Polyphenol biofortification of tea leaves by exposure to light-emitting diode (LED)

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Summary

Cuttings of tea plant (*Camellia sinensis* cv. Yabukita) grown in pots were exposed to light-emitting diode (LED) or fluorescent light as a control, subjected to extraction with H_2O:MeOH:AcOH=100:100:1, and then applied to metabolomic analysis (Agilent 6510 Accurate-Mass Q-TOF LC-MS/MS and Waters UPLC-TOF-MS). The content of coumarin, caffeine, naringenin, catechin or epigallocatechin-gallate nearly doubled with blue LED illumination for 24 hours. The content of coumarin, caffeine, naringenin or catechin increased 1.2- to 1.4-fold with red LED treatment from 48 hours up to one week. When exposed to red LED for two weeks, the caffeine content increased by 1.2 times, catechin, kaempferol and quercetin content decreased to 0.8, 0.8 and 0.5 times, respectively, and 280 constituents increased markedly while 227 compounds decreased.

Introduction

Light is an essential requirement for the growth of plants, and is utilized as an energy source for photosynthesis to assimilate CO_2 into sugars and also as a signal for the development and differentiation of cells through photoreceptors such as phytochromes, cryptochromes and phototropins (Taiz and Zeiger, 2010). It has been reported that blue light stimulates the expression of genes for phenylalanine ammonia-lyase and chalcone synthase, resulting in the accumulation of flavonoids (Kubasek *et al.*, 1992; Cominelli *et al*., 2008; Taiz and Zeiger, 2010). The use of *Arabidopsis thaliana* is advantageous given its completely sequenced genome, and has facilitated the use of DNA microarrays for the analysis of gene expression under exposure to blue or red light. The technology to produce light-emitting diodes (LEDs) has greatly advanced and these devices are now available at lower costs. This has encouraged us to develop a strategy utilizing LEDs for the biofortification of plants. The effect of blue or red light on the expression genes of enzymes involved in the biosynthesis of polyphenols or saponins differs (Kobayashi, 2007; Kobayashi *et al*., 2008a and 2008b; Ogawa *et al.*, 2009). With careful analysis of the overall results of the microarrays, we were able to generally predict which metabolic events were influenced by blue or red light in plants. On the basis of the knowledge and experience acquired with *Arabidopsis*, we have applied the use of LED illumination on tea plants, which are intensively cultivated in this prefecture in Japan.

Materials and methods

One to three young leaves of cuttings of tea plant (*Camellia sinensis* cv. Yabukita) grown in pots were exposed to irradiance generated by blue (470 nm, up to 200 μmol m^{-2} s^{-1}) or red (660 nm, up to 100 μmol m^{-2} s^{-1}) LED. White fluorescent light (up to 100 μmol m^{-2} s^{-1}) was employed as a control throughout the investigation. Following exposure to LED, leaves were ground using a mortar and pestle in the presence of liquid N_2, extracted with H_2O:MeOH:AcOH=100:100:1, subjected to sonication, and then filtered using a Millex-LG (Millipore). Compounds were analyzed by mass spectroscopy using an Agilent 6510 Accurate-Mass Q-TOF LC-MS/MS and Waters UPLC-TOF-MS with the respective software, such as MarkerLynx, and then subjected to a search using the Chemspider database.

Results and discussion

The content of phenylalanine, coumarin, caffeine, naringenin, catechin or epigallocatechin-gallate nearly doubled with blue LED exposure for 24 hours, while that of coumarin, caffeine, naringenin or catechin increased 1.2- to 1.4-fold with red LED treatment from 48 hours up to one week. When exposed to red LED for two weeks, the caffeine content increased by 1.2 times, catechin, kaempferol and quercetin content decreased to 0.8, 0.8 and 0.5 times, respectively, and 280 constituents increased markedly while 227 compounds decreased. It is concluded that exposure to monochromatic blue or red light affected the content of functional components as follows: (1) blue light enhanced the accumulation of major polyphenols, (2) red light increased the amount of caffeine, and (3) red light tended to suppress the amount of polyphenols. These observations may be applicable in strategies related to the production of tea in terms of controlling the content of antioxidant components such as polyphenols and the tea flavor determined by coumarin.

