

# Preliminary studies on some chlorophyll fluorescence parameters in Crassulaceae species of different leaf characters under water stress

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**ABSTRACT** Chlorophyll fluorescence quenching parameters of *Kalanchoe daigremontiana*, *K. fedtschenkoi* cv. *marginata*, *K. tomentosa*, *Crassula multicava* and *Cotyledon ladysmithiensis* were compared in well watered and water deficient plants. The maximum quantum yield (Fv/Fm) of photosystem II (PSII) proved to be a very stable parameter in all species and water treatments. The EQY, NPQ, qP and ETR differed in *Crassula multicava* from those of the other plants. The allocation of absorbed light by PSII that is utilized to photosynthetic electron transport and thermal dissipation was also calculated. Analysis of the chlorophyll fluorescence parameters demonstrated, that *Crassula multicava* was the only plant, which exhibited a higher proportion of excitation energy allocated to PSII photochemistry under water deficit at 1200  $\mu\text{mol m}^{-2}\text{s}^{-1}$  actinic light (AL) intensity.

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## KEY WORDS

Crassulaceae,  
water deficit,  
fluorescence quenching

Plasticity in CAM expression has been extensively explored in the Crassulaceae family (Teeri et al. 1981; Kluge et al. 1993). Experimental results on the mechanisms and ecological significance of CAM derive principally from the studying of *Kalanchoe* genus. In other plants in this family, however, there are considerable differences in the growth habit, in the epidermal appendages and in the rate of succulence. These species with small, thick leaves, and hairy epidermis are problematic test objects for the conventional techniques, thus there are no or few information on their anatomical and photosynthetic features. Studies of the CAM photosynthesis by traditional gas-exchange techniques are limited because of the stomatal closure during the light period. The objectives of this work were to compare chlorophyll fluorescence quenching parameters of the selected species of different morpho-anatomical characters at two light exposure (80 and 1200  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ).

## Materials and Methods

### Plant material

The following plants were involved in our experiments: *Kalanchoe daigremontiana* Raym-Hamet & Perrier, *K. fedtschenkoi* cv. *marginata* Raym-Hamet & Perrier, *K. tomentosa* Baker *Crassula multicava* Lem. and *Cotyledon ladysmithiensis* Poelln. The leaf of *Cot. ladysmithiensis*. and *K. tomentosa* bear hairy epidermis. the other species have got hairless surface with thick cuticle. On the abaxial surface of *C. multicava* there are numerous pores of hydattodes.

The succulence index (the ratio of leaf length and leaf

thickness) is the highest in *Cot. ladysmithiensis* and lowest in *C. multicava*.

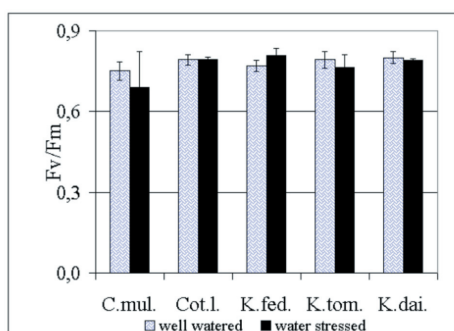
### Growth conditions

Plants were vegetatively propagated and grown in pots 9 cm in diameter in greenhouse at Botanic Garden of the University of Szeged (Hungary). The growing medium was 1:1:1 peat, compost and sand. The incident light intensity was on average 720  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Plants were watered in every three days (well watered treatment) and every 14 days (water stressed treatment). The average relative air humidity during the day was 65%. The daily average temperature was 25°C.

### Chlorophyll fluorescence measurements

In order to characterize these parameters in the photosynthesizing leaves, chlorophyll a fluorescence data were collected by means of modulated measurement technique. Chlorophyll fluorescence quenching analysis was carried out with a Dual Channel Modulated Fluorimeter (Hansatech, England) to examine photosynthetic responses of plants to various water treatments. All measurements were performed in the 730 nm emission region on the upper surface of intact leaves. Leaves to be measured were dark adapted for 20 min before the start of the measurements. AL intensities were 80 and 1200  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . The following parameters were calculated: maximum quantum yield (Fv/Fm), effective quantum yield (EQY), light induced non-photochemical and photochemical quenching coefficient (NPQ, qP) and the apparent linear electron transport rate (ETR) according to Shreiber et al. (1986) and Genty et al. (1989). The water stressed plants were measured just

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**Figure 1.** Chlorophyll fluorescence quenching parameter of Fv/Fm. Abbreviations: C.mul.: *Crassula multicaeva*, Cot. l.: *Cotyledon ladysmithiensis*, K. fed.: *Kalanchoe fedtschenkoi* cv marginata, K. tom.: *Kalanchoe tomentosa*, K. dai.: *Kalanchoe daigremontiana*.

on every 14<sup>th</sup> days, the well watered plants were measured between waterings.

