5.57e</td>
<td>3.1d</td>
<td>2.47d</td>
</tr>
<tr>
<td>Kasseb</td>
<td>71.8a</td>
<td>0.203a</td>
<td>4.51g</td>
<td>2.36g</td>
<td>2.15f</td>
</tr>
<tr>
<td>Bochra</td>
<td>66.9b</td>
<td>0.163a</td>
<td>6.52d</td>
<td>3.77a</td>
<td>2.75b</td>
</tr>
<tr>
<td>FLIP 96-114 C</td>
<td>72.5a</td>
<td>0.096a</td>
<td>4.54g</td>
<td>2.44l</td>
<td>2.1i</td>
</tr>
<tr>
<td>FLIP 88-42 C</td>
<td>74.9a</td>
<td>0.188a</td>
<td>4.74d</td>
<td>2.50g</td>
<td>2.24e</td>
</tr>
<tr>
<td>ILC 3279</td>
<td>64.3b</td>
<td>0.186a</td>
<td>7.21a</td>
<td>3.52b</td>
<td>3.68a</td>
</tr>
</tbody>
</table>

The values of the same column followed by the same letters are not significantly different at 5% level (Student-Newman-Keuls test). The values in fat are the extreme values.; RWC: Relative water contents; FI: Foliar index; Chl(a), Chl(b) and Chl(a) + Chl(b): Chlorophylls quantities a, b and total (mg.g⁻¹ of the FM); Chl(a)/Chl(b): photosynthetic effectiveness system; F0: initial fluorescence; Fv: variable fluorescence; Fm: maximum fluorescence Fv/Fm: quantum yield; Fv/F0: chlorophyll efficiency or effectiveness of the PSII.
<table>
<thead>
<tr>
<th>AI</th>
<th>Genotypes</th>
<th>RWC</th>
<th>FI</th>
<th>Chl(a)+Chl(b)</th>
<th>Parameters of chlorophyllian fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F0</td>
</tr>
<tr>
<td>I</td>
<td>Béja I</td>
<td>77.6</td>
<td>1.88</td>
<td>7.09</td>
<td>411</td>
</tr>
<tr>
<td></td>
<td>Amdoun I</td>
<td>77.3</td>
<td>2.11</td>
<td>9.56</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>Nayer</td>
<td>70.6</td>
<td>2.64</td>
<td>8.42</td>
<td>446</td>
</tr>
<tr>
<td></td>
<td>Kasseb</td>
<td>75.3</td>
<td>3.33</td>
<td>5.24 (m)</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td>Bochra</td>
<td>70.5</td>
<td>2.74</td>
<td>9.43 (m)</td>
<td>506</td>
</tr>
<tr>
<td></td>
<td>FLIP96-114 C</td>
<td>72.0</td>
<td>1.26</td>
<td>6.39 (l)</td>
<td>574</td>
</tr>
<tr>
<td></td>
<td>FLIP88-42 C</td>
<td>77.3</td>
<td>4.08</td>
<td>5.20 (m)</td>
<td>567</td>
</tr>
<tr>
<td></td>
<td>ILC 3279</td>
<td>73.5</td>
<td>3.64</td>
<td>10.20 (m)</td>
<td>550</td>
</tr>
<tr>
<td>II</td>
<td>Béja I</td>
<td>75.4</td>
<td>1.52</td>
<td>6.71 (l)</td>
<td>577</td>
</tr>
<tr>
<td></td>
<td>Amdoun I</td>
<td>69.3</td>
