DROUGHT STRESS EFFECTS ON PHOTOSYNTHESIS, CHLOROPHYLL FLUORESCENCE AND WATER RELATIONS IN TOLERANT AND SUSCEPTIBLE CHICKPEA (CICER ARIETINUM L.) GENOTYPES

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In order to evaluate morphological and physiological traits related to drought tolerance and to determine the best criteria for screening and identification of drought-tolerant genotypes, we grew two tolerant genotypes (MCC392, MCC877) and two sensitive genotypes (MCC68, MCC448) of chickpea under drought stress (25% field capacity) and control (100% field capacity) conditions and assessed the effect of drought stress on growth, water relations, photosynthesis, chlorophyll fluorescence and chlorophyll content in the seedling, early flowering and podding stages. Drought stress significantly decreased shoot dry weight, CO2 assimilation rate (A), transpiration rate (E), and PSII photochemical efficiency (Fv/Fm) in all genotypes. In the seedling and podding stages, PSII photochemical efficiency was higher in tolerant genotypes than in sensitive genotypes under drought stress. Water use efficiency (WUE) and CO2 assimilation rate were also higher in tolerant than in sensitive genotypes in all investigated stages under drought stress. Our results indicated that water use efficiency, A and Fv/Fm can be useful markers in studies of tolerance to drought stress and in screening adapted cultivars of chickpea under drought stress.

Key words: Chlorophyll fluorescence, chickpea (Cicer arietinum L.), drought stress, photosynthesis.

INTRODUCTION

Chickpea (Cicer arietinum L.) is an important food legume crop which is grown in semi-arid regions. Generally, legumes are highly sensitive to water deficit stress (Labidi et al., 2009). Water deficit affects many morphological features and physiological processes associated with plant growth and development (Toker and Cagirgan, 1998). These changes include reduction of water content (RWC), diminished leaf water potential (Ψw) and turgor loss, closure of stomata and a decrease of cell enlargement and plant growth. Drought stress reduces plant growth by affecting photosynthesis, respiration, the membrane stability index (MSI) and nutrient metabolism (Jaleel et al., 2008a). The morphological and physiological changes in response to drought stress can be used to help identify resistant genotypes or produce new varieties of crops for better productivity under drought stress (Nam et al., 2001). The reactions of plants to drought stress depend on the intensity and duration of stress as well as the plant species and its stage of growth (Parameshwarappa and Salimath, 2008).

In drought stress conditions, plants close their stomata to avoid further water loss. Decreasing internal CO2 concentration (Ci) and inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase enzyme activity and ATP synthesis lead to a decrease of net photosynthetic rate under drought stress (Dulai et al., 2006). Reduced inhibition of photosynthesis under drought stress is of great importance for drought tolerance (Zlatev and Yordanov, 2004).
The effect of drought stress on CO₂ assimilation rate (A), transpiration rate (E) and water use efficiency (WUE) has been investigated in many crops such as *Zea mays* (Ashraf et al., 2007), *Brassica napus* L. (Kauser et al., 2006) and mungbean genotypes (Ahmed et al., 2002).

Another plant response to drought stress is change in photosynthetic pigment content. Photosynthetic pigments play important roles in harvesting light. The content of both chlorophyll a and b changes under drought stress (Farooq et al., 2009). The carotenoids play fundamental roles and help plants to resist drought stress (Jaleel et al., 2009). Drought stress inhibits Chl a/b synthesis and decreases the content of Chl a/b binding proteins, leading to reduction of the light-harvesting pigment protein associated with photosystem II (Sayed, 2003). The effects of drought stress on chlorophyll and carotenoid content have been investigated in cotton (Mssacci, 2008) and *Catharanthus roseus* (Jaleel et al., 2008a).

