

## Water and Nitrogen Stress on Maize Photosynthesis

A. Sepehri and S.A.M. Modarres Sanavy  
Tarbiat Modarres University, Tehran, Iran

**Abstract:** Two varieties of *Zea mays* (L.) from Iran differing in their growth and development have been used to study the effect of water and nitrogen stress on some of their photosynthetic performance. Chlorophyll content and fluorescence, photosynthetic CO<sub>2</sub> assimilation of leaves and total leaf water potential were measured. The results indicated that photochemical efficiency of photosystem II ( $F_v/F_m$ ) was severely reduced by nitrogen and water deficiency. Whereas, leaves of low-nitrogen plants had earlier and more prolonged reduction in  $F_v/F_m$  compared to leaves of high-nitrogen grown plants of the same water treatment. Chlorophyll content and photochemical efficiency of photosystem II as the key parameters determining photosynthetic were reduced in KSC 108 and 301 cultivars. KSC 301 was able to perform better, because showed higher  $F_v/F_m$  ratios when subjected to water stress. Net photosynthetic rates and total leaf water potential were lower in the leaves of the nitrogen deficit treated plants under water stress. The rates of net photosynthetic assimilation were decreased during drought in both varieties, but KSC 108 was more affected. The results showed that although rewatering ameliorated reduction in  $F_v/F_m$  and net photosynthetic rates, not completely eliminate depression in these parameters for low nitrogen grown plants.

**Key words:** Chlorophyll content, fluorescence, water stress, nitrogen efficiency, maize

### Introduction

Plants are exposed to a multitude of natural biotic and abiotic stressors. Almost all stressors affect either directly or indirectly the photosynthesis performance of leaves (Harmut and Babani, 2000). Differences in photosynthetic rates are most likely to be observed under conditions of environmental stress (Earl and Tollenaar, 1999). Chlorophyll fluorescence has been shown to be a useful tool in identifying previsual strain (Zarco-Tejada *et al.*, 2000). The amount and kinetics of chlorophyll fluorescence emitted from leaves upon illumination with actinic light may be used as a probe of the primary photochemistry of photosynthesis. In particular, the ratio of variable fluorescence ( $F_v$ ) to maximum fluorescence ( $F_m$ ) has been used as a measurement of the photochemical efficiency of photosystem II (Björkman and Demmig, 1987). The ratio has been correlated with photosynthetic quantum yield in a variety of species (Björkman and Powles, 1984) which indicates that  $F_v/F_m$  may be used to evaluate the response of photosynthetic machinery

to environmental factors in the field. Quenching analysis using the saturation pulse technique (Schreiber *et al.*, 1986) provides semi quantitative information about photochemistry in intact leaves. This method has been used extensively to investigate the response of plants to environmental stress (Fracheboud *et al.*, 1999).

Environmental stress such as water stress (Björkman and Powles, 1984; Jagtap *et al.*, 1998; Osborne *et al.*, 2002), nitrogen deficiency (Osmond, 1983; Ferrar and Osmond, 1986; Henley *et al.*, 1991; Osborne *et al.*, 2002), high and low temperatures (Ludlow and Bjorkman, 1984; Öquist and Örgen, 1985), enhance photoinhibition (Powles, 1984). Water availability is a major limiting factor for crop production, while low available soil nitrogen can be an additional yield reduction factor (Osborne *et al.*, 2002). Nitrogen is an integral part of chlorophyll, which is the primary absorber of light needed for photosynthesis. Combination of water stress and nitrogen deficiency is known to severely inhibit photosynthesis and hence crop productivity (Kao and Forseth, 1992; Osborne *et al.*, 2002).

The objective of this experiment was to study the effect of combined nitrogen and water deficiency in the photosynthesis performance of two maize hybrids from Iran and whether the impact of nitrogen and water stress could identify at a young stage of vegetative growth in order to reactivate the plant vitality by suitable countermeasures.

#### **Materials and Methods**

Maize (*Zea mays* L. cv. KSC 108 and 301 ) plants were grown outdoors in plastic pots (25 cm in diameter, 40 cm tall) from May to July, 2002. The pots were filled with loam, peat and sand in 7:3:2 volume ratios and were fertilized with dissolved ammonium nitrate at N rate 30 and 90 mg kg<sup>-1</sup> soil. Seeds were surface-sterilized with 0.02% (W/V) HClO<sub>4</sub> and then germinated at 25°C. Plant seedlings were the placed in pots (one plant per pot) in a randomized complete block experiment with three replications. The average temperature for day/night was 30/25°C, the relative humidity was 75-90% and the maximum photosynthetically active radiation was about 1500 μmolm<sup>-2</sup>s<sup>-1</sup>. All plants were watered daily. Water stress was imposed at the six-leaf stage by withholding water from plants for 3 d. Nonstressed treatments were watered to field capacity once each day.