<td>1.36</td>
<td>5.97 (m)</td>
<td>448</td>
</tr>
<tr>
<td></td>
<td>Nayer</td>
<td>71.9</td>
<td>2.23</td>
<td>5.49 (m)</td>
<td>433</td>
</tr>
<tr>
<td></td>
<td>Kasseb</td>
<td>73.8</td>
<td>2.45</td>
<td>4.69 (g)</td>
<td>485</td>
</tr>
<tr>
<td></td>
<td>Bochra</td>
<td>76.2</td>
<td>2.31</td>
<td>6.67 (g)</td>
<td>478</td>
</tr>
<tr>
<td></td>
<td>FLIP96-114 C</td>
<td>81.1</td>
<td>1.28</td>
<td>4.55 (m)</td>
<td>463</td>
</tr>
<tr>
<td></td>
<td>FLIP88-42 C</td>
<td>77.3</td>
<td>1.61</td>
<td>4.95 (m)</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>ILC 3279</td>
<td>70.5</td>
<td>1.69</td>
<td>6.46 (g)</td>
<td>467</td>
</tr>
<tr>
<td>III</td>
<td>Béja I</td>
<td>75.2</td>
<td>0.87</td>
<td>5.33 (d)</td>
<td>437</td>
</tr>
<tr>
<td></td>
<td>Amdoun I</td>
<td>74.7</td>
<td>1.82</td>
<td>5.82 (d)</td>
<td>344</td>
</tr>
<tr>
<td></td>
<td>Nayer</td>
<td>75.9</td>
<td>1.35</td>
<td>4.52 (d)</td>
<td>453</td>
</tr>
<tr>
<td></td>
<td>Kasseb</td>
<td>72.6</td>
<td>1.55</td>
<td>4.69 (d)</td>
<td>457</td>
</tr>
<tr>
<td></td>
<td>Bochra</td>
<td>68.2</td>
<td>0.74</td>
<td>5.14 (m)</td>
<td>393</td>
</tr>
<tr>
<td></td>
<td>FLIP96-114 C</td>
<td>68.3</td>
<td>1.29</td>
<td>4.60 (d)</td>
<td>505</td>
</tr>
<tr>
<td></td>
<td>FLIP88-42 C</td>
<td>75.9</td>
<td>1.25</td>
<td>4.83 (d)</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>ILC 3279</td>
<td>54.2</td>
<td>0.99</td>
<td>6.25 (l)</td>
<td>386</td>
</tr>
<tr>
<td>IV</td>
<td>Béja I</td>
<td>62.2</td>
<td>0.55</td>
<td>4.77 (d)</td>
<td>526</td>
</tr>
<tr>
<td></td>
<td>Amdoun I</td>
<td>66.7</td>
<td>0.81</td>
<td>4.21 (d)</td>
<td>445</td>
</tr>
<tr>
<td></td>
<td>Nayer</td>
<td>50.0</td>
<td>0.23</td>
<td>3.85 (d)</td>
<td>416</td>
</tr>
<tr>
<td></td>
<td>Kasseb</td>
<td>65.0</td>
<td>0.81</td>
<td>3.79 (d)</td>
<td>482</td>
</tr>
<tr>
<td></td>
<td>Bochra</td>
<td>59.9</td>
<td>0.86</td>
<td>4.92 (d)</td>
<td>419</td>
</tr>
<tr>
<td></td>
<td>FLIP96-114 C</td>
<td>68.2</td>
<td>1.54</td>
<td>3.20 (d)</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>FLIP88-42 C</td>
<td>68.2</td>
<td>0.72</td>
<td>4.27 (d)</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>ILC 3279</td>
<td>59.4</td>
<td>1.16</td>
<td>5.91 (l)</td>
<td>443</td>
</tr>
</tbody>
</table>

