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Effect of dithiothreitol (DTT) and diethylditiocarbamate (DDC) on watered and water stressed bean leaves.

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Introduction

Photoinhibition resulting from down regulation of photosynthesis or damage, not only depends on the absorbed light intensity, but most importantly on the extent of the energy absorbed in excess. This means that moderate light intensities are likely to induce reductions in quantum yield if they are accompanied by other environmental restrictions to carbon assimilation, such as low temperature and water stress. Transient water vapour deficits induce stomatal closure, and a reduction in the CO_2 concentration at the carboxylation sites in chloroplasts, therefore, inducing an overexcitation of the photosynthetic apparatus, particularly photosystem II (PSII) and to an increased NADPH/NADP ratio (Giardy et al. 1996). Both effects might be responsible for the formation of reactive oxygen species like excited singlet oxygen and superoxide, as a result of energy donation from excited triplet chlorophylls at the PSII level and reduction of oxygen at the PSI electron acceptor side (Foyer et al. 1994, Asada, 1999)

Potosynthetic organisms have evolved mechanisms to protect themselves against photodamage. Non-photochemical energy dissipation has been described as the most important form of protection, resulting in a lower quantum yield of photosynthesis, similar to photoinduced damage, except that no maximal photosynthetic rate reduction is observed. Also, scavenging of active oxygen species has been informed as an important strategy to avoid damage of the photosynthetic apparatus. Other mechanisms for protection have been decribed, however, like chlorophyll concentration changes in order to reduce the extent of the absorbed light; chloroplast movements, reducing the organelle and photosynthetic complexes exposure to light; and leaf movement or paraheliotropism, avoiding direct exposure to sun, therefore avoiding light and heat. Beans are a sensitive species to many environmental constraints, being water availability decisive on sowing season and geographical area for production. It is commonly observed that water stress in the field is accompanied by high irradiance and high temperatures, exacerbating the water vapour deficit and, therefore, resulting in stomatal closure and low capacity for CO_2 reduction. Both, high temperature and high incident light are common to bean production areas.

Infiltration of dithiothreitol (DTT) and diethylditiocarbamate (DDC) in watered and water sterssed bean leaves have been used as a means to preliminary evaluate the importance of some of the protective mechanisms described under water stress conditions and moderate light.

Materials and methods

Bean plants, cv. Orfeo-Inia, were grown in a controlled chamber with a 12 hour photoperiod under 500 µmol m^{-2} s^{-1} PAR light, 24/18 °C day/night temperature and 50% relative humidity. Plants were watered with Hoagland - 1 solution until the second trifoliate leaf reached the third of his full size. Then, some plants continued receiving water in the same volume and frequency than nutritious solution, while the rest received a third of that amount. The former reached, after two weeks, a total leaf water potential of -0.6 MPa (watered plants), and the latter –1.2 MPa (water stressed plants). Leaves dark-adapted for 24 hours were infiltrated with solutions of either, 3 and 5 mM DTT, 10 mM DDC or distilled water. For fluorescence measurements, leaf discs from infiltrated leaves were placed inside a temperature controlled chamber, at 24 °C and illuminated for 20 min with 120 µmol m^{-2}s^{-1} PAR or 850 µmol m^{-2}s^{-1} PAR actinic light from a cold lamp. Chlorophyll fluorescence was monitored with a modulated fluorimeter (FMS1, Hansatech) and quenching parameters determined every 5 min by means of high intensity light pulses. After illumination, pulses were continued for further 20 min to determine the recovery of maximal fluorescence. CO_2 assimilation was also assessed by means of an IRGA (ADC-i) under the same light intensities than for fluorescence.
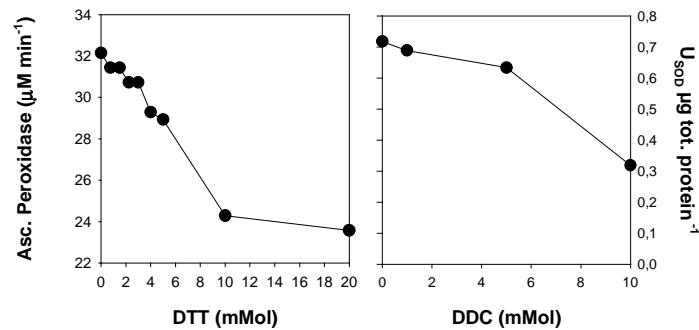
Results and discussion

Fig. 1: Effect of DTT and DDC infiltration on the activity of the enzymes ascorbate peroxidase and Superoxide dismutase from bean leaves.

DTT inhibits the violaxanthin deepoxidase which, together with the protonation of the thylakoid lumen, modulates the thermal dissipation processes in PSII complexes known as an effective protective mechanism against excess light. Results from DTT infiltration must be interpreted carefully though, since it interferes with sulphur groups. As shown in Fig. 1, concentrations of DTT used, reduced the activity of ascorbate peroxidase in 5 and 10 % at 3 and 5 mM DTT respectively. Also in Fig. 1, the activity of Cu-Zn SOD is reduced in a 56% upon infiltration with 10 mM DDC, one of the key enzymes for scavenging of superoxide.