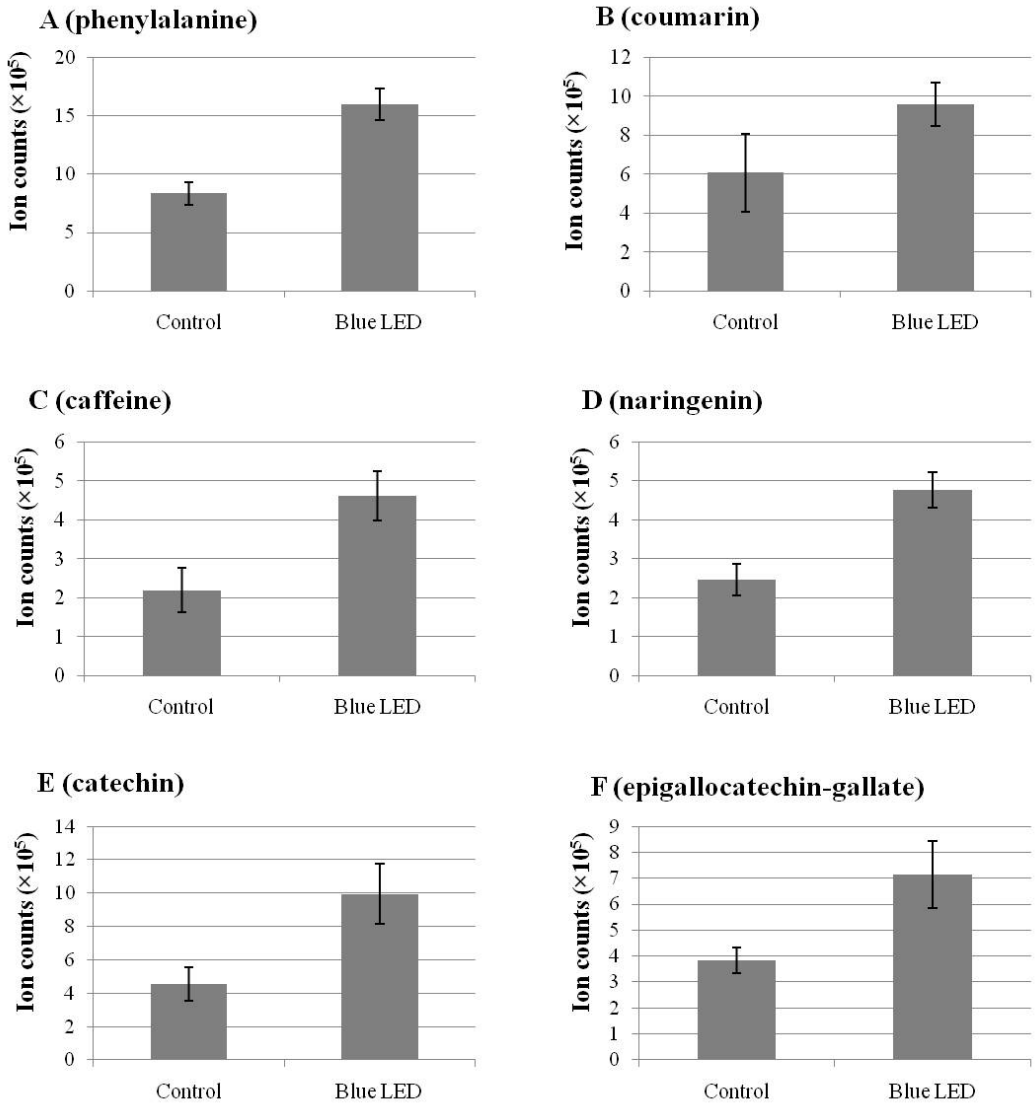

Figure 1. Compounds induced by blue LED in tea leaves. Details concerning the treatment of tea plants and determination of the content of compounds are described in the Materials and methods. A, phenylalanine; B, coumarin; C, caffeine; D, naringenin; and E, epigallocatechin-gallate (EGCG).

