BRIEF COMMUNICATION

# Photosynthetic behaviour of *Arabidopsis* plants with a Cap Binding Protein 20 mutation under water stress

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## Abstract

Under non-stressed conditions the net photosynthetic rate ($P_N$) of the mutant plants *cbp20* of *Arabidopsis* was similar to that of the wild type (WT). In response to water deprivation, however, $P_N$ started to decrease later in the mutants and remained substantially higher. Thermoluminescence measurements showed that the lipid peroxidation induced by severe water stress was also less pronounced in the mutant than in the WT. Both soil gravimetric and plant water potential data showed that *cbp20* mutants lose water more slowly than the WT plants. The drought-induced decline in $F_v/F_m$, the quantum efficiency of photosystem 2, and photochemical quenching parameters also started later in the *cbp20* mutants than in the WT plants. Thus the restricted gas exchange in the *cbp20* mutants does not impair the photosynthetic performance of the plant; however, under drought improved water retention provides significant protection for the photosynthetic apparatus.

*Additional key words*: drought; fluorescence induction; thermoluminescence; water potential.

Water stress is one of the most important factors limiting the spread of plants. The first impact of water stress on photosynthesis is caused by the closure of the stomata (Cornic 2000, Dias and Brüggemann 2007), a process mediated *via* an abscisic acid-dependent pathway (Sen and Chawan 1978). Besides this effect there are several other non-stomatal limitation factors which contribute to the reduction in photosynthesis under drought (Kovács *et al*. 2007), including a decline in the efficiency of ribulose-1,5-bisphosphate carboxylation and regeneration (Wise *et al.* 1991) and, especially under severe stress possibly leading to photoinhibitory effects, a reduction in the rate of photosynthetic electron transport. Drought may also induce the generation of reactive oxygen species, causing lipid peroxidation leading to membrane injury, protein degradation, and the disruption of nucleic acid strands (El-Tayeb 2006). These processes may lead to growth inhibition, reflected in a reduction in the dry matter yield.

Plants are able to develop several physiological adaptation mechanisms to tolerate drought conditions. When screening for *Arabidopsis* mutants with altered stress responses, a T-DNA insertion mutant displaying pleiotropic alterations was recently identified. In this mutant the *Cap Binding Protein 20* (*cbp20*) gene that encodes the 20 kDa subunit of the nuclear mRNA cap binding complex (nCBC) was disabled (Papp *et al*. 2004). Plants homozygous for the recessive *cbp20* mutation show mild developmental abnormalities, such as serrated rosette leaves and slightly delayed development. Loss of the *CBP20* function also confers hypersensitivity to abscisic acid, leading to a significant reduction in stomatal conductance and enhanced tolerance to water stress (Papp *et al*. 2004). This suggests that the isolation of the gene carrying this mutation could provide a practical tool for the development of transgenic crop plants

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*Abbreviations*: CBP20 – Cap Binding Protein 20; Chl – chlorophyll; $F_v$, $F_m$ – variable and maximum fluorescence in the dark-adapted state, respectively; $F_m'$, $F_s$ – maximum and steady state fluorescence in the light-adapted state, respectively; GWC – gravimetric water content; $P_N$ – net photosynthetic rate; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; $q_N$ – non-photochemical quenching; $q_P$ – photochemical quenching; TL – thermoluminescence.
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with enhanced drought tolerance. Therefore, the aim of the present study was to characterize the photosynthetic properties of *cbp20*-mutant plants under control and drought conditions. The photosynthetic processes in the *cbp20* mutant were compared to those of the wild type (WT) under non-stressed conditions, and also the protective role of the mutation against water stress was demonstrated.

*Arabidopsis thaliana* cv. Columbia (Col-0; WT) and *cbp20*-mutant plants were grown under short-day (10 h light) and, from the fifth week onwards, long-day (16 h light) conditions. In order to compensate for the somewhat slower growth rate of the mutant, 8-week-old WT and 9-week-old mutant plants (at the flowering stage) were used in the experiments. The temperature cycle was 22/20 °C (day/night), with a PPDF of 120 μmol $m^{-2}$ $s^{-1}$. On the first day of the measurements the soil was saturated with water to field capacity. In order to induce water stress, no further irrigation was provided for the duration of the experiment.