Total chlorophyll content was measured in 80% (V/V) acetone extracts by the method of Arnon (1949). In all the analysis leaf discs were taken from fully expanded leaves of comparable physiological age, there by eliminating developmental effects.

Chlorophyll fluorescence was measured with a PSM fluorometer (Plant Stress Meter, Biomonitor, Sweden). Prior to the measurements, small parts of the leaves were kept in the dark for 30 min in covets for dark adaptation. A 5 slight pulse at 400 μmolm<sup>-2</sup>s<sup>-1</sup> was used. Photochemical efficiency of photosystem II (PSII) can be estimated by the F<sub>v</sub>/F<sub>m</sub> ratio (Demmig and Bjorkman, 1987). The rate of photosynthetic assimilation was measured with a portable photosynthesis system (between LCA-3, Analytical development Hoddesdon, England), operating into open mode. The total leaf water potential of the leaves was measured using a scholander pressure chamber (PMS Instrument Co, Corvallis, OR). As there is possibly a difference in the

structure and physiological function from base to tip in a maize leaf, the middle segment of the leaf was chosen for measurements of chlorophyll, Chlorophyll fluorescence and net photosynthetic rate.

Statistical analysis was performed using Analysis of Variance. Significant differences from values were determined at  $P < 0.05$  according to Duncan's multiple range test (DMRT).

### Results

Chlorophyll content was not differ in varieties at the beginning of water deprivation, but differences were observed between nitrogen levels in both varieties. Table 1 shows that after water stress, chlorophyll content per unit leaf area decreased in plants. Water stress decreased chlorophyll content by 8-20% in compared with control. The drop in total chlorophyll was due both to decreases in Chl a and Chl b (data not shown). The different nitrogen utilization had shown significant effect on chlorophyll content under water stress. The lowest chlorophyll content was belong to water stressed plant with low-nitrogen levels.

Nitrogen and water availability affected the rates of net photosynthetic assimilation (A) and the total leaf water potential of plants (Fig. 1). The rates of net photosynthetic assimilation were decreased during drought in both varieties. Leaves of high nitrogen with that continuously irrigated plants had the highest A and leaves of low nitrogen with 3 d water deprivation had the lowest A, leaves of high nitrogen with water deprivation and low nitrogen with well-watered had intermediate net photosynthetic assimilation values.

The total leaf water potential in well-watered control plants was almost constant through out the period of the experiment. In contrast, the total leaf water potential of the leaves of the droughted plants measured 3 h into the photoperiod decreased sharply after 3 d of water deprivation (Fig. 1). Net photosynthetic rates and total leaf water potential were lower in the leaves of the nitrogen deficit treated plants under water stress. The ratio of variable to maximum fluorescence was used to estimate photochemical efficiency of PSII. As seen in Table 1, two

Table 1: The means of net photosynthtic CO<sub>2</sub> assimilation ( $\mu\text{molm}^{-2}\text{s}^{-1}$ ), Chlorophyll content ( $\mu\text{g}/\text{cm}^2$ ),  $F_v/F_m$ , Total leaf potential (-Mpa) by leaves of two varieties at two levels of nitrogen after 3 d of water deprivation

Variety	Nitrogen	Water stress	Chlorophyll Content	$F_v/F_m$	CO <sub>2</sub> assimilation	Total leaf water Potential
108	100	+	26.9e	0.435e	8.66e	-1.42c
108	100	-	29.4de	0.585b	14.40bc	-0.50a
108	200	+	31.7cd	0.541cd	11.20d	-1.12b
108	200	-	39.8a	0.732a	18.96a	-0.38a
301	100	+	27.8e	0.515d	12.30cd	-1.05b
301	100	-	32.8bc	0.572bc	15.13b	-0.50a
301	200	+	35.2b	0.603b	13.30bcd	-0.95b
301	200	-	40.7a	0.715a	19.50 a	-0.44a

Means followed by similar letters in each column are not significantly different (5%)