The values of the same column followed by the same letters are not significantly different at 5% level (Student-Newman-Keuls test). The values in fat are the extreme values. AI: Amounts irrigation (I, II, III and IV are respectively 100, 75, 50 and 25% of the WREU); RWC: Relative water contents; FI: Foliage index; Chl(a) + Chl(b): Chlorophylls total quantity ((mg g⁻¹ of the FM); F0: initial fluorescence; Fv: variable fluorescence; Fm: maximum fluorescence Fv/Fm: quantum yield; Fv/F0: chlorophyll efficiency or effectiveness of the PSII.
Table 5. Drought stress tolerance index.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>RWC</th>
<th>Chlorophylls content (mg g−1 of the FM)</th>
<th>Ratio: Chl(a)/Chl(b)</th>
<th>Parameters of chlorophyllian fluorescence</th>
<th>DSTI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chl(a)</td>
<td>Chl(b)</td>
<td>Chl(a)+Chl(b)</td>
<td>F0</td>
</tr>
<tr>
<td>ILC 3279</td>
<td>0.81</td>
<td>0.74</td>
<td>0.47</td>
<td>0.58</td>
<td>1.58</td>
</tr>
<tr>
<td>Béja I</td>
<td>0.80</td>
<td>0.63</td>
<td>0.74</td>
<td>0.67</td>
<td>0.86</td>
</tr>
<tr>
<td>Nayer</td>
<td>0.71</td>
<td>0.47</td>
<td>0.44</td>
<td>0.46</td>
<td>1.06</td>
</tr>
<tr>
<td>Bochra</td>
<td>0.84</td>
<td>0.46</td>
<td>0.65</td>
<td>0.53</td>
<td>0.70</td>
</tr>
<tr>
<td>Amdoun I</td>
<td>0.86</td>
<td>0.36</td>
<td>0.58</td>
<td>0.44</td>
<td>0.61</td>
</tr>
<tr>
<td>Kasseb</td>
<td>0.86</td>
<td>0.63</td>
<td>0.86</td>
<td>0.72</td>
<td>0.73</td>
</tr>
<tr>
<td>FLIP 88-42 C</td>
<td>0.89</td>
<td>0.56</td>
<td>1.22</td>
<td>0.82</td>
<td>0.46</td>
</tr>
<tr>
<td>FLIP 96-114 C</td>
<td>0.95</td>
<td>0.35</td>
<td>0.71</td>
<td>0.47</td>
<td>0.50</td>
</tr>
</tbody>
</table>

RWC: Relative water contents; Chl(a): chlorophyll (a); Chl(b): chlorophyll (b); F0: initial fluorescence; Fv: variable fluorescence; Fm: maximum fluorescence; Fv/Fm: quantum yield; Fv/F0: chlorophyll efficiency or effectiveness of the PSII.

The foliar index

The foliar index (FI) variance analysis showed that there is a highly significant difference (P<0.001) between the amounts of irrigation. Genotypic variability and the interaction (genotype × amounts of irrigation) are not significant. The coefficient variation is 51.3% (Table 2).

The foliar index is proportional to the amounts of irrigation and varies from 0.84 to 2.73. The mean comparison showed that there are three homogeneous groups. The highest foliar index is recorded with the amount 100% of the WREU; whereas the feeble indices are recorded on the amounts 25 and 50% of the WREU with similar indices varying from 0.096 to 0.203 (Table 3). These foliar index low values combined with the lack of genotypic variability indicate that these climatic factors. Gate (1995) announced that the water quantity transpired by the plant is a function of the foliar index which, itself, depends on the seedling development stage and its growth state. With the drought stress pressure increase, the foliar index as well as the water use efficiency will be reduced. During the vegetative period, it would be useful to decrease the demand for water in order to preserve part of the useful reserve in the ground for the subsequent phases, of strong water needs, namely flowering and grain filling. Amigues et al. (2006) recommended rationing of the culture vegetative development, which consists in limiting the development of the leaf area and thus transpiration. On the other hand this strategy is in competition with another, which consists in seeking a fast development of vegetable cover to reduce the ground evaporation and to control the adventitious, and thus to favor an early growth and a strong foliar index.

The chickpea genotypes showed similar foliar indices varying from 0.096 to 0.203 (Table 3). These foliar index low values combined with the lack of genotypic variability indicate that these
genotypes manifested the lack of water by the reduction of their leaf areas. In fact, Amigues et al. (2006) announced that the genotypes with very early flowering and/or maturity, generally, less water demanding and equipped with short biological cycles, can avoid the final drought by ensuring a fast cover of the ground and by presenting a feeble foliar index.