In low intensity light, no significant differences were observed in CO_2 assimilation rates between watered and water stressed plants (Fig. 2) in any infiltration treatment. Stomatal conductance affected net photosynthesis in stressed plants only in high light (data not shown) resulting in lower rates compared to the watered. Diffrences were significant only in control and 3DTT plants (Fig. 2). Even though no significant
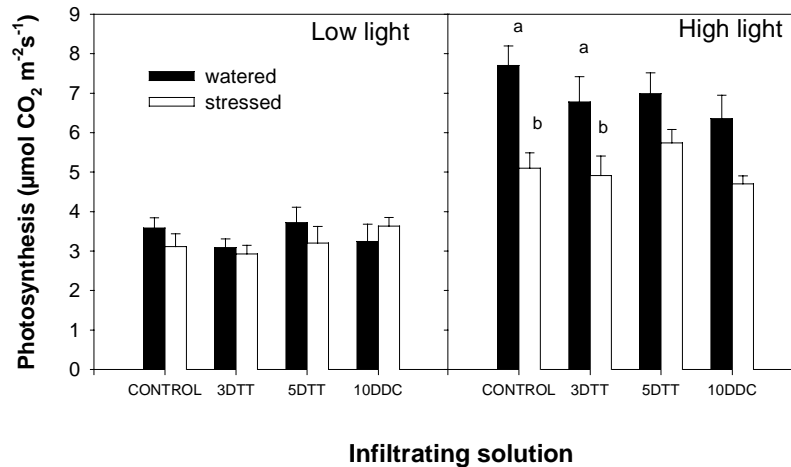

Fig. 2. CO_2 assimilation rates in low light (left) and high light intensity (right) in infiltrated leaves from watered and water stressed plants.

differences were observed in high light between watered plants, lower rates were observed in the infiltrated leaves compared to control.

As for qN, in watered leaves, only the higher DTT concentration in high light, reduced the extent of the parameter (Fig 3), while in the stressed, both 3 and 5 mM DTT reduced qN formation. Interestingly, in low light stressed leaves, DDC increased the parameter at the end of the illumination period compared to control.
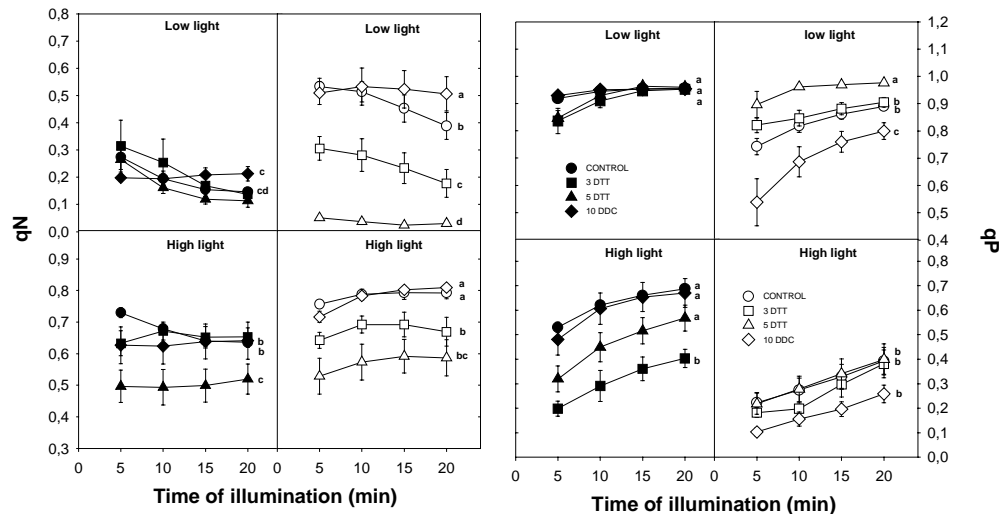

Fig. 3. qN (left) and qP (right) in watered and water stressed leaves illuminated for 20 min. with low (upper panels) and high (lower panels) intensity light, upon different infiltration treatments. Symbols as shown in qP plots.

Even though no changes in qN were observed in high light watered plants, after 3 mM DTT, qP was strongly reduced, even further than the 5mM DTT. In this case, a balance between de-epoxidation and thylakoid lumen protonation might be involved. The low qP value in stressed leaves was not further reduced by DTT in high light, instead, the steady state fluorescence increased (data not shown). In the low light stressed plants, qP is either maintained or increased by DTT at the end of the 20 min illumination period (Fig. 3). This, together with the higher recovery of Fm' in the same plants (Fig. 4), suggests that in low light, qN could interfere with the activation of assimilatory enzymes, which in turn could limit linear electron transport.

Fig. 4. Fm´measured after the 20 min illumination, for a period of 1200 s in watered (left panels) and stressed (right panels) leaves. Values were normalized to Fm measured at the begining of the illumination. Symbols as in Fig. 3.
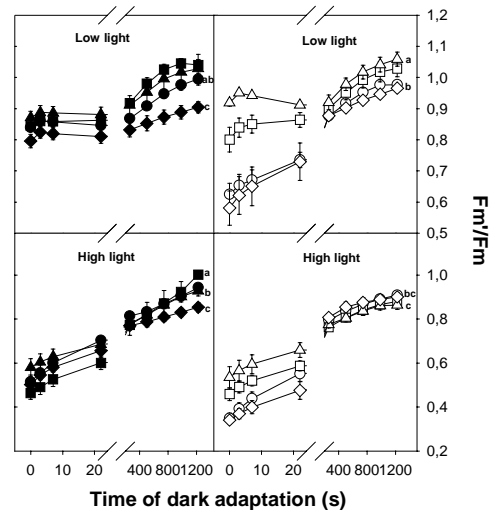
In watered plants, DDC negatively affected the Fm´ recovery after 20 min darkness (Fig. 4) in high and low intensity light, but not in the stressed leaves, reaching similar values than the observed for control. The light intensities used, for 20 min, were not enough to affect the capacity of leaves to recover the maximum fluorescence value upon infiltration with 3 and 5 mM DTT. However, a mild decrease in the activity of the Cu-Zn SOD, resulted in lower Fm´ values in the watered plants. The results, suggest that watered plants are more sensitive to inhibition of SOD inhibitors compared to stressed plants.

References

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