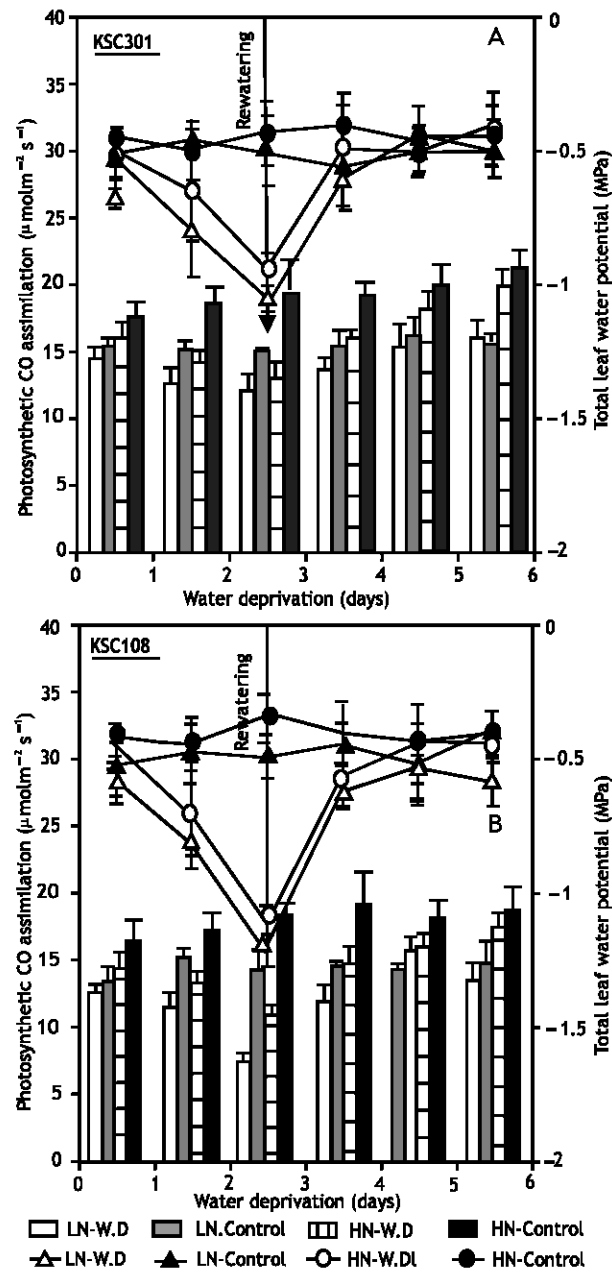


Fig. 1: The rate of net photosynthetic assimilation ( $\mu\text{molm}^{-2}\text{s}^{-1}$ ) (Bars) and total leaf water potential (-MPa) (Broken lines) of two varieties (KSC 301[a], KSC 108 [b]) of maize plant continuously irrigated (closed symbols) and imposed by withholding water for 3d then rewatered (open symbols) in two nitrogen rates HN-WD=high nitrogen-water deprivation, HN-Control=high nitrogen-well watered, LN=low nitrogen-water deprivation, LN-Control=low nitrogen-well watered, SE is indicated for each value

varieties showed about 14-20% decreases in the  $F_v/F_m$  ratios under nitrogen deficiency. When exposed to water stress for 3 d, inhibition in the photochemical efficiency was 13% in KSC 301, 25% in KSC 108.

### **Discussion**

The chlorophyll content is potentially one of the most important indicators of vegetation strain and changed the proportion of light-absorbing pigment and leading to less overall absorption (Zarco-Tejada *et al.*, 2000). The result showed that under water stress similar reductions of chlorophyll content were observed in two varieties, but leaves of high-nitrogen grown plants had more chlorophyll content compared to leaves of low-nitrogen grown plants at the same water treatment. The chlorophyll reduction of nitrogen and water stressed plants reported in different species (Fanizza *et al.*, 1991; Jagtap *et al.*, 1998).

The ratio of variable to maximum fluorescence decreased in two varieties under water deprivation for 3 d, the decrease was more pronounced in cv. KSC 108, indicating a larger level of photoinhibition in KSC 108 than in KSC 301. This parameter is the most sensitive fluorescence to water stress. Similar results were also found by Jagtap *et al.* (1998) during water stress treatment in *Sorghum* varieties. Changes in chlorophyll function frequently precede changes in chlorophyll content, hence changes in chlorophyll fluorescence observed before leaves become chlorotic (Zarco-Tejada *et al.*, 2000). Net photosynthetic rates were decreased during drought, Change in chlorophyll fluorescence confirm that non-stomatal limitation may have been responsible for reduction in photosynthetic assimilation rates under water stress. Photosynthetic  $CO_2$  assimilation was comparable in all plants when water was resupplied to the droughted plants on 3 d of the experiment. The photosynthetic apparatus is known to be relatively resistant to water stress (Cornic *et al.*, 1989; Chaves and Pereira, 1992). This appears to be true in maize leaves, in which photosynthesis quickly recovered when plants were rehydrated, especially in high-nitrogen grown plants. Under suboptimal nitrogen status the photosynthetic apparatus was found to be more sensitive to water stress (Ögren, 1988). There are have been reports of drought stress-induced damage to the photosynthetic apparatus (Kaiser, 1987; Van Rensburg and Krueger, 1993), this damage was thought to depend on other additional factors which become inhibitory to photosynthesis under condition of water stress. Nitrogen-deficient maize plants had a greater reduction in the ratio of variable to maximum fluorescence when exposed to short-term water stress than non-nitrogen deficient plants. Although rewatering ameliorated reduction in  $F_v/F_m$  and net photosynthetic rates, they did not completely eliminate depression in these parameters for low nitrogen grown plants.

In summary, exposure to a water stress under nitrogen deficit induced chlorophyll loss and marked decline in the photochemical efficiency of photosystem II and caused considerable degradation of photosynthetic assimilation rates in both varieties, but cv. KSC 108 was more affected.

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