Is a cumulative exposure to UV-C more harmful than a single one on romaine lettuce chlorophyll *a* fluorescence?

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Abstract

In this work we aimed to compare the effect of the exposure to a single dose of UV-C and the multiple doses on the romaine lettuce chrlorophyll *a* fluorescence. After harvest, the romaine lettuces were divided into 3 batches, a control one which not receive UV-C radiation, the second was exposed to the dose of 8.57 $kJ.m^{-2}$ and the third was exposed to a cumulative dose of 8.57 $kJ.m^{-2}$ for one week reaching the dose of 59.99 $kJ.m^{-2}$. Our results of the Chlorophyll *a* fluorescence showed a significant decrease in the 3rd batch.

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Introduction

The development of the life of terrestrial plants is possible thanks to the development of the ozone layer which plays the role of a screen face toUV radiation at the level of the

stratosphere. This layer, acting as a UV filter, absorbs UV-C sun rays (whose wavelength is less than 280 nm and part of the UV-B solar radiation (whose wavelength varies between 280 nm and 315 nm).The interest in the study of UV effects on plants is becoming more and more important. These wavelengths are biologically active but they are also the most aggressive with respect to the changes that the ozone layer undergoes. It is for these reasons that the UV levels perceived by plants depend on the latitudes and duration of exposure in other words the intensity of exposure (Houghton et *al* 2001). A radiation dose of UV- A is less effective than UV-B and UV-C in order to induce plant reactions (Barta et *al* 2004). The dose or fluence depends on the lamp power and the exposure time. The dose is obtained simply by multiplying the power by the time.

Photosynthesis, common to all chlorophyll plants, is affected by this hole in the ozone layer. UV rays cause the photosynthesis to slow down and thus endanger the growth of the plant. Exposure to long-term UV radiation has irreversible effects on plant growth, enzymatic activity, carbohydrate content. The most serious effect is on chloroplasts, which would explain the photosynthesis. However, the application of UV-C on post-harvest fruits and vegetables is intended to prolong their life, increase their resistance to pathogens and improve their nutritional quality. The effects of UV-C vary according to the applied doses and the plant species studied. It is therefore necessary to test different doses of UV-C to choose a suitable dose and the effects of which do not alter the conservation of the plant product**.**

Materials and methods

Plant material and growing conditions

Lactuca sativa L. is an annual herbaceous plant belonging to the *Compositae* (*Asteraceae*), one of the largest and most diverse families of flowering plants, comprising one-tenth of all known angiosperm species. Lettuces were grown under cover in a tunnel covered with 200 micron made polythelene film. Mulching was used and was micro- and macroperforated with a density of 14 plant.m^{-2}

UV-C treatment

The UV-C treatment was performed with a UV-C lamp delivering 254 nm to a bank of two 60-W germicidal UV lamps (VL-215 C 254 nm, Vilbert Lourmat, France) equipped with a

254-nm bandpass filter at 1.4 mW. cm^{-2} mounted at a distance of 10 cm from plants. The intensity of irradiation was determined using a photo-radiometer (HD 2102.2; Delta OHM, Padova, Italy). After treatment, control and UV-C-treated plants were stored in a growth chamber at a day/night temperature of 22/16 °C for 16 and 8 h, respectively.

Chlorophyll *a* fluorescence measurement

Fast kinetics of chlorophyll a fluorescence of photosystem II were established on dark-adapted leaves of the control plant material using a Handy PEA (Hansatech, Norfolk, U. K). Dark adaptation lasted 20 min and was followed by exposure to a saturating flash of 3000 µmol photons m^{-2} s^{-1}. In addition to maximal quantum efficiency of dark-adapted leaves (Fv/Fm), we determined the Performance Index on absorption basis (PI) and its constituents, RC/ABS, the amount of active photosystem II (PSII) reaction centres per absorbed energy flux, Fv/F0, the quantum yield of primary photochemistry, and [(1-VJ)/VJ] which represents the efficiency with which a PSII trapped electron is transferred from QA to QB (Srivastava and Strasser 1999; Stirbet 2011; Strasser et *al* 2000; Strasser et *al* 2004). The PI, based on the analysis of the fast fluorescence rise, measured from 50 ms to 1 s upon illumination of photosynthetic samples, is considered as a much more sensitive and discriminating stress indicator than Fv/Fm (Lee 2007).

Results

The Handy PEA makes possible to measure the state of the plants by measuring the two stress indicators, the maximum quantum yield (FV / Fm) on the one hand and the PI, with its three components (RC / Abs) , (1-Vj) / Vj and Fv / F0.

The maximum quantum yield (Fv / Fm) is the criterion most commonly used to characterize the state of stress. It actually provides information on the reduction rate of the plastoquinone pool (PQ). Fv / Fm only accounts for stresses affecting photosystem II (PSII).

Measurements showed a decrease in Fv / Fm, but only more pronounced in the leaves exposed to the cumulative doses of UV-C during 7 days. This reduction, thus causes a reduction in the photo protection of the plant.

The other parameter for assessing stress is PI, the photosynthetic performance index. Our results show clearly that exposure to UV-C irradiations make a general perturbation of the photosynthetic machinery, but only in the third batch, the concentration of active (RC / Abs) reaction centers (Tab. 1), the quantum yield of primary photochemistry (Fv / F0) (Tab.1), and a decline in electron transfer by trapped photon ((1-Vj) / Vj) in response to these UV-C doses. These effects lead to a decrease in the index of photosynthetic performance (PI), which supports the hypothesis of the installation of a state of stress in these plants.

