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Plasticity of young *Moringa oleifera* L. plants to face water deficit and UVB radiation challenges

Márcia Araújo [a], Conceição Santos [a], Maria Costa [b,c], José Moutinho-Pereira [d], Carlos Correia [d], Maria Celeste Dias [b,e,*]

[a] Department of Biology & GreenUP/CITAB-UP, Faculty of Sciences, University of Porto, Rua Campo Alegre, 4169-007 Porto, Portugal
[b] QOPNA and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Ministério da Educação, Dili, Timor-Leste
[d] CITAB & Department of Biology and Environment, University of Trás-os-Montes e Alto Douro, Apartado 1013, 5001-801 Vila Real, Portugal
[e] Centre for Functional Ecology (CEF), Department of Life Science, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

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ABSTRACT

Climatic change challenges are predicted to increase combined episodes of drought, heat and higher UVB-radiation, which will impact crops/plants production. *Moringa oleifera* is a major source of food/economy in underdeveloped regions, and its tolerance to high UVB radiation (UVB) and water deficit (WD) remains unclear. We exposed two-month old plants to: a) WD for 4 days; b) UVB radiation for 3 days (26.3 kJ m^{-2}); c) WD + UVB in combination for 4 days (the UVB stress was imposed at the 2nd day of WD). One and ten days after the end of stresses the following parameters were measured: plant growth, water-status, chlorophyll *a* fluorescence, leaf gas-exchange, pigments, carbohydrates, cell-membrane-stability, phenols, malondialdehyde content and antioxidant capacity (including antioxidant enzymes). Stress exposure did not affect plant water status. One day after the end of the stresses, net CO_2 assimilation rate dropped in all treatments, but after 10 days an overall recovery was observed, except in plants exposed to UVB treatment. The plants exposed to UVB showed, in general, more severe effects, increasing pigment content and MDA, while no changes were observed in the total antioxidant capacity (after 1 day). Our data suggest that young *M. oleifera* plants present some tolerance to WD or UVB radiation and that the combination of the two stresses led to lower stressful responses than the UV stress imposition alone (photosynthesis, pigments, starch and antioxidant capacity). The lower outcome shown in the combined stresses suggest that this species is able to cope multiple stresses, and that a previous acclimation (possibly activating the antioxidant capacity) to one of the stresses may play an important role in this tolerance.

1. Introduction

Since Industrial Revolution, anthropogenic activity increasingly releases to atmosphere gases with greenhouse effect (e.g. CO_2 and other gases), increasing the risk of global warming together with damages on the stratospheric ozone layer. This combination of increasing global warming and ozone degradation may lead to drought episodes together with an increase of exposure to ultraviolet B (UVB) radiation [1].

In general, when exposed to osmotic stress (e.g. drought, salinity) plants have to adjust their metabolism, which may involve decrease of growth, changes in morphology or even lead to leaf decay [2–4]. Photosynthesis is particularly sensitive to abiotic stresses, with implications at chloroplast structure and function as well as at stomatal aperture and mesophyll conductance [5]. This impairment decreases plant nutrition and carbon assimilation and, consequently, plant productivity [2]. A primary cell target of most abiotic stresses (e.g. UV, heat, drought stress) is the increase of reactive oxygen species (ROS), and therefore, the antioxidant system is in general also affected. This was well shown for plants under water deficit (WD) [2,5], salinity [6], metals [7] and UVB radiation [8,9]. This overproduction of ROS stimulates the cellular antioxidant system, including enzymes like superoxide dismutase (SOD), catalase (CAT) or glutathione reductase (GR). When the antioxidant battery is insufficient to impair ROS-induced damages, an unwanted and excessive oxidation of macromolecules may then occur. For example, a first target of lipid peroxidation may include cell membrane, whose damage may lead to cell death [10].

