BRIEF COMMUNICATION

Effects of reddening of cotton (*Gossypium hirsutum* L.) leaves on functional activity of photosynthetic apparatus

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Abstract

Strong inhibition of rates of CO_2 assimilation and transpiration, stomatal conductance, and water use efficiency as well as photosystem 2 (PS2) photochemical activity were related to the severity of reddening. The inhibition of photosynthesis in red cotton leaves was due to both decreased photochemical activity and stomatal limitation. Lowered photosynthetic capacity could be one of the main factors of reduced yield in reddening cotton.

Additional key words: chlorophyll fluorescence; net photosynthetic rate; stomatal conductance; transpiration rate; water use efficiency.

Red colouring of cotton leaves (known as "red leaf disease") is a phenomenon observed in some regions of Sudan and India (Dastur *et al.* 1960, Dhopte 1990). In the last ten years cotton growers in the Aegean region of Turkey are also facing this "disease" occurring in late summer and leading to economic losses: the cotton yield is reduced and the quality of fibre is damaged. The phenomenon is provoked by abiotic stress related to disturbed soil ion salinity equilibrium, leading to shortage of K in the plant compensated by an important accumulation of Na ions in cotton leaves (Hakerlerler, unpublished). Other authors also observed K deficiency in cotton during the late summer season, which was related to lowered boll mass and poorer fibre quality, as well as to a smaller leaf area index and reduced photosynthetic capacity (Minton and Ebelhar 1991, Pettigrew and Meredith 1997, Bednarz and Oosterhuis 1999). Various natural abiotic stress factors, characteristic for late summer conditions, such as low night temperature, mild water deficit, and high irradiance could also induce leaf reddening (Dodd *et al.* 1998, Chalker-Scott 1999).

No abundant data are available on the biochemical and physiological mechanisms underlying leaf reddening in cotton. A dramatic accumulation of anthocyanins, drop of chlorophyll (Chl) content, and increase of peroxidase activity and proline content in red cotton leaves were reported, these changes being indicative of a stress situation in which anthocyanins could play a protective role (Edreva *et al.* 2002).

The aim of the present work was to investigate the effect of reddening in cotton on the functional activity of photosynthetic apparatus and the effectiveness of photosystem 2 (PS2) by following net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), water use efficiency (WUE), and Chl fluorescence kinetics. The non-invasive approaches used in this work allow achieve a characteristic relevant to a stress situation in plant.

Cotton (*Gossypium hirsutum* L.) cv. Nazilli 84 plants were grown in three locations of the Aegean region (Söke, Menemen, and Bergama). Plants at flowering-boll formation stage were used. Measurements were carried out in the middle of September when leaf reddening (formation of large, deeply red-coloured spots) was fully expressed. Intact upper leaves bearing symptoms of reddening were used. Two stages of reddening were assayed: light symptoms (presence of small, slightly red-coloured

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spots taking no more than 1/10 of leaf lamina; we refer to these leaves as "light red", LR) and severe symptoms (formation of large, diffuse, deeply red coloured spots covering about 1/2 of leaf lamina; we refer to these leaves as "strong red", SR). Green upper leaves of plants of the same age without symptoms of reddening were used as controls.

P_N, g_s, and E were measured by a portable system for photosynthetic measurements *LI-6000* (*LI-COR*, Lincoln, NE, USA). Experiments were carried out with intact leaves at sunlight, leaf temperature of 32-37 °C, and CO_2 concentration in the leaf chamber of about 400 μmol mol^{-1}. Chl fluorescence parameters were measured using a Plant Efficiency Analyser (*P.E.A.*, *Hansatech*, King's Lynn, UK). After 30 min of dark adaptation, the initial fluorescence yield (F_0) and maximal fluorescence yield (F_m) emitted during a saturating red radiation (630 nm) pulse (5 s, over 5 000 μmol m^{-2} s^{-1} PFD) were determined. Photochemical efficiency of PS2 was estimated as the ratios F_v/F_m and F_v/F_0. In the three locations surveyed measurements were performed on 30 uniformly developed plants in each of the three variants studied: controls and LR- and SR-plants. Significance of differences is given by the *t*-test of Student at $p = 0.05$.

Significant changes of the photosynthetic gas exchange parameters were found in both LR and SR cotton leaves in all regions investigated. P_N of red cotton leaves was inhibited, more strongly in SR leaves, being only 6 % of the control value in the Söke region (Fig. 1*A*). In the Menemen and Bergama regions P_N in SR leaves was 12 and 34 % of the control green plants, respectively. Also E and g_s were more significantly affected in SR than in LR leaves (Fig. 1*B,C*). No strong correlation was established between the decrease in P_N and g_s in SR leaves. Thus not only stomatal factors were involved in photosynthetic inhibition. Significant reduction of WUE was observed in reddening cotton leaves in all regions (Fig. 1*D*).