On the interaction (Genotype X Amounts of irrigation), the foliar index varies from 0.23 to 4.08 (Table 4). Although the difference is not significant, the mean comparison made it possible to detect four homogeneous groups which are interfered. The highest foliar index is recorded at FLIP88-42C with the irrigation amount 100% of the WREU; whereas the lowest index is recorded at Nayer with the amount 25% of the WREU. The first group, characterized by high FI, is composed of Béja1, Amdoun1, Nayer, Kasseb, Bochra, FLIP88-42C and ILC3279 with the irrigation amount 100% of the WREU and Nayer, Kasseb, Bochra with the amount 75% of the WREU. The last group, characterized by the lowest FI, is composed of the whole of the genotypes under the various amounts of irrigation except for FLIP88-42C, Kasseb and ILC3279 with the amount 100% of the WREU.

With the irrigation amount 25% of the WREU, the genotypes ILC3279, FLIP96-114C and Bochra presented the highest foliar indices; whereas FLIP88-42C, Béja1 and Nayer presented the lowest indices (Table 4). It seems that the genotypes ILC3279, FLIP96-114C and Bochra have shown more tolerance with the lack of water than the remaining genotypes.

Chlorophylls content

The variance analysis of the total chlorophylls content (Chl (a)+Chl (b)), chlorophyll a (Chl (a)), chlorophyll b (Chl(b)) and of the photosynthetic effectiveness (Chl (a)/Chl (b)) have shown that there is a highly significant difference between the amounts of irrigation, the genotypes and their interactions. The coefficients variation vary from 1.1 to 3.9% (Table 2).

The total chlorophylls content, Chl (a), Chl(b) and the photosynthetic effectiveness are proportional to the amounts of irrigation (Figure 3a, b and c). Water deficit has a significant negative effect on the plant contents of Chl(a), Chl (b) and on the photosynthetic effectiveness (Estill et al., 1991; Ashraf et al., 1994; Garg et al., 1998). Heller et al. (1996) reported that there are narrow correlations between the content chlorophylls and the intensity of the photosynthetic activity.

The means comparison of the chlorophylls contents showed that there are four homogeneous groups. The highest contents are recorded on unstressed witness; whereas the lowest contents are recorded on the amount 25% of the WREU (Figure 3a and b). These results indicate that the amounts of irrigation lower than the culture water requirement have negative effects on the concentration of the chlorophylls chickpea genotypes. Belabed et al. (1997) announced that a reduction in the content chlorophylls was observed at water stressed durum wheat seedlings. According to Impens (1989) cited by Bettaieb et al. (2008), under favorable growth conditions, in a healthy chlorophyll cells, new chlorophyll
molecules are synthesized whereas other molecules degenerate. However, under stressed conditions, this balance is disarranged and chlorophylls are destroyed as soon as they are elaborated. The synthesis of the chlorophyll pigments is thus reduced. Nevertheless, other research tasks showed that the chlorophylls concentration increases in response to a water deficit (Poorter and Evans, 1998). Although photosynthesis is simultaneously reduced with the reduction of the amounts of irrigation, the chlorophylls concentration in the leaves is clearly increased. With low amounts of irrigation the chlorophylls concentration in the leaves is considerably higher than with amounts of irrigation superior to 100% of ETc (Bhattarai, 2005).

With the various amounts of irrigation, the content of Chl(a) is higher than that of Chl(b) (Figure 3b). These results conform to those of Villarepos (2000) which noticed that at the vegetable species, Chl (a) mean concentration is higher than that of Chl(b). Generally, at the prochlorophyts, in particular, algae and green plants, the Chl(b) tissue content represents the third of that Chl(a) (Folly, 2000).

The chickpea accessions showed a genotypic variability for the total chlorophylls content, Chl(a), Chl(b) and the photosynthetic system effectiveness. The genotypes ILC3279, Bochra and Amdoun1 are richest in Chl(a), Chl(b) and total chlorophylls. On the other hand FLIP88-42C, FLIP96-114C and Kasseb are poorest (Table 3). The photosynthetic system effectiveness of the chickpea genotypes varies from 1.03 to 1.4. It is the highest at Béja1, Amdoun1 and Bochra and lowers at FLIP88-42C, Kasseb and ILC3279 (Table 3).

