of sandy-textured soil with gravimetric water content at a field capacity of 8%. The soil surface was covered with a 4 cm gravel layer in order to avoid water losses by evaporation. No water restriction was applied to the plants for about 40 days after sowing. During this period, the plants were watered with Hoagland nutrient solution once a week. Subsequently, plants were randomly distributed within two groups, to which different water treatments (60 pots per treatment) were applied: (a) "Control" plants were watered to saturation every 7 days, and (b) "Water stress" was initiated by withholding water for 22 days. Soil water content, growth, water relations, glycinebetaine, and soluble sugar concentrations were evaluated 1, 8, 15 and 22 days after the treatment was initiated.

Soil water content

Soil gravimetric water content was defined as $\theta = Ww/DWs \times 100$ (where Ww is the weight of the water contained in a soil sample, and DW is the dry weight of the sample). It was measured by the method of responding to changes in apparent dielectric constant using the ThetaProbe soil moisture sensor type ML1 (Delta-T Devices Ltd., England) using the calibration procedure previously described (Martinez et al., 2003). It was measured at the same time in control and stressed plants, that is, 24 h after watering control plants to make sure that pots were at field capacity. θ was determined using 20 plants per treatment.

Plant growth and water relations

Plant growth was estimated by measuring the plant heights and leaf numbers of 20 plants every 3 days. The number of leaves was determined by recording all the leaves on the main stem with blades longer than 1 cm. Plant growth was also measured on the basis of shoot dry weight (shoot DW) per plant. Shoot DW (after 48 h in an oven at 80°C) was determined using 10 plants per treatment.

Leaf relative water content (RWC) and osmotic potential (Ψs) were measured between 12:00 and 14:00. The fully expanded leaves of five plants (four leaves per plant) were harvested on the main stem. RWC was calculated as: $RWC = [(FW-DW)/(TW-DW)] \times 100$, where FW is the fresh weight, TW is the turgid weight measured after 24 h of saturation (when leaf weight reached a plateau, Martinez, 2001) on deionised water at 4°C in the dark, and DW is the dry weight determined after 48 h in an oven at 80°C. For Ψs, leaves were quickly collected, cut into small segments, then placed in Eppendorf tubes perforated with four small holes and immediately frozen in liquid nitrogen. After being encased individually in a second intact Eppendorf tube, they were allowed to thaw for 30 min and centrifuged at 15,000*g* for 15 min at 4°C. The collected tissular sap was analysed for Ψs estimation. Osmolarity (c) was assessed with a vapour pressure osmometer (Wescor 5500) and converted from mosmoles kg^{-1} to MPa using the formula: $\Psi s\,(MPa) = -c\,(mosmoles\,kg^{-1}) \times 2.58 \times 10^{-3}$ according to the Van't Hoff equation. For the measurement of osmotic potential at full turgor (Ψs^{100}), leaves of stressed and control plants were rehydrated in deionised water for 24 h at 4°C in the dark. Total OA (OA^{tot}) was calculated as the difference of osmotic potential at full turgor between control (Ψs_c^{100}) and water stress (Ψs_s^{100}) treatments for each population (Blum, 1989; Zhang et al., 1999):

$$OA^{tot} = \Psi s_c^{100} - \Psi s_s^{100}.$$

Solute concentration resulting from changes in non-osmotic volume due to insoluble polymer accumulation at full hydration OA^{conc} was estimated using a modified formula (Girma and Krieg, 1992) from changes in TW/DW between control (c) and water stressed (s) plant leaves:

$$OA^{conc} = [(TW/DW)^c - (TW/DW)^s] / [(TW/DW)^c]OA.$$

The contribution of net solute accumulation (OA^{acc}) was calculated as:

$$OA^{acc} = OA^{tot} - OA^{conc}.$$

For determination of RWC, Ψs, OA^{tot}, OA^{acc} and OA^{conc}, 10 plants per treatment were used.

Organic solute quantification

Old and young leaves of the main stem were analysed separately on 20 plants per treatment, for both levels containing the same number of leaves (when the main stem bore an uneven number of leaves, the median leaf was attributed to old leaves). However, for all analysed compounds except starch, there was no significant impact of the leaf age on endogenous concentrations and most data presented hereafter are pooled for both levels. All samples were frozen in liquid nitrogen and stored at −80°C until analysis.