Drought stress affects photosystem efficiency (Fv/Fm) and decreases the electron transport rate (ETR) and the effective quantum yield of photosystem II photochemistry (Y) (Ahmed et al., 2002). The Fv/Fm ratio is a parameter which allows detection of any damage to PSII and possible photo-inhibition (Ahmed et al., 2002). Changes in the proportion of photochemical and energy-dependent quenching lead to alteration of fluorescence kinetics under drought stress (Zlatev and Yordanov, 2004). Chlorophyll fluorescence emitted from the chloroplast thylakoid membrane is often used as a very sensitive intrinsic indicator of the photosynthetic reaction in photosystem II (Ahmed et al., 2002). Analysis of chlorophyll fluorescence and measurement of the Fv/Fm ratio can be useful in determining damage to light reaction systems in photosynthetic mechanisms under drought stress.

The effects of drought stress on MSI, RWC and leaf water potential have also been investigated in many studies (Siddique et al., 2000; Jinmin and Huang, 2001; Terzi and Kadioglu, 2006; Bayoumi et al., 2008). It is believed that leaf water potential and RWC are reliable parameters for quantifying the plant drought stress response (Siddique et al., 2000; Bayoumi et al., 2008).

In this study we measured the early responses of certain parameters associated with photosynthesis and the involvement of various factors in photosynthetic damage in chickpea plants under drought stress. We assessed the effects of drought stress on leaf water potential, relative water content and membrane stability in sensitive and resistant chickpea genotypes to find a fast and easy technique for screening chickpea genotypes for drought tolerance.

**MATERIALS AND METHODS**

**PLANT MATERIALS**

Seeds of two tolerant genotypes (MCC392, MCC877) and two sensitive genotypes (MCC68, MCC448) were grown in pots containing 3 kg soil mixture composed of sand and farmyard manure (2:3) under drought stress (25% field capacity) and control conditions (100% field capacity) at the Research Center for Plant Science, Ferdowsi University of Mashhad, Iran. Three seeds were planted in each pot in a growth chamber. They were kept under a 12.5 h photoperiod (21°C day/8°C night) for the first month and under a 13 h photoperiod (27°C day/12°C night) the second month, similar to normal field situations in the chickpea growing region. Morphological and physiological indices were measured in the seedling, early flowering and podding stages in order to find reproducible, fast and easy techniques for screening chickpea genotypes for drought tolerance.

**PHYSIOLOGICAL MEASUREMENTS**

**Gas exchange measurement**

Photosynthetic gas exchange was measured from non-detached young and fully expanded leaves using a portable infrared gas analyzer (IRGA, LCA4, ADC Bio. Scientific Ltd., Herfordshire, UK): leaf surface area 1 cm², ambient CO₂ concentration 370 μmol mol⁻¹, and PPFD 200 μmol m⁻²s⁻¹. The leaf internal CO₂ concentration (Ci), CO₂ assimilation rate (A), and transpiration rate (E) were recorded between 09.00 and 11.00 a.m. Water use efficiency (WUE) was calculated from the A/E ratio (Piper et al., 2007).

**Chlorophyll fluorescence**

Photosystem photochemical efficiency (Fv/Fm) was measured using a portable chlorophyll fluorometer (OS5-FL modulated chlorophyll fluorometer, ADC Bio Scientific Ltd., Hoddesdon, Hert, EN11 0DB England). Minimal fluorescence (F₀) was determined by applying weak modulated light (0.4 μmol m⁻²s⁻¹) and maximal fluorescence (Fm) was induced by a short pulse (0.8 s) of saturating light (8000 μmol m⁻²s⁻¹). Measurements were made from the same leaf used for gas exchange determination, after 20 min dark adaptation (Maxwell et al., 2000). All physiological measurements used four or more plants from each treatment under drought stress and control conditions.

**Chlorophyll content**

Fresh leaves (0.1 g) were extracted with 15 ml 80% acetone and centrifuged at 5000× g for 10 min. The absorbance of the supernatant was read at...
663, 647 and 470 nm and calculated for chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content according to Arnon (1949). The chlorophyll stability index (CSI) was determined according to Sairam et al. (1997) and calculated as follows:

$$\text{CSI} = \frac{\text{total chlorophyll under stress}}{\text{total chlorophyll under control}} \times 100$$

Leaf water potential ($\Psi_w$)

Leaf water potential ($\Psi_w$) of control and stressed plants was measured using a pressure chamber (ARIMAD 3000, MRC), from the terminal leaflet of the uppermost fully expanded leaf of each plant (Gindaba et al., 2004).