Soil water content was monitored by the gravimetric method. The pots were weighed in the early afternoon on the days indicated during the measurement period. At the end of the experiment the contents of the pots were kept at 80 °C for 24 h to achieve the total desiccation of the soil, and the gravimetric water content (GWC) was calculated as follows: GWC [%] = [(M – DM)/(FM – DM)] × 100, where; M – mass of pot at the time point indicated, DM – mass of pot after desiccation, FM – mass of pot at field capacity. Plant water status was characterized by measuring water potential in a pressure bomb (*PMS610*, *PMS Instrument*, Corvallis, OR, USA). All measurements were done in the early afternoon on the day indicated. Five leaves from two different pots were measured for each data point. All the experiments were repeated three times unless otherwise stated, with one representative result shown.

The net photosynthetic rate ($P_N$) of the fully expanded leaves was measured using an *LCi* Portable Photosynthesis System (*ADC Bioscientific*, Herts, UK). The temperature cycle and PPDF were as indicated above.

The chlorophyll (Chl) *a* fluorescence induction parameters were measured using pulse amplitude modulated system (*FMS 2*, *Hansatech*, King's Lynn, UK) at room temperature after at least 30 min dark adaptation. Quenching parameters were determined after 5 min actinic irradiation (PPFD = 40 μmol $m^{-2}$ $s^{-1}$) according to Schreiber *et al*. (1986). The nomenclature of van Kooten and Snell (1990) was used.

Thermoluminescence (TL) measurements were performed using the laboratory-made apparatus and software described earlier (Ducruet and Miranda 1992, Ducruet *et al*. 1998, Janda *et al*. 2000). Luminescence was detected with a *Hamamatsu H5701-50* photomultiplier linked to an amplifier. A 4×4 cm Peltier element (*Marlow Instruments*, Dallas, Texas, USA) was used for temperature control. The leaf sample was gently pressed against the plate using a rubber ring and a *Pyrex* window, with the addition of 100 $mm^3$ water for better thermal conduction. The plants were dark-adapted for 2 h before the measurements. The samples were cooled to 0.1 °C within a few seconds and irradiated *via* a fibre optic by far-red radiation provided by a *PAM 102-FR* light source (*Walz*, Effeltrich, Germany) for 30 s (setting: 11) prior to the measurement. The rate of heating during measurement was 0.5 °C $s^{-1}$.

The results were the means of at least 5 measurements and were statistically evaluated using the standard deviation and T-test methods. Representative curves are shown for TL.

WT and *cbp20* mutant plants were water stressed by withholding irrigation. Signs of wilting became visible after 4–5 d of water deprivation. $P_N$ in unstressed plants was slightly, but statistically not significantly lower in the mutants than in the WT plants (Fig. 1*A*). In the WT plants $P_N$ started to decrease after 4 d of water deprivation. In the mutant plants this decrease was significant only after the 6$^{th}$ day. The rate of assimilation remained significantly higher in the *cbp20* mutants under moderate and severe drought. Hence reduced gas exchange in the mutant did not impair photosynthetic performance under well-watered conditions.

Stomatal closure is the most efficient way to reduce transpirational water loss (Cornic 2000, Lawlor and Cornic 2002). Abscisic acid mediates stomatal closure under stress (Schroeder *et al*. 2001, Shinozaki *et al*. 2003, Fan *et al*. 2004). Changes in the water content of the soil were represented as a decrease in the mass of the pots. Both soil gravimetric (Fig. 2*A*) and plant water potential data (Fig. 2*B*) showed that *cbp20 Arabidopsis* mutants, which have recently been characterized as abscisic acid-hypersensitive, with significantly lower stomatal conductivity than the WT (Papp *et al.* 2004), lost water more slowly than the Col-0 WT plants. The mutants evaporated less water right from the beginning of the experiments.