Abbreviations: ABTS, 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); CAT, catalase; Chl, chlorophyll; C_i/C_a, ratio between intercellular and atmospheric CO_2 concentration; F_v/F_m, maximum efficiency of photosystem II; FW, fresh weight; F_0, minimal fluorescence yield; GR, glutathione reductase; MDA, malondialdehyde; PPFD, photosynthetic photon flux density; ROS, reactive oxygen species; RWC, relative water content; SOD, superoxide dismutase; TSS, total soluble sugars; UVB, ultraviolet B radiation; WD, water deficit; WD + UVB, water deficit and ultraviolet B radiation.

* Corresponding author at: Centre for Functional Ecology (CEF), Department of Life Science, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.
E-mail address: celeste.dias@uc.pt (M.C. Dias).

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UVB radiation is important to plant photomorphogenic signalling and normal development [9], but excessive levels can lead to uncontrolled oxidative stress and to damages of the photosynthetic apparatus and/or genotoxicity [11–13]. Besides leaf thickness and waxes deposition, plant cells have developed other defence mechanisms to deal with UVB radiation, including UV-screening pigments, increased antioxidant battery, and/or accumulation of phenolic compounds [8,13–15].

Moringa oleifera (also known as *Moringa pterygosperma* Gaertn) belongs to the Moringaceae family [16]. This species is native from south Himalaya region [17]. *M. oleifera* can be found, native or introduced, in several countries of tropical and subtropical areas [10,18], currently under environmental pressure of increased drought and high UVB radiation (e.g. Africa, South-east Asian regions). This species is particularly beneficial due to its multiple uses, including as food source and ethnobotany [16], being mostly known for its high nutritional value and antioxidant properties [17]. *M. oleifera* represents an important crop for local populations in regions with high vulnerability to desertification and is an important commercial species for agro-pharmaceutical industry. To our knowledge, the degree of tolerance of this species to climate changes, including combination of WD and UVB, remains unknown. However, a recent study showed that young plants of *M. oleifera* have some tolerance degree to WD by maintaining a high relative water content (RWC) and water use efficiency, increasing the levels of photosynthetic pigments and balancing the antioxidant system [10]. In this study, we hypothesized that young *M. oleifera* plants may cope with stresses related to climate changes, such as WD, UVB and WD + UVB, by adjusting carbon metabolism and antioxidant battery.

2. Material and Methods

2.1. Plant Material and Experimental Conditions

M. oleifera seeds, provided by the Ministry of Agriculture of East Timor, were soaked in 10% commercial bleach (NaClO < 5%) for 15 min, washed 3 times, and then embedded in distilled water for another 15 min before sowing. Seeds were germinated in a mixture of turf and perlite (2:1 w/w) in plastic pots (≈300 ml). During four weeks, cultures were maintained in a greenhouse with approximately 150 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) with a photoperiod of 16 h/8 h (day/night) and 20 ± 3 °C. Plants were then transferred to a chamber with the same photoperiod, at 23 ± 1 °C, relative humidity of 40% and a PPFD of 150 μmol m^{-2} s^{-1}. Juvenile plants with the same height (36 ± 5 cm) and age (≈2 months) were, prior the treatments, watered to field capacity (≈100 ml).

For the treatments, plants were assigned between four conditions: 1) well-watered without ultraviolet radiation (Control); 2) water deficit (WD) — plants were not irrigated for four days; 3) ultraviolet B radiation (UVB) — plants were irrigated as in control and exposed for three days to a total UVB irradiation of 26.3 kJ m^{-2}; 4) WD combined with UVB radiation (WD + UVB) for four days — plants were exposed to WD in the first day and in the following three days were also exposed to a total UVB irradiation of 26.3 kJ m^{-2} (Table 1). For all UVB exposures, ten UVB lamps (Model G8T5E, Sankyo Denki, Kanagawa, Japan) with an energy spectrum of 280–320 nm and a maximum emission at 306 nm were used as UVB source. UVB exposure was monitored by using a VLX 312 radiometer equipped with a UVB sensor (Vilber Lourmat, Marne-la-Vallée Cedex, France).