Relative water content (RWC)

Relative water content was determined according to Barr and Weatherley (1962). Fresh weight of the young fully expanded leaf was determined within 2 h after excision. Turgid weight was obtained after soaking the leaf for 16 to 18 h in distilled water. After soaking, the leaves were quickly and carefully blotted dry with tissue paper prior to determination of turgid weight. Shoot dry weight was obtained after drying the leaf sample for 72 h at 70°C. Relative water content was calculated from the following equation:

$$\text{RWC} = \frac{[\text{fresh weight} – \text{dry weight}]/(\text{turgid weight} – \text{dry weight})]}{100}$$

Membrane stability index (MSI)

Leaf samples (0.1 g) of leaf material were taken in 10 mL double-distilled water in glass vials and kept at 40°C for 10 min. Initial conductivity ($C_1$) was recorded with a conductivity meter after bringing the sample to 25°C. The samples were kept at 100°C for 30 min and cooled to 25°C, and final conductivity ($C_2$) was recorded according to Premachandra et al. (1990) as modified by Sairam (1994). The membrane stability index (MSI) was calculated as

$$\text{MSI} = [1 – (C_1/C_2)] \times 100$$

### STATISTICAL ANALYSIS

The study was conducted as a factorial experiment based on a completely random design with four replicates. The data were analyzed by ANOVA and the significance of differences between treatment means was checked with Duncan’s multiple range test ($p<0.05$).

### RESULTS

**SHOOT DRY WEIGHT**

Drought stress decreased shoot dry weight in all genotypes in the three investigated stages (Fig. 3b), but the effects of drought stress on shoot dry weight were significant only in the podding stage. There were no significant differences between genotypes in either drought or control conditions in the seedling and early flowering stages (Fig. 3b). In the podding stage, however, shoot dry weight was higher in one tolerant genotype (MCC392) than in one sensitive genotype (MCC68) in both drought and control conditions (Fig. 3b). Drought stress decreased shoot dry weight in the podding stage more than in other stages. In the podding stage, genotype MCC877 had lower shoot dry weight than the other genotypes under drought stress (Fig. 3b).

### TABLE 1. Total chlorophyll content (Total Chl) (mg g$^{-1}$ FW), internal CO$_2$ concentration ($C_i$) (vpm), CO$_2$ assimilation rate ($A$) ($\mu$mol m$^{-2}$s$^{-1}$), transpiration rate ($E$) (mmol m$^{-2}$s$^{-1}$) and leaf water potential (MPa) in the seedling stage of chickpea genotypes in drought and control conditions

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Total Chl</th>
<th>$C_i$</th>
<th>$A$</th>
<th>$E$</th>
<th>$\Psi_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCC392</td>
<td>control</td>
<td>17.7a</td>
<td>817.0a</td>
<td>24.4a</td>
<td>3.9bcd</td>
<td>-1.2a</td>
</tr>
<tr>
<td>MCC68</td>
<td>control</td>
<td>22.1a</td>
<td>775.0a</td>
<td>17.9a</td>
<td>7.4a</td>
<td>-0.99ab</td>
</tr>
<tr>
<td>MCC448</td>
<td>control</td>
<td>14.7a</td>
<td>862.9a</td>
<td>25.2a</td>
<td>6.2ab</td>
<td>-0.91b</td>
</tr>
<tr>
<td>MCC392</td>
<td>drought</td>
<td>14.5a</td>
<td>868.7a</td>
<td>18.2a</td>
<td>2.7cd</td>
<td>-1.01ab</td>
</tr>
<tr>
<td>MCC68</td>
<td>drought</td>
<td>14.5a</td>
<td>882.3a</td>
<td>15.2a</td>
<td>6.2ab</td>
<td>-0.88b</td>
</tr>
<tr>
<td>MCC877</td>
<td>drought</td>
<td>16.4a</td>
<td>817.4a</td>
<td>18.3a</td>
<td>2.3d</td>
<td>-1.08ab</td>
</tr>
<tr>
<td>MCC448</td>
<td>drought</td>
<td>22.1a</td>
<td>800.4a</td>
<td>14.4a</td>
<td>2.6cd</td>
<td>-0.90b</td>
</tr>
</tbody>
</table>

Values with the same letter within column do not differ significantly ($p<0.05$).
GAS EXCHANGE

**Leaf internal CO₂ concentration (Cᵢ)**

Under both drought stress and control conditions, in the seedling stage there were no significant differences in leaf internal CO₂ concentration between genotypes (Tab. 1).