The far-red irradiation of leaves at low but non-freezing temperatures induces a B TL band peaking below 30 °C, together with an afterglow (AG) band peaking at around 40-45 °C (Miranda and Ducruet 1995). The intensity of the AG band indicates the NADPH+ATP potential (Krieger *et al.* 1998). The AG band is more sensitive to various stress treatments than is the flash-induced B band and, as a consequence, it could act as a sensitive stress indicator (Janda *et al.* 1999, 2000). Heat or drought stress may cause a shift in the peak position of the AG band towards higher temperatures, as observed in maize plants. However, this up-shift cannot always be seen in plants where the AG band appears at a relatively high temperature (Ducruet *et al*. 1997). Measurement of the TL curve induced by far-red radiation in *Arabidopsis* plants showed that there was no significant difference between the unstressed WT and *cbp20* plants in the peak position and amplitude of the AG and B bands

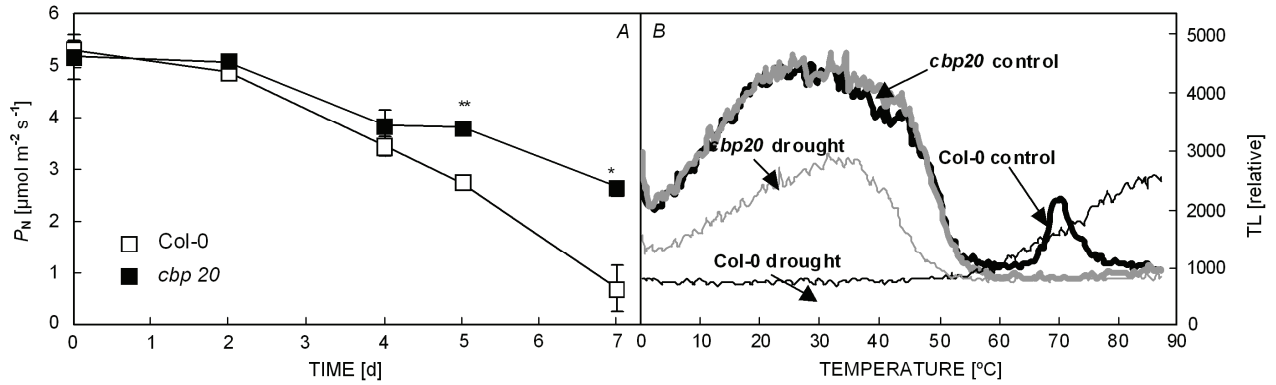


Fig. 1. *A*: Changes in the net photosynthetic rate ($P_N$) in wild-type (WT) and *cbp20*-mutant *Arabidopsis* plants during water deprivation. $^{*}$, $^{**}$ represent significant differences between the WT (*open symbols*) and mutant plants (*closed symbols*) at the $p \leq 0.05$ and $p \leq 0.01$ levels, respectively. *B*: Thermoluminescence (TL) curves induced by 30 s far-red radiation measured before (*thick lines*) or after (*thin lines*) 9 d of water deprivation in Col-0 WT (*black lines*) and *cpb20*-mutant (*grey lines*) *Arabidopsis* plants.