The total chlorophylls mean content varies jointly according to the amounts of irrigation and the chickpea genotypes from 3.02 to 10.2 mg.g\(^{-1}\) of the FM. The mean comparison revealed twenty homogeneous groups. The first group, composed of genotype ILC3279, represents the highest total chlorophylls content with the amount of irrigation 100% of the WREU. The last group is composed of the genotype FLIP96-114C which contains the lowest total chlorophylls content with the amount of irrigation 25% of the WREU (Table 4).

Under the stressed treatment, 25% of the WREU, the genotypes: ILC3279, Bochra and Béja1 kept the highest total chlorophylls concentrations. On the other hand Nayer, Kasseb and FLIP96-114C presented the lowest concentrations (Table 4). It seems that the chlorophylls content is a genetically controlled character revealing rich chlorophylls genotypes and other poor chlorophylls. In a vegetable tissue, the chlorophylls quantity is largely surplus and a significant reduction in this substance content appears only after one severe drought stress. In
case of prolonged water stress, the majority of the plants adapt to the environment conditions by the reduction of their leaf areas while keeping higher chlorophylls concentrations (Heller et al., 1996; Kotchi, 2004).

Chlorophyll fluorescence

The variance analysis of the chlorophyll fluorescence parameters (F0, Fm, Fv, Fv/Fm and Fv/F0) showed that there are highly significant differences between amounts of irrigation, the chickpea genotypes and the interaction (Genotype X amounts of irrigation). The coefficients of variation vary from 1.2 to 5.7% (Table 2).

Initial fluorescence (F0) varies from 372 to 502. It is proportional to the amounts of irrigation (Figure 4a). The F0 means comparison showed that there are four distinct homogeneous groups relating to the four amounts from irrigation. Similar results are obtained by Dekkaki et al. (2000) on "Karim" durum wheat variety. They noticed that initial fluorescence decreases under the effect of the water stress. They attribute this reduction to the senescence of the vegetable material and the low chlorophylls (a and b) content. Other explanations stipulate that the reduction in F0 could reflect damage of the external processes of regulation of the PSII reactional center and the weakening of the photoprotectors processes which facilitate the dissipation of leaves excessive energy (Angelopoulous et al., 1996; Hong and Xu, 1999). On the other hand, Havaux (1992) noted that seedlings leaves of durum wheat, tomato, potato, barley and of triticale irrigated had an initial fluorescence lower than those of the stressed seedlings. He attributed this phenomenon to the action of the water stress which caused a deterioration of the PSII primary reaction of the structure centers and generated an increase in F0. Armond et al. (1980) noticed that, under abiotic stress conditions, the increase in F0, observed at several vegetable species, could be the result of a physical dissociation of the PSII luminescent complex core. Havaux (1993) interpreted the increase in F0 as being a constant reduction of the trapping rate energy by the PSII reactional centers. Whipped et al. (1995) announced that the increase in F0 at the stressed seedlings represented the reduction of the electrons capture faculty and the transfer of the energy to the reactional centers and a beginning of denaturation of the primary photosynthesis acceptors. Other comparable results, obtained by Eyletters and Bourrié (1986), Percival and Sheriffs (2002), and Bounaqba (1998), noted that with low dose of irrigation, the seedlings submitted to water stress which generated a decrease of the chlorophylls content in the seedlings tissue and an increase in the initial fluorescence. Percival and Sheriffs (2002) concluded that the increase or the reduction in F0 indicates that the photosynthetic apparatus is damaged.

Fm and Fv parameters vary respectively from 2154 to 1510 and 1652 to 1138. They are proportional to the amounts of irrigation (Figure 4b and c). Similar results were obtained by Havaux (1992), Bounaqba (1998), Royo et al. (2000) and Dekkaki et al. (2000). The comparison of Fm and Fv means showed that there are
four distinct homogeneous groups relating to the four amounts of irrigation. Bounaqba, (1998) observed a stability of Fm at wheat and triticale green leaves which are in photosynthetic activity. The chlorophylls losses and the leaves yellowing result in Fm reduction.