Free proline was specifically quantified according to Bates et al. (1973). For glycinebetaine,

lyophilised material (50 mg) was extracted three times with 750 µl of 1 N HCl at 4°C. After centrifugation at 12,000*g* for 20 min at 4°C, the three supernatants were combined. The extract was filtered and dried by vacuum distillation. It was then dissolved in D_2O to perform glycinebetaine determination through H^1-NMR spectroscopy according to Jones et al. (1986), and using tert-butyl alcohol as an internal reference. NMR spectroscopy measurements were carried out with a 500.13 MHz Bruker AM 500 spectrometer. Presaturation of water was performed at room temperature with a recycling time of 7.7 s (5 s relaxation delay and 2.7 s acquisition time). The integrated intensities of the methyl resonances of the two compounds were identical within the experimental error (5%). Fourier transform conditions were: 3 kHz spectral width, 16 K data points, 5 µs (45°) pulse width, 7.7 s recycle time, and 32 scans. A line broadening of 0.4 Hz was applied to the free induction decays before Fourier transformation. The solvent signal was suppressed by presaturing the H_2O resonance.

Soluble sugars were extracted in 80% ethanol from 1 g of fresh leaf tissue. After centrifugation for 10 min at 8000*g*, the pellet and the supernatant were stored up to analysis. Total soluble sugar content was determined in leaves of ten plants per treatment by the classical anthrone method (Yemm and Willis, 1954) using a spectrophotometer (Beckman DU® 640, USA). Starch content was evaluated by a modified method of McCready et al. (1950). Starch remaining in the residue from ethanol extraction was hydrolysed to glucose using 16 ml 1 N HCl and incubated in a water bath at 95°C for 2 h. After filtration (Miracloth), pH was neutralised by adding 1 N NaOH and each sample volume was adjusted to 25 ml with deionised water, and starch was then determined colorimetrically using glucose as a standard.

Since the water stress had a significant effect on the RWC of the plants (see Fig. 2), proline, glycinebetaine, and sugar contents were adjusted to the RWC of unstressed plants (Z) according to $X \times Y/Z$, where X is the solute content and Y is the RWC of the stressed plants.

Statistical analysis

Data were analysed using a three-way analysis of variance (ANOVA) at a significance level of $P \leqslant 0.05$(*) or $P \leqslant 0.01$(**). The model is defined on the basis of fixed effects and hierarchal classification criterion. Main effects were considered to be population, treatment, and time, as well as their interaction. For starch, leaf age was considered as another source of variation. When the ANOVA was significant at $P \leqslant 0.05$, Duncan multiple range test was used for mean comparison. The data were analysed by a MSTACT statistical package. The statistical analysis showed that there were no significant differences between the results of two independent experiments. Data presented hereafter are taken from one experiment.

Results

Soil water content and plant growth

During the development of water stress, soil water content (θ, %) decreased progressively and similarly in pots of the two populations ($F = 2502.1$ **). At the end of the experiments, θ of the stressed plants was about 3%, as compared to 24% in control plants.

In control conditions, the shoot elongated during the experimental period, reaching a maximum length of 29 cm in Kairouan and 34 in Tensift (Fig. 1). In water stress conditions, a significant reduction was observed in both populations ($F = 40.2$ **) (Fig. 1). It was detected earlier and was more marked in Tensift than in Kairouan. In contrast, leaf number at the end of the experimental period was similar in the two treatments for both populations, indicating that leaf production was not affected by the stress up until the time the experiment was discontinued (Fig. 1). Shoot DW increased similarly in unstressed plants of both populations (Fig. 1). This increase was slowed down by the water deficit after the 8th day of treatment, but the difference between control and stressed plants was significant only at the 22nd day ($F = 14.04$ **). Tensift was significantly less affected than Kairouan by the water stress.

Water relations

In controls, leaf RWC and Ψs remained relatively constant during the experimental period (Fig. 2). In stressed plants, RWC and Ψs declined markedly in both populations after the 8th day ($F = 36.20$ ** and $F = 30.44$ **, respectively) (Fig. 2); Ψs reduction was larger in Kairouan than in Tensif ($F = 4.14$*) at the end of the experiment (Fig. 2). TW/DW in unstressed plants decreased significantly throughout the experimental period whatever the population. However, in stressed plants, the reduction of TW/DW was more marked in Kairouan than in Tensift after 22 days of water withholding (Fig. 2). Our results also show that during the period of water stress, OA^{tot} increased in both