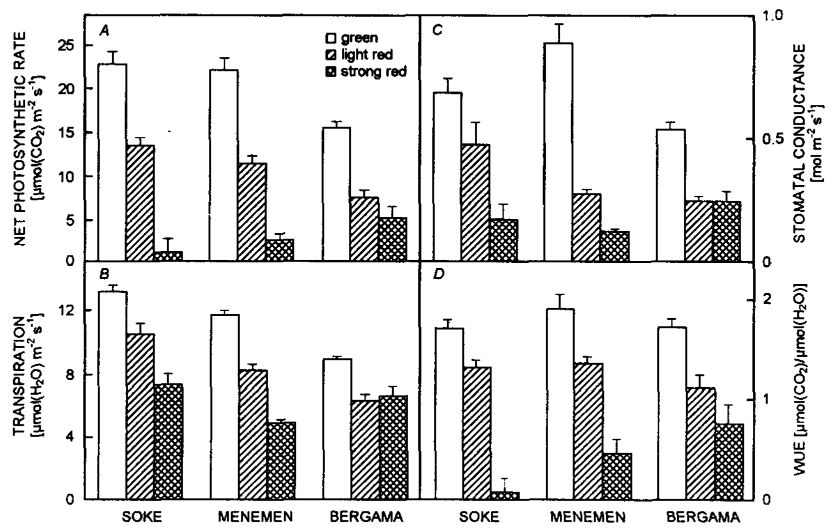

Fig. 1. Net photosynthetic rate (*A*), transpiration rate (*B*), stomatal conductance (*C*), and water use efficiency (WUE) (*D*) in green cotton leaves (controls) and leaves in different stages of reddening: light red and strong red. Samples were taken from three cotton growing locations in Aegean region: Söke, Menemen, and Bergama. Means of 30 measurements for each of the three variants studied (controls, light red, and strong red) in every location. Bars indicate ± S.E.

The Chl fluorescence parameter F_0 increased significantly upon reddening, particularly in SR leaves, in all regions investigated (Fig. 2*A*). In SR leaves F_0 increased to 163 (Söke), 133 (Menemen), or 135 (Bergama) % in comparison to the controls. A decline in F_m and F_v was recorded in reddening cotton, this being more clearly expressed in leaves with severe symptoms. More pronounced changes occurred in the variable component of Chl fluorescence: the decrease of F_v in SR leaves amounted to 66, 70, and 87 % of the controls in Söke, Menemen, and Bergama, respectively (Fig. 2*B,C*). The F_v/F_m ratio (region Söke) in LR and SR leaves decreased to 90 and 75 % of the control, respectively. A similar response was recorded in the other two regions (Fig. 2*D*). The F_v/F_0 ratio decreased considerably in reddening leaves. It declined to 43 and 65 % of the control in SR- and LR-leaves, respectively (Söke region). Similar pattern of changes was found for Menemen and Bergama (Fig. 2*E*).

Hence in cotton plants with red coloured leaves photosynthetic gas exchange and Chl fluorescence parameters were strongly affected. P_N, g_s, and E were lowered. E was reduced relatively less than P_N and this led to a lower WUE of the cotton plants (Fig. 1*A-D*). Our results are in line with previous reports on the effects of abiotic constraints in cotton (Ackerson 1980, Lu *et al.* 1998, Zhao *et al.* 2001). Stomatal factors were mainly responsible for the dramatically reduced P_N in red cotton leaves (Fig. 1*C*). Non-stomatal factors, such as the decreased PS2 photochemical efficiency, also contributed to P_N decline (Fig. 2*D,E*). g_s was more limiting to photosynthesis since its decrease was stronger (in the range of 15-45 % of controls in SR leaves) than the decrease of PS2 activity (43-67 % of controls for F_v/F_0, and 75-90 % of controls for F_v/F_m in SR leaves). Of course, biochemical factors may also be involved in lowering photosynthesis.

Decreased P_N in red cotton leaves was also observed in K-deficient cotton plants (Pettigrew and Meredith 1997, Bednarz and Oosterhuis 1999, Zhao *et al.* 2001).