Quantum yield (Fv/Fm) is proportional to the amounts of irrigation and varies from 0.681 to 0.765 (Figure 4d). Govindjee et al. (1981) indicated that quantum yield is, generally, used to appreciate the light use effectiveness by the PSII for photochemical conversion. Berry and Bjorkman (1980), noticed, that in case of drought stress, quantum yield decreases. They attributed this reduction to a slowing down of the primary photochemical reactions localized in the membrane system of the thylacoids and implied in the PSII inhibition. According to Bounaqba (1998), the chlorophylls losses in the leaves which present a beginning of yellowing translated in the chlorophylls fluorescence kinetic by a reduction in the PSII quantum yield (Fv/Fm). Eyletters and Bourrié (1986) stated that Fv/Fm is about 0.8 for the healthy plants and decreases in case of stress. Havaux (1992) showed that the water stress has little effect on the PSII photosynthetic activity and that quantum yield is negatively affected only if the dehydration of tissues is high and the RWC is lower than 45%. According to Bounaqba, (1998), the plants developed under severe climatic conditions appear water stress tolerant and maintained a quantum yield near to 0.8. Flexas et al. (2002) indicated that, under drought stress conditions, the quantum yield of the young vines is about 0.8. Zanella et al. (2004) found that Fv/Fm of a bean culture is not affected by the water stress and varies from 0.78 to 0.81.

PSII photochemical effectiveness (Fv/F0), considered as a good indicator of the PSII activity (Govindjee et al., 1981), varies from 2.84 to 3.55. It is the highest with the amount of irrigation 50% of the WREU and the lowest with the amount 25% of the WREU. With the amounts of irrigation 100 and 75% of the WREU, it has intermediate and similar values compared to the two other values (Figure 4e). Havaux (1992) announced that all the environment constraints affect negatively Fv/F0. In addition, Zanella et al. (2004) found that the photochemical efficiency varies from 3.8 to 4.3 and is not affected by the water stress. They indicated that, although photosynthesis is deteriorated by the water stress, the values of Fv/F0 showed that the photosynthetic apparatus is not damaged. Other studies showed the PSII resistance to the water deficit in leaves tissues (Nogue’s and Baker, 2000; Cornic and Fresneau, 2002). Following a study of the tolerance of winter triticale genotypes to water stress, Hura et al. (2007) did not find significant differences between the PSII photochemical effectiveness of stressed and not stressed genotypes. According to same authors, in case of dryness, the photochemical efficiency measures did not provide enough information on the PSII because a reduction in the PSII activity could be the result of the of photosynthesis inhibition. In addition, Zlatev and Yordanov (2004) noticed that this inhibition is not due only to the deterioration of the thylacoidienne membrane, which is responsible for the electrons transport and the reactions for Kelvin cycle, but also to other factors.

Abiotic stress installation at the sensitive genotypes is accompanied by chlorophylls losses in the leaves which present a yellowing beginning. On the level of the chlorophylls fluorescence kinetic, a reduction in F0, Fm, Fv and PSII quantum yield (Fv/Fm) is recorded. The reduction in Fv results in a reduction in the number of active reactional centers and electrons transfer is more and more blocked (Bettaieb et al., 2008).

On the chickpea genotypes, the initial florescence varies from 4.91 to 3.69. The means comparison showed five homogeneous groups. The first group is composed of Béja1 and Kasseeb which have high and similar F0 values. The last group is composed of FLIP88-42C which has the lowest F0 (Table 3).

Variable fluorescence varies according to the chickpea genotypes from 2155 to 1211. The means comparison showed seven homogeneous groups. The first group, relating to the highest Fv, is composed of Nayer. On the other hand, the last group is formed of FLIP96-114C which has the lowest Fv (Table 3).

Maximum fluorescence varies according to the genotypes from 2155 to 1211. The means comparison showed seven homogeneous groups. The first group is formed of Béja1 and Nayer which have high and similar Fm values. The last group is composed of FLIP96-114C with the lowest Fm (Table 3).