### Statistical Analyses

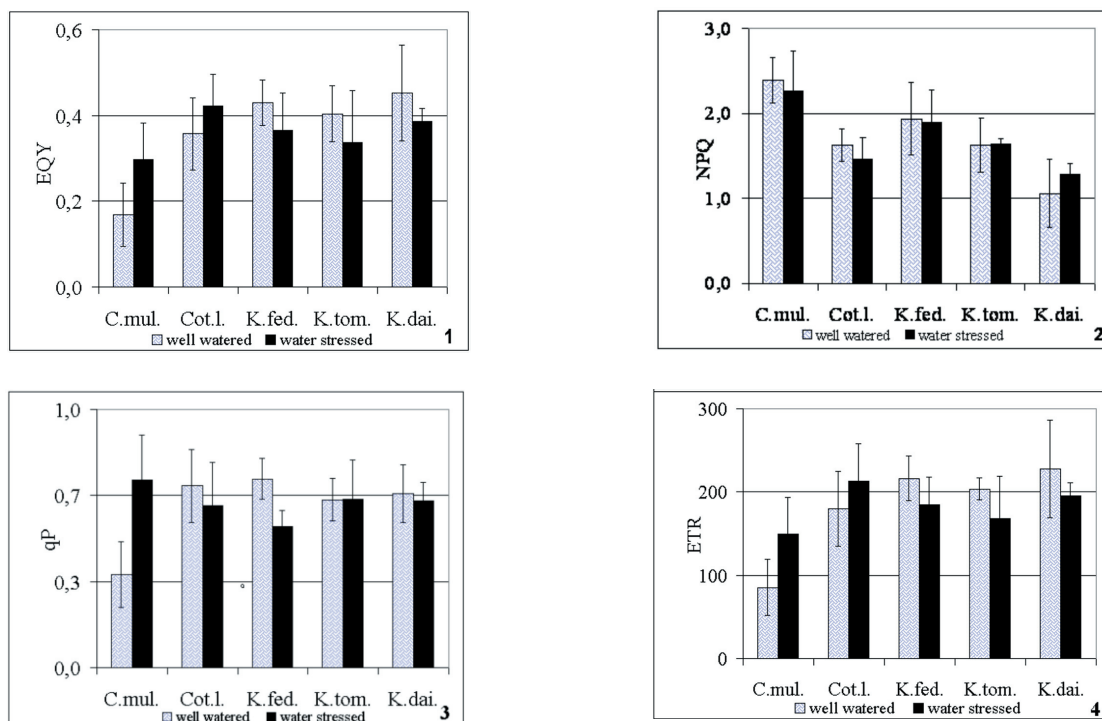
Differences among the five species and between the two water treatments were analysed by a two-way factorial ANOVA, and post hoc comparisons were performed by Newman-Keuls tests in STATISTICA 6.0.