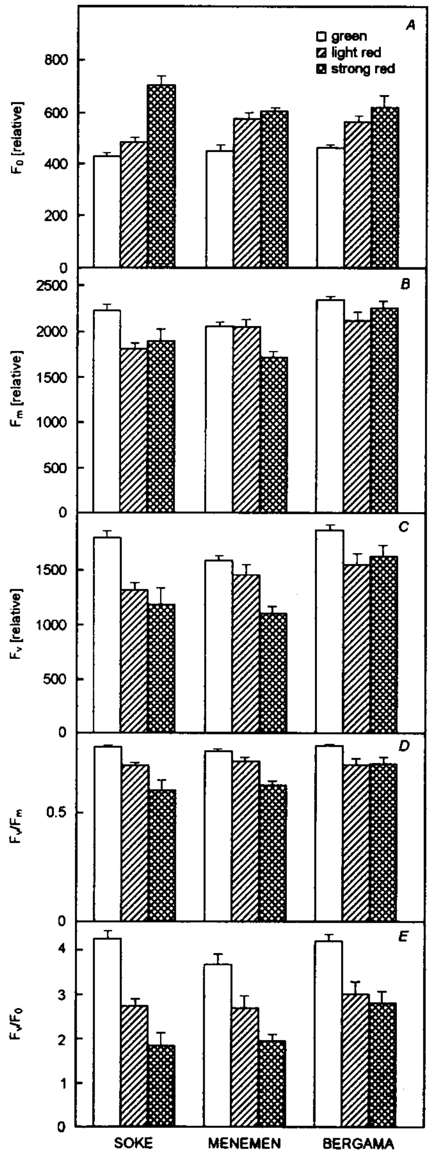

Fig. 2. Chlorophyll fluorescence parameters – initial, F_0 (*A*), maximal, F_m (*B*), and variable, F_v (*C*), and their ratios F_v/F_m (*D*) and F_v/F_0 (*E*) in green cotton leaves (controls) and leaves in different stages of reddening: light red and strong red. For other explanations see Fig. 1.

Bednarz *et al.* (1998) concluded that during a mild K deficiency increased stomatal resistance results first in a decrease in P_N and, as the deficiency becomes more acute, biochemical factors may also contribute.

The strongly reduced P_N upon reddening of cotton could be one of the main reasons for low boll yield. Moreover, disturbed saccharide distribution in K-deficient plants could influence the supply of photosynthates to reproductive organs and thus lead to lowered yield. According to Pettigrew (1999) the elevated saccharide contents remaining in source tissue, such as leaves, is part of the overall effect of K-deficiency in reducing the amount of photosynthates available for reproductive sinks and thereby producing reductions in lint yield and fibre quality seen in cotton. Accumulation of sucrose was observed in leaves of K-deficient plants, this being associated with reduced entry of sucrose into the transport pool or decreased phloem loading (Zhao *et al.* 2001). Along with K-deficiency, other abiotic stress factors may reduce P_N in red cotton plants (Huner *et al.* 1998, Lu and Zhang 1998, Saccardy *et al.* 1998, Hong and Xu 1999, Savitch *et al.* 2000, Xu *et al.* 2000, Xin and Browse 2000).

Chl fluorescence analysis indicated that photochemical activity significantly decreased in red cotton leaves (Fig. 2). Increase in F_0 could be partly due to the reduced plastoquinone acceptor, Q_A^-, not being completely oxidised because of interruption of the electron flow through PS2. DCMU, a photosynthetic inhibitor, binding the Q_B site of the D_1 protein of the PS2 reaction centre, changes F_0 in the same manner (Krause and Weis 1991). Besides, the F_0 increase may also be due to the separation of light-harvesting chlorophyll *a/b* protein complexes of PS2 from the PS2 core complexes, which is similar to high temperature effects (Schreiber and Armond 1978). Along with an increase in F_0, a reduction in F_m and F_v was observed. The drop in F_m under unfavourable environment is usually associated with: enhanced transfer of excitation energy from LHC2 to PS1 (Schreiber and Armond 1978), inactivation of the O_2-evolving system and formation of P_{680}^+, which quenches Chl states (Katoh and San Pietro 1967), and denaturation of Chl-protein complexes (Yamane *et al.* 1997). The established reduction of F_v might indicate the decreased ability of PS2 to reduce plastoquinone.

In reddening cotton leaves the PS2 photochemical activity was lowered. The ratio F_v/F_0 decreased more considerably than F_v/F_m (Fig. 2*D,E*). The more significant response of F_v/F_0 corresponds to the dramatic decline in CO_2 assimilation; hence F_v/F_0 ratio appears more informative for photosynthetic activity than F_v/F_m. This is in accordance with Nauš *et al.* (1992) pointing that F_v/F_0 is more suitable for detection of stress injury in plants.

The damage of photosynthetic activity upon reddening may be caused by oxidative stress due to K shortage and corresponding accumulation of Na ions in red cotton leaves (Hakerlerler, unpublished), the latter resulting in overproduction of OH• free radicals (Alia *et al.* 1993). Other stress factors typical for late summer season when cotton reddening becomes spectacularly expressed (low night temperature, high irradiance, mild water deficit) are also reported to induce oxidative stress (Bohnert and Sheveleva 1998). Abundant data point to the inhibiting effect of OH• and other active oxygen species on photosynthetic efficiency and Calvin cycle enzymes (Smirnoff 1998).

The state of oxidative stress in reddening cotton may be partly counteracted by the strong accumulation of anthocyanins having protective antioxidant and anti-radical functions. The lack of structural membrane damage in red cotton leaves (Edreva *et al.* 2002) supports this assumption.

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