In the early flowering stage, Cᵢ decreased in all genotypes versus the levels in control plants, but these differences were not significant (Tab. 2). Genotype MCC877 (tolerant) had significantly higher Cᵢ than genotype MCC448 (sensitive) in control conditions. Cᵢ was highest in genotype MCC877 in both control and drought-stressed plants.

In the podding stage, Cᵢ decreased in MCC392, MCC877 and MCC448 but increased in MCC68 under drought stress as compared to the control (Tabs. 1–3). In the seedling stage, A was highest in the tolerant genotypes (MCC877, MCC392) in both drought and control conditions.

In the early flowering stage, A was highest in MCC392 and lowest in MCC68 in both drought-stressed and control plants (Tab. 2).

In the podding stage, A had less reduction in MCC392 and MCC877 genotypes under drought stress, but it showed more reduction in MCC68 and MCC448 genotypes under drought stress as compared to control conditions (Tab. 3). In the podding stage, A fell less in MCC392 and MCC877 under drought stress versus the control than it did in MCC68 and MCC448 (Tab. 3). In both the early flowering and the podding stages, A was higher in the tolerant (MCC392, MCC877) than in the sensitive (MCC68, MCC448) genotypes under drought stress.

**CO₂ assimilation rate (A)**

In all investigated stages the CO₂ assimilation rate decreased in all genotypes under drought stress as compared to the control (Tabs. 1–3). In the seedling stage, A was highest in the tolerant genotypes (MCC877, MCC392) in both drought and control conditions.

In the early flowering stage, A was highest in MCC392 and lowest in MCC68 in both drought-stressed and control plants (Tab. 2).

In the podding stage, A had less reduction in MCC392 and MCC877 genotypes under drought stress, but it showed more reduction in MCC68 and MCC448 genotypes under drought stress as compared to control conditions (Tab. 3). In the podding stage, A fell less in MCC392 and MCC877 under drought stress versus the control than it did in MCC68 and MCC448 (Tab. 3). In both the early flowering and the podding stages, A was higher in the tolerant (MCC392, MCC877) than in the sensitive (MCC68, MCC448) genotypes under drought stress.

**Transpiration rate (E)**

In all three investigated stages, the transpiration rate decreased in all genotypes under drought stress.
stress versus the control (Tabs. 1–3). In the seedling stage, E was highest in MCC68 and lowest in MCC392 in control plants. Sensitive genotypes (MCC68, MCC448) had higher E than tolerant genotypes (MCC392, MCC877) under drought stress (Tab. 1).

In the early flowering stage, MCC392 had significantly lower E than MCC68, and MCC877 had significantly lower E than MCC448 in control plants. It decreased by 82% in MCC68, by 80% in MCC877, by 55% in MCC392 and by 46% in MCC448 in drought-stressed plants. In the podding stage, E significantly decreased in all genotypes under drought stress, but there were no significant differences between genotypes in either drought stress or control conditions. The decrease was greatest in MCC877 (92%) under drought stress.

**WATER USE EFFICIENCY (WUE)**

Water use efficiency significantly increased from the seedling to the early flowering stage and then decreased in the podding stage.

**CHLOROPHYLL FLUORESCENCE**

**PSII photochemical efficiency (Fv/Fm ratio)**

The Fv/Fm ratio decreased in all genotypes and in all three stages under drought stress (Fig. 1b). In the seedling stage the Fv/Fm ratio was highest in MCC877 (tolerant) and lowest in MCC68 (sensitive) in control plants; under drought stress it was highest in MCC392 (tolerant) and lowest in MCC448 (sensitive) (Fig. 1b). Under drought stress, in this stage the decrease in the Fv/Fm ratio versus the control was significant in MCC877 and MCC448 (p<0.01) (Fig. 1b).