Tab. 1. Parameters derived from chlorophyll fluorescence measurements performed on dark adapted leaves of lettuce plants exposed to three doses of UV-C.. Data represent means ± standard errors. *n*=12. Different letters indicate significant differences at the p = 0.05 threshold. Differences for data in italics were significant at the p = 0.1 threshold

UV-C doses, $kJ.m^{-2}$	0	8.57	59.99
F_v/F_m	0.84 ± 0.01^a	0.83 ± 0.03^a	0.79 ± 0.04^b
PI	2.27 ± 0.08^a	2.1 ± 0.01^b	1.9 ± 0.1^b
F_v/F_0	5.41 ± 0.37^a	5.31 ± 0.11^a	5.16 ± 0.06^b
RC/ABS	0.73 ± 0.02^a	0.88 ± 0.02^b	0.89 ± 0.02^b
$(1-V_J)/V_J$	0.54 ± 0.1^a	0.53 ± 0.06^a	0.49 ± 0.06^b

Discussion

The effect of UV-C radiations on the photosynthetic status is not very investigated in scientific literature unlike UV-B radiations which are highly studied. In fact, studies on *Sorghum*, *Sorghum vulgare*, which was field-grown under ambient levels and increased UV-B radiation (the additional daily dose corresponds to a 20% reduction of the stratospheric ozone column). In order to evaluate the impact of the increase in UV-B radiation, several parameters were determined. They determined the biomass, the levels of photosynthetic pigments, flavonoids and ascorbic acid, as well as the activities of peroxidase and catalase. The analysis of gas exchange indicates that the decrease in photosynthesis comes from a stomatal

limitation. The plants were exposed to UV-B radiation for 60 days, which resulted in a reduction of chlorophyll pigments and carotenoids (Ambasht et *al* 1998) However, others suggest that UV-B irradiation in pea leaves, *Pisum sativum*, has no specific effect on enzymes in the biosynthetic pathway of chlorophyll, but rather influences the genetic regulation of Chlorophyll thus causing its destruction. During this study, the pea plants were either illuminated by visible light supplemented by UV-B light doses, or illuminated by a control light after a short exposure to UV-B for a few hours. (Strid et *al* 1992). Indeed, in plants, UV-B radiation affects several points by their harmful effects especially chloroplasts which are organelles containing chlorophyll, localized in the cytoplasm of cells of green plants. The chloroplast is known as the seat of photosynthesis. In the chloroplast, the thylakoid membrane is much more sensitive to UV-B radiation than the photosynthetic activity of the components within this membrane several components of the thylakoid membrane are affected by UV radiation -B. The activity of the ATP synthase and the performance of the photosystem II are affected by UV-B radiation, in fact there is a decrease in the ATP synthase activity and an irreversible dysfunction of the photosystem II activity..The activity of 1.5 Ribulose Biphosphate carboxylase is affected by UV-B radiation and in the majority of cases the effect of these radiation is harmful (Jordan et *al* 1992). UV-B radiation is a minor component of the solar spectrum, but it has the potential to disproportionately affect metabolic processes in animals, humans, plants and microorganisms. In plants, UV-B can interfere with growth, development, photosynthesis, flowering, pollination and transpiration (Roezma et al 1997).

However, these UV rays inhibit the production of fresh material. This inhibiting effect on fresh material production and leaf number may be due to damage to photosynthetic machinery (Krizek et *al* 1998). Other more recent studies suggest that this is not the case. Indeed, other explanations are needed, such as the hypothesis that the anthocyanin biosynthesis which absorbs in the spectrum of photosynthesis reduces the photosynthetic capacity of the leaves. Another explanation may be that the Production of secondary metabolites acts in direct competition for the assimilation of the carbon necessary for the growth of the plant. (Tsormpatsidis et al 2007).

The application of UV-C on fruits and vegetables postharvest aims to delay their decay, increase their resistance against pathogens (Ouhibi et *al* 2014) and improve their nutritional quality (Artés-Hernándeza et *al*,. 2010). The effects of UV-C vary with applied doses and plant species studied. It is therefore necessary to test different doses of UV-C to select a suitable dose and whose effects do not affect neither the conservation of plant product nor its photosynthetic performance. But recently, one work was carried out to (*1*) apply different

doses of UV-C on leaves of Romaine lettuce variety once a day for a week, (*2*) monitor indicators of stress using a Handy PEA, and (*3*) visually assess the appearance of leaves. The monitoring the visual appearance of the leaves shows that only plants exposed to a dose of 0.85 $kJ.m^{-2}$ have an appearance similar to that of control plants. In contrast, plants exposed to doses of 1.71 $kJ.m^{-2}$ and 3.42 $kJ.m^{-2}$ have leaves with many necrotic spots from the third day. Because of their highly aggressive, 1.71 and 3.42 $kJ.m^{-2}$ doses can not be maintained for the rest of our experiences while 0.85 $kJ.m^{-2}$ appears to represent a suitable dose (Ouhibi et *al* 2013). This dose was studied to enhance also the resistance of romaine lettuce on *Botrytis cinerea* and *Sclerotinia minor* (Ouhibi et al 2014).

Our results show clearly that either the UV-C dose applied (8.57 $kJ.m^{-2}$) or the cumulative doses (59,99$kJ.m^{-2}$), can not be used to enhance the nutritionnal status of romaine lettuce during storage since they alter the photosynthetic machinery.

Conclusion

Our results show clearly that a cumulative effect of UV-C doses alters the status of chlorophyll fluorescence, and this is mainly by affecting the photosynthetic index.

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