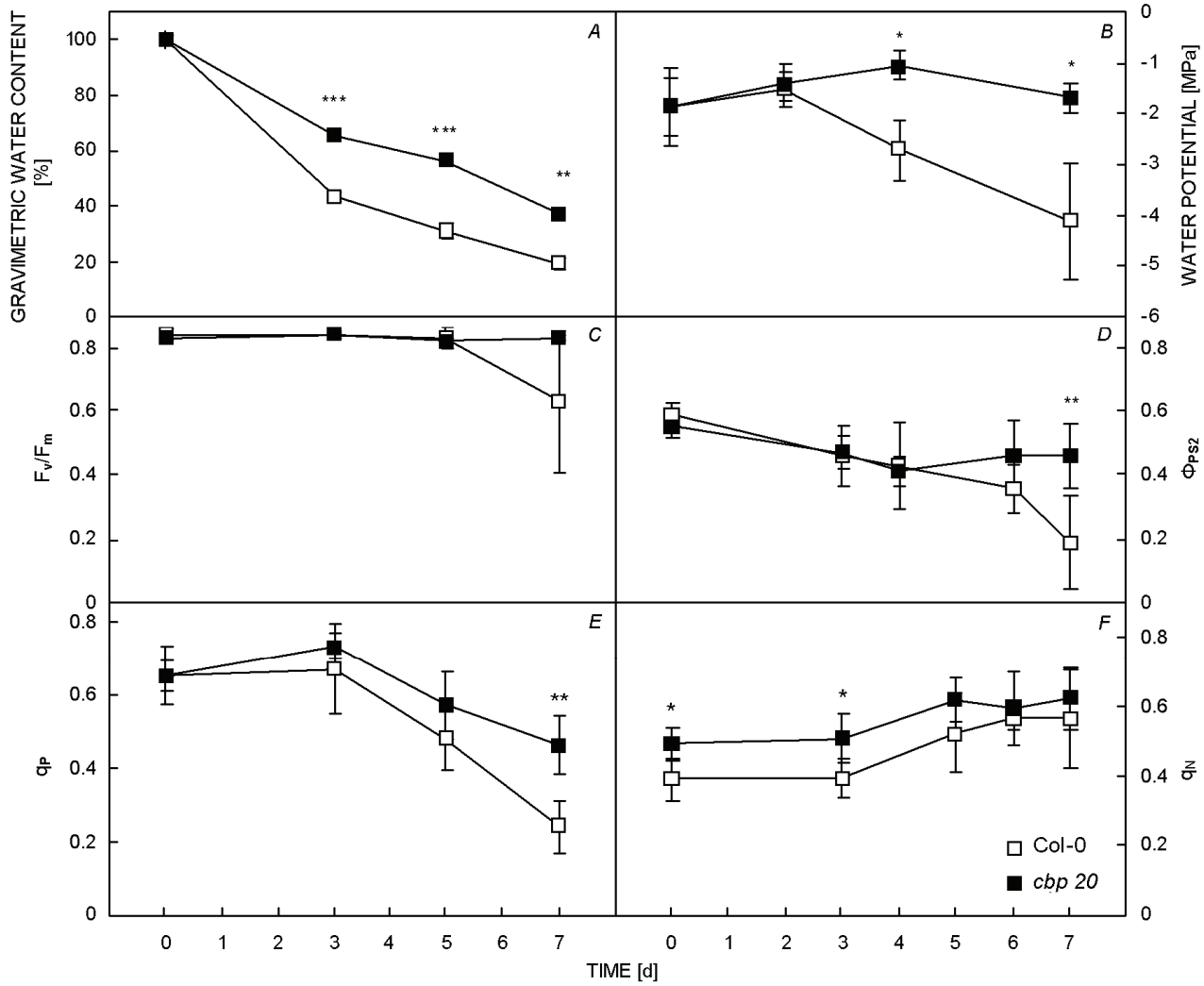


Fig. 2. Changes in the gravimetric water content (GWC: *A*), water potential (*B*), and in the chlorophyll *a* fluorescence induction parameters $F_v/F_m$ (*C*), quantum yield of PS2 ($\Phi_{PS2}$: *D*), photochemical quenching ($q_P$: *E*), and non-photochemical quenching ($q_N$: *F*) in Col-0 wild-type (*open symbols*) and *cbp20*-mutant (*closed symbols*) *Arabidopsis* plants in response to water deprivation. $^{*}$, $^{**}$, $^{***}$ represent significant differences between the wild-type and mutant plants at the $p \leq 0.05$, 0.01, and 0.001 levels, respectively.