In the early flowering stage, MCC68 had the highest and MCC392 the lowest Fv/Fm ratio under drought stress (Fig. 1b), but there were no significant differences between genotypes in either drought stress or control conditions (Fig. 1b).

In the podding stage, drought stress significantly decreased the Fv/Fm ratio in all genotypes (Fig. 1b). The Fv/Fm ratio was highest in MCC877 under drought stress and in MCC392 in the control. The Fv/Fm ratio was significantly higher in the tolerant genotypes (MCC392, MCC877) than in the sensitive genotypes (MCC68, MCC448) in both seedling and podding stages under drought stress (Fig. 1b).

**Chlorophyll content**

In the seedling stage under drought stress, total chlorophyll content decreased in MCC392 (by 18%), MCC448 (3%) and MCC68 (30%), and increased in MCC877 (12%) (Tab. 1); carotenoid content decreased in MCC392, MCC448 and MCC68, and increased in MCC877 (Fig. 2b).

MCC448 had the highest chlorophyll a and b and carotenoid content under drought stress in all stages (Fig. 2a–c). In the seedling stage, the chlorophyll a/b ratio in all genotypes was significantly lower under drought stress than in control conditions (Fig. 2d).

In the early flowering and podding stages, chlorophyll a and b content significantly increased in all genotypes under drought stress (Fig. 2a, c). In the early flowering stage, chlorophyll b and carotenoid content in MCC877 was significantly lower than in the other genotypes in control conditions (Fig. 2b, c). In MCC392, MCC68 and MCC877 the chlorophyll a/b ratio in drought conditions was significantly higher than in control conditions (Fig. 2d).
In the podding stage, carotenoid content decreased in MCC877 and increased in MCC448 and MCC68 (Fig. 2b), so the chlorophyll a/b ratio was highest in MCC392 and lowest in MCC68 under drought stress (Fig. 2d).

Chlorophyll content and carotenoid content were highest in seedlings and lowest during early flowering.

The three investigated stages differed significantly in the chlorophyll stability index (CSI). It was highest in the podding stage and lowest during early flowering (Fig. 2e).

There were significant positive correlations between chlorophyll a and chlorophyll b in all stages: seedling ($r^2=0.60$), early flowering ($r^2=0.76$), and podding ($r^2=0.23$), and also between total chlorophyll and carotenoids ($r^2=0.74$, 0.95 and 0.94 respectively) ($p<0.01$).

LEAF WATER POTENTIAL
In the seedling stage, MCC392, MCC68 and MCC448 had higher and MCC877 had lower leaf water potential under drought stress than in control conditions (Tab. 1). Drought stress significantly affected $\Psi_w$ in the seedling and flowering stages. The tolerant genotypes had significantly lower $\Psi_w$ than the sensitive genotypes under drought stress ($p<0.05$) (Tab. 1).

In the early flowering and podding stages, drought stress decreased $\Psi_w$ versus the control ($p<0.05$) (Tabs. 2, 3). In early flowering, MCC392 had the highest and MCC877 the lowest $\Psi_w$ under drought stress (Tab. 2). In the early flowering and podding stages, one sensitive genotype (MCC68) had lower $\Psi_w$ than one tolerant genotype (MCC392), but MCC877 and MCC448 did not differ significantly under drought stress, nor in control conditions (Tabs. 2, 3).
Drought stress decreased the relative water content of all genotypes in all three stages (Fig. 3a). In the seedling stage, there were no significant differences in RWC between genotypes in control conditions. MCC68 (sensitive) had significantly lower RWC than MCC448 (sensitive) under drought stress (Fig. 3a). It was lowest in MCC68 under drought stress (Fig. 3a). In the early flowering and podding stages, RWC was higher in the tolerant genotypes (MCC392, MCC877) than in one sensitive genotype (MCC448) under drought stress. RWC was highest during early flowering in all genotypes under drought stress (Fig. 3a).