Quantum yield varies according to the chickpea genotypes from 0.797 to 0.487. The means comparison revealed six homogeneous groups. The first group is composed of Amdoun1, Nayer, and Bochra which have higher and similar quantum yields. The last group is composed of FLIP96-114C which has the lowest Fv/Fm (Table 3).

Chlorophyll efficiency varies according to the chickpea genotypes from 3.94 to 1.54. The means comparison revealed four homogeneous groups. The first group is composed of Amdoun1 and Nayer which have high and similar Fv/F0. The last group is composed of FLIP96-114C with the lowest chlorophyll efficiency (Table 3). Chlorophyll fluorescence parameters Fv; Fm; Fv/Fm; Fv/F0 appear the highest at the genotypes Nayer, Béja1 and ILC3279 and the lowest at FLIP 96-114C, Kasseeb and FLIP 88-42C.

Initial fluorescence varies, simultaneously, according to the chickpea genotypes and of the amounts of irrigation from 43 to 577 (Table 4). The means comparison showed that there are fourteen homogeneous groups. The first group, characterized by the highest F0, is formed of genotypes Kasseeb, FLIP96-114C, FLIP88-42C and ILC3279 with the amount of irrigation 100% of the WREU and Béja1 at the amount 75% of the WREU; whereas,
the last group, characterized by the lowest F0, is composed of the genotype FLIP88-42C with the amount of irrigation 25% of the WREU.

Fm varies simultaneously, according to the chickpea genotypes and of the amounts of irrigation from 155 to 2354 (Table 4). The means comparison showed that there are fifteen homogeneous groups. With the amounts of irrigation 100 and 25% of the WREU, the genotype Béja1, characterized by the highest maximum fluorescence, represents the first group. The last group, provided with the lowest maximum fluorescence, is represented by the genotype FLIP88-42C with the amount of irrigation 25% of the WREU.

Variable fluorescence (Fv) varies, jointly, according to the chickpea genotypes and the amounts of irrigation from 48 to 1943 (Table 4). The means comparison showed that there are twenty-one homogeneous groups of which the first, equipped with the highest Fv, is represented by Béja1 with the amount of irrigation 100% of the WREU; whereas the last group, characterized by the lowest Fv, is composed of FLIP96-114C with the amount 25% of the WREU. Drought stress installation at the sensitive genotypes was accompanied by a reduction in Fv which results in a reduction in the number of active reactional centers and the transfer of the electrons is more and more blocked.

Quantum yield varies, simultaneously, according to the chickpea genotypes and the amounts of irrigation from 0.194 to 0.825 (Table 4). The means comparison showed that there are thirteen homogeneous groups. The first group, characterized by the highest quantum yield, is represented by the genotypes: Béja1, Amdoun1 and Nayer with the amount 100% of WREU, Nayer, FLIP88-42C and ILC3279 with the amount 75% of WREU, Béja1, Amdoun1, Nayer, Kasseb, Bochra and ILC3279 with the amount 50% of the WREU, and Nayer and Bochra with the amount 25% of the WREU. Whereas the last group, is characterized by a low Fv/Fm and is represented by the genotype FLIP96-114C with the amount 25% of the WREU.

Chlorophyll efficiency or PSII photochemical effectiveness varies simultaneously, according to the chickpea genotypes and the amounts of irrigation from 0.24 to 4.7 (Table 4). The means comparison showed that there are twelve homogeneous groups. The first group, with the highest Fv/F0, is represented by Béja1 and Amdoun1 with the amount of irrigation 100% of the WREU and by Amdoun1, Bochra and ILC3279 with the amount 50% of the WREU. The last group is represented by FLIP96-114C with the amount of irrigation 25% of the WREU.