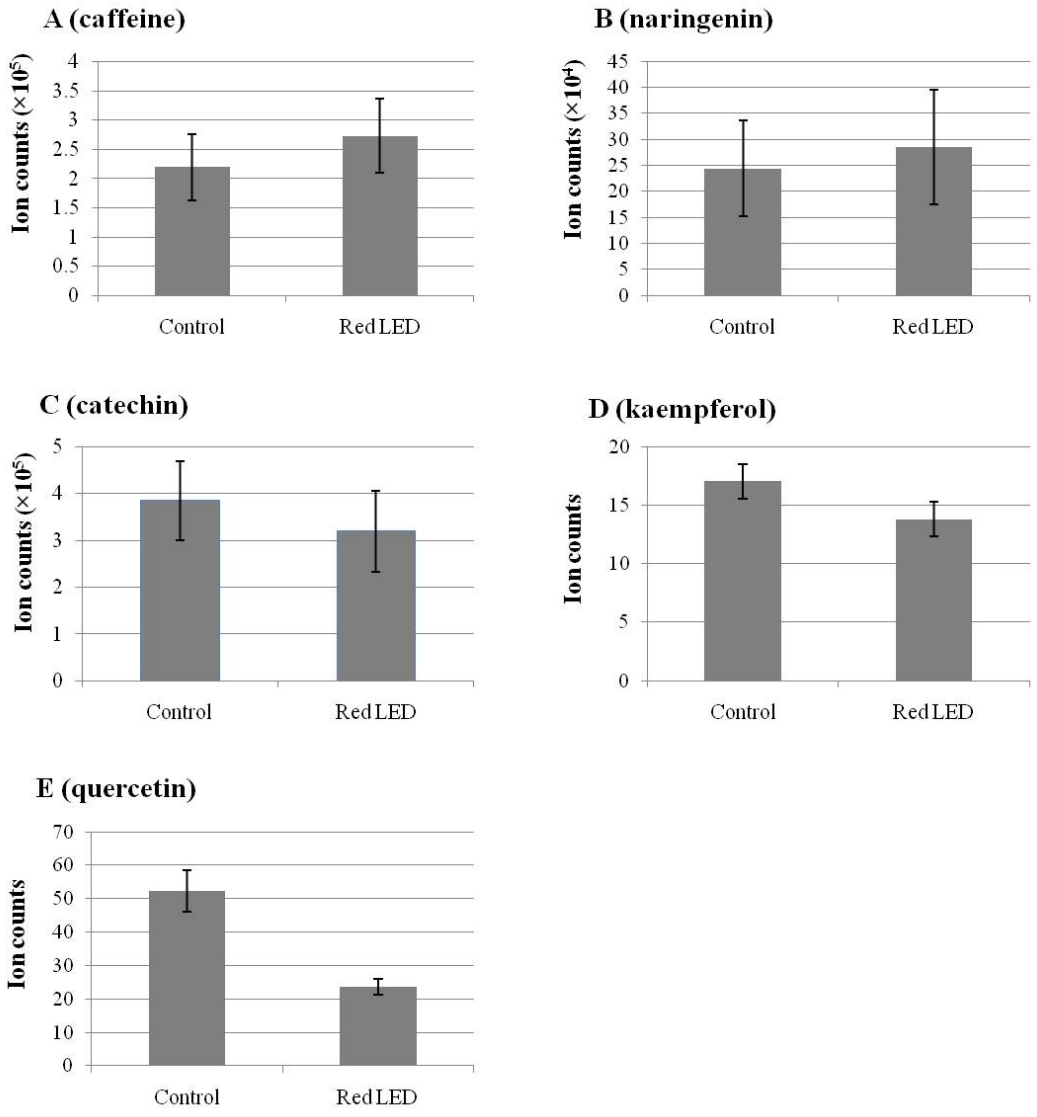

Figure 2. Effects of red LED on the content of compounds in tea leaves. Details concerning the treatment of tea plants and determination of the content of compounds are described in the Materials and methods. A, caffeine; B, naringenin,; C, catechin; D, kaempferol, and E, quercetin.

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