One day after the end of the stress exposure (1 d post stress) plant height, RWC, cell membrane permeability, gas-exchange and chlorophyll fluorescence were measured in plants of all treatments, and additionally, leaf samples were also collected, frozen in liquid nitrogen and maintained at −80 °C for further determination of lipid peroxidation, antioxidant enzyme activities, phenol content and the total antioxidant activity. Immediately after that, plants from treatments WD and WD + UVB were re-watered by adding water to the soil until field capacity was reached. Then, every two days, plants were irrigated to field capacity during 10 days. After this period of recovery (10 days post stress) all the measurements and sample storage described previously for day 1 were also performed at day 10.

Table 1
Experimental conditions. Plant distribution between groups.

After stress	Treatment	*n*	Water supply	UVB exposure
1 day	Control	8	Yes	No
	WD	8	No	No
	UVB	8	Yes	Yes
	WD + UVB	8	No	Yes
10 days	Control	8	Yes	No
	WD	8	No	No
	UVB	8	Yes	Yes
	WD + UVB	8	No	Yes

WD: 4 days without watering; UVB total irradiance: 26.3 kJ m^{-2}/3 days.

For each parameter (except chlorophyll *a* fluorescence and gas exchange) samples of all conditions were weighed and the values normalized for weight unit. For chlorophyll *a* fluorescence and gas exchange the same leaf areas were used in all conditions, and the values normalized for area unit.

Concerning the control treatment, two groups of control plants were used, one for day 1 and other for day 10 (Table 1). Since no significant differences were observed between these groups for the two sampling days, all data were gathered (day 1 and 10) and only one control group is presented.

2.2. Plant Growth and Water Status

Plant shoot height (cm) was measured before (BE) and after (AE) the experiments. Relative growth increment was calculated as the growth rate $(\%) = (\frac{(AE-BE)}{BE}) \times 100$ [19].

Leaf fresh weight (FW), turgid weight (TW) and dry weight (DW) were also determined. Leaf relative water content (RWC) was calculated as RWC $(\%) = \frac{(FW-DW)}{(TW-DW)} \times 100$ [20].

2.3. Carbon Metabolism

2.3.1. Chlorophyll a Fluorescence and Gas Eexchange

Chlorophyll *a* fluorescence was measured on *M. oleifera* leaves using a pulse amplitude modulation system (FMS 2, Hansatech Instruments, Norfolk, England). Minimum fluorescence (F_0) was measured in 30 min dark-adapted leaves by applying a weak modulated light, and maximum fluorescence (F_m) was measured after applying a 0.7 s saturating pulse of white light (>1500 μmol m^{-2} s^{-1}). The maximum quantum efficiency of photosystem II was calculated as $^{Fv}/_{Fm} = \frac{(Fm-F0)}{Fm}$ [20]. Leaf gas exchange measurements were performed using a portable infrared gas analyser (LCpro+, ADC, Hoddesdon, England) under the same conditions of growth. Net CO_2 assimilation rate, stomatal conductance, ratio between intercellular and atmospheric CO_2 concentration (C_i/C_a) and transpiration rate were determined [13].

2.3.2. Pigments Quantification

Briefly, frozen leaves powder were extracted in an acetone/50 mM Tris buffer (80:20 v/v, pH = 7.8), centrifuged during 5 min at 5000 ×*g* at 4 °C. Absorbance was read with a Thermo Fisher Scientific spectrophotometer (Genesys 10-uv S) at 470, 537, 647 and 663 nm. Concentration of Chlorophyll *a* (Chl_a), Chlorophyll *b* (Chl_b) and Carotenoids (Car) were calculated according to Sims and Gamon [21].

2.3.3. Total Soluble Sugar (TSS) and Starch Content

Soluble sugars were extracted from frozen powder leaves with 80% (v/v) ethanol, in an 80 °C bath over an hour [22], and then centrifuged at 5000 ×*g* during 10 min at 4 °C. The supernatant was incubated with an anthrone solution during 10 min at 100 °C. The mixture was