With the amount of irrigation 25% of the WREU, the genotypes ILC3279, Bochra and Nayer showed the highest quantum yields and PSII photochemical effectiveness; whereas the genotypes FLIP96-114C, Kasseb and FLIP88-42C recorded of the lowest Fv/Fm and Fv/F0 (Table 4). Berry and Bjorkman (1980) and Dekkaki et al. (2000) indicated that quantum yield and PSII photochemical effectiveness are high at the drought tolerant genotypes and low at the sensitive genotypes. Probably, the genotypes FLIP96-114C, Kasseb and FLIP88-42C submitted water stress which generated deterioration of their photosynthetic processes (Bounaqba, 1998) and disturbance of their electrons transfer apparatuses (Flexas et al., 2002). Meinander et al. (1996) announced that under water stress conditions, Fv/Fm and Fv/F0 followed similar reduction tendencies and that this reduction, generated by dehydration, can be used like criterion of selection for the drought tolerance.

Drought stress tolerance index

Under various amounts of irrigation, 100, 75, 50 and 25% of the WREU, chickpea genotypes presented variables physiological answers and chlorophyll fluorescence. These results make the identification of water stress tolerant genotypes complicated and uncertain.

Resort to drought stress tolerance indices, as defined by Fischer et al. (1983), could be a means of discrimination between studied genotypes. These indices allow direct comparison of the genotypic answers to the water stress. In fact, for each genotype, the water stress tolerance index represents the mean ratios of the parameters presenting a genotypic variability (Table 5) under amount of irrigation stressing (25% of the WREU) by those of the same parameters under non amount stressing (100% of the WREU). The low indices indicate the sensitivity; whereas, the high indices indicate the tolerance (Fischer et al., 1983).

Drought stress tolerance index varies from 0.38 to 0.95. It is the highest for the genotypes ILC3279, Béja1, Nayer and Bochra and the lowest for FLIP96-114C and FLIP88-42C (Table 5). These results testify a broad genotypic variability of this chickpea collection to water stress and indicate that ILC3279, Béja1, Nayer and Bochra are the most tolerant; whereas FLIP96-114C and FLIP88-42C are the most sensitive to water stress. They are confirmed in partly by those obtained by Slim et al. (2006) which indicated that, among the chickpea genotypes cultivated in Tunisia, Bochra and Chétoui (ILC3279) are the most tolerant water stresses; whereas Nayer and Kasseb are the most sensitive. Nevertheless, Labidi et al. (2007) announced that Kasseb, Béja1 and Chétoui are moderately sensitive to water stress. On the other hand, Amdoun1 and Nayer are extremely sensitive. Following rainfall spring chickpea culture, ILC3279 was sensitive to drought stress (Malhotra, and Johanson, 1999; Toker, 2005); whereas FLIP88-42C was tolerant (Malhotra and Johanson, 1999). These last authors concluded that FLIP88-42C represents a source of drought stress tolerance. To improve chickpea productivity in Tunisia, Slim et al. (2006) suggested deeper research on drought stress resistance mechanisms for molecular approach, through identification of one or several
resistance genes and of a biochemical approach, based on the search for proteins synthesized or inhibited at the time of abiotic stress.

Conclusions

The physiological parameters analysis, in particular, relative water contents, foliar index, chlorophylls (a), chlorophylls (b) and total chlorophylls content as well as chlorophyll fluorescence parameters revealed that the drought stress intensity is inversely proportional to the amount of irrigation and that the amount 25% of the WREU had more stressing effects on chickpea genotypes.

Genotypic variability, toward drought stress, was detected through these parameters. Nevertheless, application of relative water contents, foliar index and chlorophylls content requires destruction of the vegetable material. Moreover, it appears relatively slow and causes many handling of laboratory which can cause errors. On the other hand, the measurement of chlorophyll fluorescence has the advantage of being nondestructive, direct, easy and reliable, even in situ.

Chickpea accessions presented, according to selection parameters, variable answers of sensitivity or tolerance toward drought stress. Because of this variability, identification of drought stress tolerant genotypes proves to be complicated and dubious. Resorting to drought stress tolerance index is justified. It permits us to classify genotypes in three groups. First is formed of genotypes ILC3279, Béja1, Nayer and Bochra which appear tolerant to drought stress. Second is composed of FLIP96-114C and FLIP88-42C which seems to be the most sensitive. The third group contains the genotypes Kasseb and Amdoun1 which are fairly sensitive to water stress.

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