## Results and Discussion

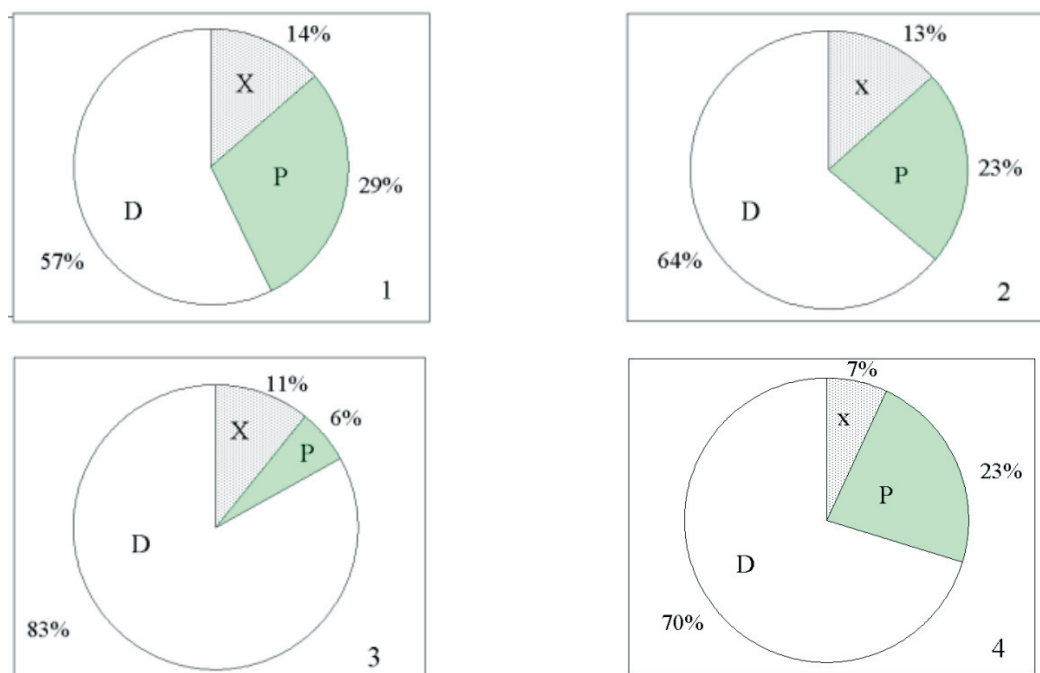
Generally, CAM plants are characterized with a four-phase framework of the CO<sub>2</sub> assimilation: it means, that the photosynthetic parameters and the organic acid fluctuations show considerable disparity during the day as a result of periodic stomata opening. In order to avoid the impact of the changes of these periods, our measurements were carried out only between 9 am and 01 pm. At 80 μmol m<sup>-2</sup>s<sup>-1</sup> AL intensity there were no significant differences in fluorescence parameters either among the species or between growing conditions. The Fv/Fm (Fig. 1) measured after 20 min dark adaptation, showed no considerable difference among the species examined, which is suggested, that the relative number of active PSII reaction centers are similar.

The EQY, NPQ, qP and ETR (Fig. 2) seemed to be not as stable feature as the Fv/Fm. In *C. multicaeva*, the well watered variety realizes lower value of EQY (p<0.05), qP (p<0.05) and ETR (p<0.05) than the other plants. In *C. multicaeva* as a result of the water deficit the qP value has raised also at a significant level (p<0.001). The NPQ of *C. multicaeva* was the same on the two water levels, and these values exceeded that of the other species.

To get more information about the divergent parameters of *C. multicaeva*, we calculated the fractions of the excitation energy absorbed in the PSII antennae allocated to PSII



**Figure 2.** Chlorophyll fluorescence quenching parameters at 1200 μmol m<sup>-2</sup>s<sup>-1</sup> in well watered and water stressed plants. 1: effective quantum yield of linear electron transport (ΔF/Fm'), 2: non-radiative energy dissipation (NPQ), 3: the photochemical quenching parameter (qP), 4: apparent linear electron transport rate of PSII (ETR). Abbreviations: see: Fig. 1.



**Figure 3.** Allocation pattern of excitation energy absorbed in the PSII antennae to PSII photochemistry (P)=(Fv'/Fm')\*qP, thermal dissipation (D=1-(Fv'/Fm')) and excess excitation (X)=1-(D+P) in the leaves. 1-2: average of the species *Cotyledon ladysmithiensis*, *Kalanchoe tomentosa*, *Kalanchoe daigremontiana*, *Kalanchoe fedtschenkoi* cv marginata 1: well watered treatment, 2: water stressed treatment 3: well watered *Crassula multica*, 4: water stressed *Crassula multica*.

photochemistry (P), thermal dissipation (D) and excess excitation (X) according to the work of Demmig-Adams et al. (1996) and Váradi et al. (2003). Although the plants except *C. multica* did not show significant differences, we calculated the average of the well watered and water stressed varieties of *Kalanchoe daigremontiana*, *K. fedtschenkoi* cv. *marginata*, *K. tomentosa* and *Cot. ladysmithiensis* and these data were compared with that of *C. multica*. Considerable differences were not observed between the average values of the four plants in water treatments (Figs. 3.1 and 3.2). In *C. multica*, however, we found that extremely low fraction of the excitation energy used for photochemistry absorbed in the PSII antennae under well watered condition (Fig. 3.3). This value greatly (three fold) increased at water deficiency (Fig. 3.4) indicating, that *C. multica* used the absorbed light energy better for photosynthesis under stress.

According to our preliminary studies, the cause of this phenomenon is not well known yet. CAM plants are believed not sensitive to water deficiency, so it was surprising that *C. multica* exhibited severe wilting and considerable differences in photosynthesis after relative short (14 days long) water deficit. Further studies are needed to understand the deeper background of its special physiological characters. It is supposed, that the other species need much more prolonged dry period for the development of the physiological symptoms of water stress.

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