induced by far-red radiation. However, severe drought stress (9 d of water withdrawal) totally suppressed both the B and AG bands in the WT plants, but not in the *cbp20* mutants, where only a significant decline appeared (Fig. 1*B*). Besides the B and AG bands another sharp band peaking at around 70 °C, which is specific to unstressed leaves of crucifers (Havaux *et al.* 2000, Ducruet 2003), could be detected in the WT plants. In the *cbp20* mutant the intensity of this band was close to the noise level of the TL signal. The origin of this band is not clear; it is not related to lipid peroxidation and could be due to the thermolysis of a crucifer-specific volatile compound (Ducruet 2003). The intensity of this band was independent of the irradiation of the sample: in contrast to the B and AG bands, it could also be detected in dark-adapted plants without previous light excitation (data not shown). Severe drought stress also eliminated this band in WT plants and only a high temperature band (HTL) peaking above 90 °C could be detected (Fig. 1*B*), indicating the stress-induced generation of lipid peroxides (Hideg and Vass 1993, Stallaert *et al*. 1995, Vavilin and Ducruet 1998, Vavilin *et al*. 1998, Ducruet and Vavilin 1999, Marder *et al*. 1999).

The Chl *a* fluorescence induction method detects the effects of various stresses in plants (Roháček and Barták 1999, Sayed 2003). To obtain further information on changes in the photosynthetic electron transport chain during the water deprivation process, several Chl *a* fluorescence induction parameters were determined. Photosystem 2 (PS2) itself is considered to be a "drought-tolerant" unit of the photosynthetic apparatus, so mild or moderate water stress usually has no direct effect on the primary photochemistry of PS2. Significant changes in the $F_v/F_m$ parameter under drought usually occur when the stress is severe and secondary symptoms caused by oxidative damage may already be visible. As illustrated by Fig. 2*C*, there was no significant change in the $F_v/F_m$ parameter representing the maximum quantum efficiency of PS2 after the first 5 or 7 d of water stress in WT and *cbp20* plants, respectively. This also means that the *cbp20* mutation provided protection against the photoinhibition induced by severe water stress.

The quantum yield of PS2 ($\Phi_{PS2}$), calculated from the $(F_m' - F_s)/F_m'$ parameter, started to decrease earlier in both genotypes than the $F_v/F_m$ parameter: there was a slight decrease after 4 d of drought in both the WT and *cbp20* plants, and the difference between the WT and mutant plants was significant after the 6$^{th}$ d of water withdrawal (Fig. 2*D*). In contrast to the $F_v/F_m$ and $\Phi_{PS2}$ parameters, $q_P$ showed a slight increase at the beginning of water withdrawal, followed by a decrease after the 3$^{rd}$ d (Fig. 2*E*). A similar tendency was reported in cold-stressed maize plants (Janda *et al.* 1994b). Generally, an increase in $q_P$ may be part of the acclimation process, when plants try to maximize the number of open reaction centres in order to minimize the harmful effect of the excitation pressure occurring in PS2 under stress (Hurry *et al.* 1992, Janda *et al.* 1994a). A decrease in $q_P$ under severe drought stress has been described in several plant species (Loreto *et al*. 1995, da Silva and Arrabaca 2004). In the present work, changes in $q_P$ also confirmed the protective role of the *cbp20* mutation against water stress in *Arabidopsis* plants, since the decline in $q_P$ was less pronounced in the mutant than in the WT. All the above-mentioned parameters of Chl *a* fluorescence induction, *i.e*. $F_v/F_m$, $\Phi_{PS2}$, and $q_P$, had similar values in both the unstressed Col-0 control plants and in the *cbp20* mutant plants. The $q_N$, however, was slightly higher in *cbp20* than in the WT even under control conditions. The $q_N$ parameter did not change in the first 4 d of water withdrawal, but after 5 d it increased in both WT and *cbp20* plants (Fig. 2*F*). Besides a decrease in quantum efficiency, another sign of the down-regulation of the photosynthetic electron transport processes under unfavourable environment is the increased dissipation of excess energy as heat, as reflected in the increase in $q_N$ (Molnár *et al.* 2004). The increased non-photochemical quenching capacity of the *cbp20* plants may also contribute to better survival in response to unfavourable changes in the environment.

In conclusion, we suggest that the restricted gas exchange in the *cbp20* mutant does not impair the photosynthetic performance of the plant, while under drought the improved water retention provides significant protection for the photosynthetic apparatus. This beneficial effect against water stress was demonstrated by all the photosynthetic parameters measured. Whether other significant parameters, such as grain yield and biomass, remained unchanged in the mutant plants is an important question for further studies.

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