Effects of quercetin and enhanced UV-B radiation on the soybean 
(Glycine max) leaves

Yao Yin An1,2, Zu Yan Qun3, *Li Yuan1,3

1 The Center for Agricultural Biodiversity Research and Training of Yunnan Province, 
Yunnan Agricultural University, Kunming 650201, P. R China
2 Chengdu Research Institute of Biology, Chinese Academy of Science, Chengdu 610041, P. R. China
3 Eco-environment Research Institute, College of Resources and Environment, Yunnan Agricultural University, Kunming 650201, P. R. China
* Corresponding author e-mail: liyuan03@yahoo.com.cn; liyuanzu@public.km.yn.cn, Fax: 0086 871 5227942

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Abstract

The possible ameliorative effects of quercetin on soybean (Glycine max (L.) Merr.) leaves exposed to UV-B radiation were conducted in greenhouse. The symmetrical leaves supplied with quercetin solution (0.2 %, 1 %) were exposed to UV-B radiation (0, 3.5, 6.5 kJ m⁻² d⁻¹). 0.2 % quercetin ameliorated leaf photosynthesis, improved leaf water content (LWC), and decreased lipid oxidation. The unfavorable effect on photosynthetic parameter was displayed in 1 % quercetin treatment. The effect of quercetin on phenylalanine ammonia lyase (PAL) activity varied with the quercetin concentration, UV-B radiation intensity and leaf development. In the later development polyphenol oxidase (PPO) activity was increased significantly by quercetin treatments. We suggested that quercetin with suitable concentration could serve as UV-B protective agent partly due to its antioxidant capacity.

List of abbreviations: UV-B ultraviolet-B; NPR Net photosynthetic rate; MDA malondialdehyde; PAL phenylalanine ammonialyase; SOD superoxide dismutase; PPO polyphenol oxidase; LWC leaf water content; ROS reactive oxygen species

Introduction

The increased levels of ultraviolet-B (UV-B) radiation reaching the Earth surface due to the depletion of the stratospheric ozone layer (Madronich et al. 1998) prompted active research on how plant organs were protected by different mechanisms (Roxema et al. 1997, Gotz et al. 1999). The plant antioxidant enzyme activities was increased to eliminate reactive oxygen species (ROS), the leaf carotenoids contents were increased to protect photosynthetic chain, and leaf flavonoid content was also induced to screen the UV-B radiation. In recent years, a series of experiments provided convincing evidence that plants subjected to UV-B radiation responded to changes in the content and ratios of different flavonoid in leaf epidermal cells, wax, hairs (Harborne et al. 2000). Some flavonoid increased much more than others (Noriaki et al. 2000), especially the flavonoid with ortho-hydroxy structure in B-ring such as quercetin and quercetin glycoside in Brassica napus and Trifolium repens (Olisson et al. 1998, Hofmann et al. 2000), luteolin in Marchantia polymorpha (Markham et al. 1998),
chlorogenic acid in *Cucumis sativus* (Norirut et al. 2000), iso-orientin acylated glucosides in *Oryza sativa* (Markham et al. 1998). The antioxidant ability of dihydroxylated flavonoids had been demonstrated in animal and *in vitro* experiment (Terao et al. 1997, Leak et al. 1997, Hertog et al. 1997). The antioxidant ability was also supposed to exist in plants (Takahama et al. 2000). Due to our lack in understanding of flavonoid function in plants, further studies would be worthwhile.

Soybean is one of the major world food crop, and the effects of enhanced UV-B radiation on the photosynthesis, plant growth, total biomass, yield and intraspecific response differences in soybean have been studied (D’surney et al. 1993, Li et al. 2002, Zu et al. 2003). Physiological functions of plants damaged by enhanced oxidative level under UV-B irradiation have been observed extensively by determination of malondialdehyde (MDA) and O$_2^-$ radical (Murphy 1990, Roxema et al. 1997). Considerable research revealed that the protective enzyme activities like PAL and peroxidase were increased under UV-B irradiation to adapt to the oxidative stress (Liu et al. 1995), and the SOD activities were changed differently according to the UV-B irradiation intensities (Tekchandani et al. 1998). In this study we grew soybean cultivars in greenhouse under different UV-B radiation with the objective to determine whether quercetin decreased leaf oxidative stress or affected antioxidant enzyme activities.

**Materials and methods**

**Plant materials and growth conditions**

The experiment was conducted in greenhouse at Yunnan Agricultural University, Kunming, P. R. China. Seeds of soybean (*Glycine max* L.) cultivars Yudou 8 were obtained from Henan Academy of Agricultural Sciences. The seeds were sowed in pot with homogeneous garden soil on March 5, 2002. Temperature was kept 20-25 °C in the day and 15-19 °C in the night, with a 14h/10h light-dark cycle. No fertilization was necessary during the experiment stage. At 25 days after sowing, plants were thinned to 5 per pot for uniformity in growth, and then the healthy seedling with first trifoliate stage was exposed to UV-B radiation for 20 days. During the growth duration, the plants received near ambient sunlight cut off UV-B radiation by greenhouse glass. The photosynthetic active radiation (PAR, 400-800 nm) at the top of the plants ranged from 150 to 400 µmol m$^{-2}$ s$^{-1}$.

**Quercetin treatment**

In the day before UV-B irradiation, the symmetric leaves of the top twig in each seedling were treated by quercetin or as its control, respectively. The leaves treated were dipped into the quercetin solution for few seconds carefully (not to injure them). The control leaves were treated with distilled water. There were two quercetin concentration: 0.2 %, 1 % (v/v), and three total daily flux of biologically effective UV radiation: 0, 3.5, 6.5 kJ m$^{-2}$ d$^{-1}$. Each quercetin treatment had its own comparison, and leaf for comparison was the symmetric leaf for the treatment. There are 4 replications for each pair of treatments (1 pot per replication). Quercetin powder was dissolved in alcohol firstly to form original solution (m/v 1:1), then diluted to 0.2 % and 1 % (v/v) by distilled water.

**UV-B radiation**

UV-B irradiation was provided by filtered Gucun brand (Gucun Instrument Factory, P R China) 30W UV lamps following the procedure outlined by Lydon et al. (1986). Lamps were suspended above and perpendicular to the pots and filtered with either 0.13 mm thick cellulose diacetate (transmission down to 290 nm) for supplemental UV-B radiation or 0.13 mm polyester plastic films (absorbs all radiation below 320 nm) as a control (Berkelaar et al. 1996). Plants under polyester-filtered lamps received no UV-B radiation and the plants beneath the cellulose diacetate filters received UV-B radiation. Cellulose diacetate filters were presolarized for 8 hour and changed weekly to ensure uniformity of UV-B transmission. The UV-B radiation was supplemented for 6 hours around noon (from 9 in the morning to 3 in the afternoon) to provide 3.5, 6.5 KJ m$^{-2}$ d$^{-1}$ biologically effective UV-B radiation (UV-B$_{BE}$). The lamp heights above the plants were adjusted weekly to maintain distance of 0.40 m and 0.60 m between the lamps and the top of the plants. The spectral irradiance from the lamps was...