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Research article

Environmental stresses induce health-promoting phytochemicals in lettuce

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ABSTRACT

Plants typically respond to environmental stresses by inducing antioxidants as a defense mechanism. As a number of these are also phytochemicals with health-promoting qualities in the human diet, we have used mild environmental stresses to enhance the phytochemical content of lettuce, a common leafy vegetable. Five-week-old lettuce (*Lactuca sativa* L.) plants grown in growth chambers were exposed to mild stresses such as heat shock (40 °C for 10 min), chilling (4 °C for 1 d) or high light intensity (800 μmol m^{-2} s^{-1} for 1 d). In response to these stresses, there was a two to threefold increase in the total phenolic content and a significant increase in the antioxidant capacity. The concentrations of two major phenolic compounds in lettuce, chicoric acid and chlorogenic acid, increased significantly in response to all the stresses. Quercetin-3-*O*-glucoside and luteolin-7-*O*-glucoside were not detected in the control plants, but showed marked accumulations following the stress treatments. The results suggest that certain phenolic compounds can be induced in lettuce by environmental stresses. Of all the stress treatments, high light produced the greatest accumulation of phenolic compounds, especially following the stress treatments during the recovery. In addition, key genes such as phenylalanine ammonia-lyase (PAL), L-galactose dehydrogenase (L-GalDH), and γ-tocopherol methyltransferase (γ-TMT) involved in the biosynthesis of phenolic compounds, ascorbic acid, and α-tocopherol, respectively, were rapidly activated by chilling stress while heat shock and high light did not appear to have an effect on the expression of PAL and γ-TMT. However, L-GalDH was consistently activated in response to all the stresses. The results also show that these mild environmental stresses had no adverse effects on the overall growth of lettuce, suggesting that it is possible to use mild environmental stresses to successfully improve the phytochemical content and hence the health-promoting quality of lettuce with little or no adverse effect on its growth or yield.

1. Introduction

Diets containing fruits and vegetables are rich in a wide variety of phytochemicals with antioxidant properties that are known to confer health-promoting benefits. This is broadly supported by many epidemiological studies that suggest that phytochemicals can prevent a number of chronic and degenerative diseases [1–4]. These findings have led to many dietary guides which recommend fruits and vegetables as part of their daily diet [5]. Thus in recent years, with the increasing awareness of the importance of fruits and vegetables in the human diet, there has been a greater impetus to improve the nutritional and health-promoting qualities of commonly consumed fruits and vegetables.

Lettuce is a popular leafy vegetable and ranks high both in production and economic value among the vegetables grown in the U.S. [6]. It is an important source of dietary antioxidants especially considering its high peroxyl radical scavenging activity [7]. A major part of the antioxidant activity in lettuce arises from a number of phenolic compounds, the dominant ones being caffeic acid derivatives and flavonols [8–10]. In addition to phenolic compounds, vitamins such as vitamin C and vitamin E also contribute to the antioxidant activity in lettuce [10].

The antioxidant content of plants varies considerably depending on their growing and management conditions [11–13]. It has been demonstrated that antioxidants in plants are part of a complex defense mechanism against a wide range of stresses and thus, accumulate in response to these stresses [14,15]. Evidently, these findings provide an opportunity to enhance the health-promoting

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Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); CTAB, cetyltrimethylammonium bromide; EDTA, ethylene diamine tetraacetic acid; L-GalDH, L-galactose dehydrogenase; HPLC, high-performance liquid chromatography; PAL, phenylalanine ammonia-lyase; PPFD, photosynthetic photon flux density; SDS, sodium dodecyl sulfate; γ-TMT, γ-tocopherol methyltransferase.

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benefits of plant-based foods by using regulated environmental stresses. However, such an approach of using environmental stresses to improve the quality of fruits and vegetables has to be considered with some caution because of their potential adverse effects on crop growth and yield.

It is not clear if the accumulation of antioxidants in plants in response to environmental stresses is as a result of activation of key genes involved in their biosynthesis. There are, however, some limited studies that support this premise, especially as an adaptive strategy to environmental stresses. For example, the gene for phenylalanine ammonia-lyase (PAL), a gateway enzyme in the biosynthesis of various phenolic compounds, is known to be activated by a number of biotic and abiotic stresses [16–18]. Similarly, gene activation of L-galactose dehydrogenase (L-GalDH) and γ-tocopherol methyltransferase (γ-TMT), key enzymes involved in the biosynthesis of antioxidants such as ascorbic acid and α-tocopherol, respectively, has been observed in response to environmental adaptation in lettuce [19]. However, it is unclear if mild environmental shocks can activate genes involved in the biosynthesis of antioxidants.

The primary objective of this study was to examine the role of various environmental stresses in producing health-promoting phytochemicals in lettuce (*Lactuca sativa* L.). We examined the accumulation of phenolic compounds and the activation of key genes involved in the biosynthesis of antioxidants including phenolic compounds, ascorbic acid, and α-tocopherol in response to mild environmental stresses while considering the impact of these stresses on plant growth and development.

2. Results

Lettuce plants were subjected to mild environmental stresses including heat shock, chilling, and high light to determine their ability to accumulate antioxidants. Total phenolic content of lettuce plants increased in response to each of the stress treatments both during and after the treatments (Fig. 1). The increase in total phenolic content in lettuce was rather rapid in response to heat shock and chilling. A significant increase in phenolic compounds occurred within 10 min of heat shock and within 1 h of chilling, with higher accumulation of phenolic compounds occurring after 3 d of recovery. Of all the stress treatments, high light produced the highest level of total phenolic accumulation in lettuce plants, which was approximately threefold higher than in the control plants after 1 d exposure to high light and remained high following the stress treatment up to 3 d.