MEMBRANE STABILITY INDEX (MSI)

In the seedling and early flowering stages, membrane stability significantly decreased versus the control in all genotypes under drought stress (Fig. 3a). MCC68 (sensitive) had significantly lower RWC than MCC448 (sensitive) under drought stress (Fig. 3a). It was lowest in MCC68 under drought stress (Fig. 3a). In the early flowering and podding stages, RWC was higher in the tolerant genotypes (MCC392, MCC877) than in one sensitive genotype (MCC448) under drought stress. RWC was highest during early flowering in all genotypes under drought stress (Fig. 3a).

DISCUSSION

Drought stress alters many physiological and metabolic processes in plants (Gunes et al., 2006). Shoot dry weight is one of the earliest plant responses to drought. In this study, drought stress decreased shoot dry weight in all three investigated stages. The tolerant genotypes (MCC392, MCC877) had higher shoot dry weight than the sensitive ones (MCC68, MCC448) under drought stress. Higher shoot dry weight in tolerant genotypes under drought stress may be related to greater root growth, which helps in uptake of water and nutrients, boosting growth under drought stress. Reduction of shoot dry weight under drought stress has been reported in Zea mays L. (Ashraf et al., 2007), Beta vulgaris L. (Hussein et al., 2008) and Cicer arietinum L. (Gunes, et al., 2006).

In this work we found that drought stress decreased the CO₂ assimilation rate, relative water content, leaf water potential and membrane stability in all investigated stages. The tolerant genotypes (MCC392, MCC877) had higher values for relative water content, the membrane stability index, CO₂ assimilation rate and water use efficiency than the sensitive genotypes (MCC68, MCC448) under drought stress in all investigated stages. These results are in agreement with Piper et al.’s (2007) findings in Nothofagus dombeyi and Nothofagus nitida; they reported that the greater drought tolerance of N. dombeyi versus N. nitida was associated with higher water use efficiency and photosynthesis under drought stress. Nageswara et al. (2008) considered water use efficiency to be an important trait for selection of drought-tolerant varieties. In mungbean plants, Ahmed et al (2002) also found that drought stress decreased the CO₂ assimilation rate and leaf water potential.

Of the three investigated stages, the CO₂ assimilation rate in drought-stressed plants was highest in the seedling stage. Zlative et al (2004) suggested that decreasing CO₂ assimilation under drought stress...
may be related to restriction of CO₂ diffusion into
the leaf, and also inhibition of biochemical process-
es such as ATP synthase and Rubisco activity.

Our results showed significant positive correla-
tions between the membrane stability index and rel-
ative water content in all genotypes in the seedling
(r²=0.184) and flowering (r²=0.12) stages (p<0.01);
genotypes that could maintain higher relative water
content had higher membrane stability and higher
tolerance to drought stress.

Decreasing membrane stability under drought
stress has been reported in wheat varieties (Simova-
Stoilova et al., 2008).

We found that relative water content decreased
under drought stress in all investigated genotypes,
but with no significant differences between tolerant
and sensitive genotypes.

Leaf water potential differed significantly
between the growth stages, and was highest in the
podding stage. The sensitive MCC68 genotype had
lower Ψₜ than the tolerant MCC392 in the flowering
and podding stages.

Most studies have shown decreased relative
water content and leaf water potential in response to
drought stress (Siddique et al., 2000; Jinmin and
Huang, 2001; Terzi and Kadioglu, 2006; Bayomi et
al., 2008).

Genotypic variation of leaf water potential may
be attributed to differences in the ability to absorb
more water from the soil and the ability to reduce
water loss through stomata (Siddique et al., 2000).
It may also be due to differences in the ability of
genotypes to maintain tissue turgor and hence phys-
iological activities (Terzi and Kadioglu, 2006). At the
cell level, plants attempt to decrease the damaging
effects of stress by altering their metabolism to cope
with stress. Genotypes that maintain higher relative
water content under drought stress are believed to be
more tolerant and give higher yield than others
(Bayomi et al., 2008). It has been observed generally
that genotypes with higher leaf water potential and rel-
ative water content have a higher photosynthetic rate
under drought stress (Siddique et al., 2000). We found
that leaf water potential correlated positively with the
photosynthetic rate in the podding stage (r²=0.047,
p<0.05), but there were no significant correlations
between the photosynthetic rate and relative water
content in any of the investigated stages.