The antioxidant capacity of lettuce plants increased in response to each of the stresses, similar to the total phenolic content. It increased readily in response to all the stresses and remained high during the 3-d recovery period following the stress treatments, compared to that in the control plants (Fig. 2). Although the data for total phenolic content and antioxidant capacity presented here are based on fresh weight, a similar overall trend in plant response was observed for dry weight as well (data not shown).

The activation of genes for phenylalanine ammonia-lyase (PAL; EC 4.3.1.5), L-galactose dehydrogenase (L-GalDH; EC 1.1.1.117), and γ-tocopherol methyltransferase (γ-TMT; EC 2.1.1.95), which are involved in the biosynthesis of phenolics, ascorbic acid, and α-tocopherol, respectively, was examined in response to environmental stresses (Fig. 3). There was a rapid increase in the transcript levels of PAL, L-GalDH, and γ-TMT in response to chilling stress. Activation of these genes was observed within 1 h of exposure to chilling and remained high during the treatment. However, the transcript levels for all the genes appear to decline during the recovery period. In addition, L-GalDH was activated in response to all the stress treatments while there was no clear response of PAL and γ-TMT to either heat shock or high light stress.

As the total phenolic content in lettuce was generally high following various stress treatments, accumulation of individual key phenolic compounds including caffeic acid and its derivatives and flavonoids was measured during the recovery, after 3 d of stress treatments (Fig. 4). The two major phenolic compounds in lettuce were chicoric acid and chlorogenic acid with minor amounts of caffeic acid. Control plants did not have measurable quantities of either quercetin-3-*O*-glucoside or luteolin-7-*O*-glucoside. The major caffeic acid derivatives, namely chicoric acid and chlorogenic

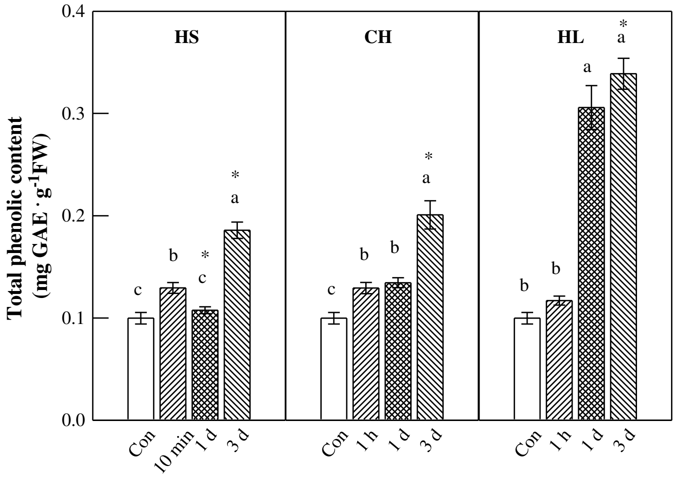

Fig. 1. Changes in total phenolic content of lettuce plants subjected to environmental shocks. Five-week-old lettuce plants were exposed to heat shock (HS, 40 °C for 10 min), chilling (CH, 4 °C for 1 d), or high light (HL, 800 μmol m^{-2} s^{-1} for 1 d) conditions. Control plants (Con) were grown at 22/18 °C (day/night) and a PPFD of 250 mmol m^{-2} s^{-1}, and under a 12 h photoperiod. Plants were allowed to recover following the stress treatments, which are indicated by asterisks. The data indicate the means ± S.E. ($n = 3$). Significant differences (at $P = 0.05$) within a treatment are indicated by letters.

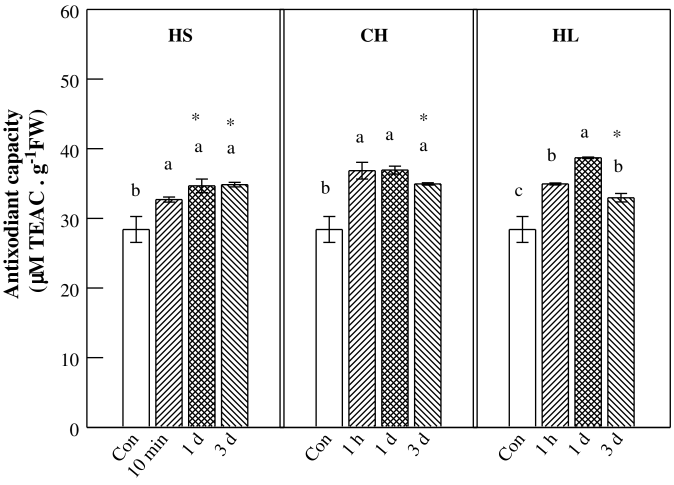

Fig. 2. Changes in antioxidant capacity of lettuce plants subjected to environmental shocks. Five-week-old lettuce plants were exposed to heat shock (HS, 40 °C for 10 min), chilling (CH, 4 °C for 1 d), or high light (HL, 800 μmol m^{-2} s^{-1} for 1 d) conditions. Control plants (Con) were grown at 22/18 °C (day/night) and a PPFD of 250 mmol m^{-2} s^{-1}, and under a 12 h photoperiod. Plants were allowed to recover following the stress treatments, which are indicated by asterisks. The data indicate the means ± S.E. ($n = 3$). Significant differences (at $P = 0.05$) within a treatment are indicated by letters.