There were significant positive correlations
between the membrane stability index and the trans-
spiration rate in all stages: seedling (r²=0.073) early
flowering (r²=0.129) and podding (r²=0.075), and
also between the membrane stability index and
shoot dry weight (r²=0.144, 0.052 and 0.091
respectively) (p<0.05). There was also a significant
positive correlation between the transpiration rate
and shoot dry weight in the early flowering (r²=0.24)
and podding (r²=0.28) stages (p<0.05).

The tolerant genotypes had higher values for
shoot dry weight, the membrane stability index and
photosynthetic rate than the sensitive genotypes.

Drought stress also had effects on the Fₚ/Fₘ
ratio. The drought-sensitive MCC68 chickpea geno-
type had lower Fₚ/Fₘ than the two drought-tolerant
genotypes in all three stages. The Fₚ/Fₘ ratio can be
used to detect damage to photosystem II and possible
photo-inhibition (Ahmed et al., 2002). Several studies
have demonstrated damage to the PSII oxygen-evolv-
ing complex and the PSII reaction centers, and, in
turn, degradation of D₁ protein under drought stress
(Lu and Zhang, 1998; Maxwell and Johnson, 2000;
Galle et al., 2002). Photo-inhibition is represented by
decreasing Fₚ/Fₘ ratio, effective quantum yield of
photosystem II photochemistry and electron trans-
port rate (Piper et al., 2007). Decreases of the Fₚ/Fₘ
ratio and electron transport rate may be the result
of Calvin cycle disturbances that delay red-oxidation
of QA and induce photosystem II down-regulation or
damage thylakoid membrane electron transport
(Galle et al., 2002).

The plants in our drought-stress treatment
showed a marked decrease of the Fₚ/Fₘ ratio. Zlate
and Yordanov (2004) found lower Fₚ/Fₘ in bean geno-
types under drought stress, suggesting chronic pho-
toinhibition due to photo-inactivation of photosystem II
centers, possibly attributable to D₁ protein damage.
Piper et al. (2007) reported that the Fₚ/Fₘ ratio
declined in Notothfagaus species under drought stress
and was higher in tolerant than in sensitive genotypes.
Similar effects on these chlorophyll fluorescence
parameters have been observed in different species,
among them Brassica napus L. (Kauser et al., 2006)
and Aegilops species (Dulai et al., 2006).

We studied the correlations between photosyn-
thesis and the Fₚ/Fₘ ratio in chickpea. There were no sig-
nificant correlations between the CO₂ assimilation rate
and the Fₚ/Fₘ ratio, but the transpiration rate corre-
lated positively with the Fₚ/Fₘ ratio in the seedling
(r²=0.09) and podding (r²=0.259) stages (p<0.05).

Drought stress can also alter the tissue concen-
trations of chlorophylls and carotenoids (Hussein et
al., 2008). In our drought-stress treatment, the
MCCC448 genotype had the highest chlorophyll and
carotenoid content. Increased chlorophyll and
carotenoid content under drought stress may be rela-
ted to a decrease in leaf area. It can be a defensive
response to reduce the harmful effects of drought
stress (Farooq et al., 2009). The chlorophyll stability
index was highest in the podding stage and lowest
during early flowering. The higher chlorophyll stable-
ity index in the podding stage showed increasing
chlorophyll content under drought stress. Reduction of
chlorophyll content has been reported in drought-
stressed cotton (Mssacci, 2008) and Catharanthus
roseus (Jaleel et al. 2008b). Chlorophylls decreased
significantly under higher water deficit in sunflower
plants (Kiani et al., 2008) and in *Vaccinium myrtillus* (Tahkokorpi et al., 2007).

In our experimental treatments the tolerant genotypes showed significantly higher values for water use efficiency, the Fv/Fm ratio and the CO₂ assimilation rate under drought stress, indicating their superior ability to osmoregulate and ensure survival. These parameters can serve as useful markers for screening chickpea genotypes and identifying drought-tolerant genotypes.

REFERENCES


Effects of drought stress on tolerant and sensitive chickpea genotypes

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