

Priming with Vitamin U Enhances Cold Tolerance of Lettuce (*Lactuca sativa* L.)

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Abstract

Priming may be an efficient pre-treatment of plants in order to enhance their ability to cope with unfavourable growth conditions, and to improve defensive metabolism through elevated levels of protective substances which may also act as health-promoting agents upon human consumption. The aim of this work was to evaluate the beneficial influence of priming with the naturally occurring, but scarcely known vitamin U (S-methylmethionine) on cold stress tolerance of lettuce (the frequently grown 'May King' cultivar). Effects on germination, photosynthetic efficiency, as well as on health-promoting carotenoid and vitamin C contents were investigated. Photosynthetic capacity, strongly related to productivity, was evaluated with parameters of induced chlorophyll fluorescence and of leaf gas exchange through stomata, using plants grown in hydroponic cultures. Priming with vitamin U significantly compensated for the delaying effect of low temperature (5 °C) on seed germination, as well as for inhibition of light-converting photochemical reactions and of carbon dioxide assimilation by cold stress. Use of vitamin U to prime lettuce plantlets for low temperature stress resulted in an elevated content of carotenoid pigments and of vitamin C in leaves, which improve the quality of consumed lettuce with respect to the health-promoting capacity. This beneficial influence of vitamin U was not proportional with its concentration (2 mM had no stronger effects than 0.25 mM), so small amounts of this substance were sufficient for a sustained efficiency in promoting hardening against chilling temperatures. This is the first report on priming of lettuce for cold tolerance by using S-methylmethionine (vitamin U), with a possible application in improvement of crop quality and productivity.

Keywords: ascorbate; biomass production; carotenoids; germination; photosynthesis; S-methylmethionine

Introduction

A relatively new approach for improvement of environmental stress tolerance of crop plants is priming with various organic or inorganic substances that accelerate or enhance protective and compensatory mechanisms of antistress defense. This pre-treatment may be useful against a great variety of adverse developmental conditions, such as drought, high salinity, hypoxia, air pollution, extreme temperatures, pest attack etc. (Ahmad *et al.*, 2010; Conrath, 2011). Several agents have been assayed as priming agents, some of these being harmful chemicals with role in stress

signaling (e.g. hydrogen peroxide, hydrogen sulfide, propiconazole), while most of them are natural metabolites involved in defense mechanisms and in regulation of growth (e.g. proline, ascorbic acid, salicylic acid, polyamines, quercetin, biotine, thiamin). These are usually applied as foliar sprays, seed soaking solutions or additives to the nutrient solution (Filippou *et al.*, 2013; Aranega-Bou *et al.*, 2014). Because of the promising efficiency (low quantities, short treatment periods and multiplied physiological effects), more and more priming agents are tested for various crop plants, in different developmental phases. The main condition for a priming agent is to induce a physiological state in which plants respond more rapidly and more intensely to low levels of adverse stimuli, and exhibit a

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stronger defense reaction upon exposure to abiotic and biotic stressors (Beckers and Conrath, 2007; Rejeb *et al.*, 2014).

Vitamin U or S-methylmethionine is a natural derivative of the universal amino acid methionine, being produced by all angiosperms. Its highest quantities were measured in members of the Brassicaceae family. It is a sulfur- and methyl group-containing molecule, translocated mainly through the phloem sap and accumulated mostly in young organs with intense metabolism. Its main sources are the fully developed leaves. Even though it is not demonstrated that, like other vitamins, it is essential for humans and represents a dietary requirement, S-methylmethionine is a biologically active natural compound with beneficial metabolic effects. This is the reason why, in analogy with other vitamins, it can be also considered a vitamin, being named vitamin U (McRorie *et al.*, 1954; Miret and Munne-Bosch, 2014). Although it is a ubiquitous intermediate product of plant metabolism, at present its physiological functions are mostly unknown, and literature concerning its roles in plants is very scarce. Being a constituent of the futile S-methylmethionine (SMM) cycle, it plays a role in regulation of methionine pool homeostasis, as well as in methylation reactions needed for biosynthesis of specific plant metabolites, such as vitamin E, vitamin H, flavonoids, polyamines (Ranocha *et al.*, 2001; Roje, 2006; Zagorchev *et al.*, 2013). It also serves for temporary storage of reduced sulfur. There is no available information about S-methylmethionine content of lettuce leaves, but it was identified in several flowering plant species in micromolar concentrations. It was demonstrated that in wheat phloem sap its amount may be 1.5-fold that of glutathione, constituting about 2% of free amino acids translocated through the phloem (Bourgis *et al.*, 1999). For reducing cell membrane damage in several crop plants exposed to low-temperature stress, plants grown in hydroponic cultures were supplied with 0.5 mM S-methylmethionine in the culture medium (Racz *et al.*, 2008). Usually, when natural compounds are used as priming agents (only at the beginning of the experimental period), they are applied in higher concentrations than those that exist in plants under normal growth conditions (Aranega-Bou *et al.*, 2014). In studies performed during the last decade, it was demonstrated that externally administrated vitamin U, which is translocated through the aqueous solution of the xylem sap, enhances tolerance to environmental stress conditions, such as hypoxia, heavy metal toxicity and mosaic virus infection (Menegus *et al.*, 2004; Fodorpataki *et al.*, 2010; Ludmerszki *et al.*, 2011). In maize seedlings, it protects chloroplast structure, reduces cell membrane damage and stimulates the phenylpropanoid pathway upon exposure to low temperature stress (Racz *et al.*, 2008; Szego *et al.*, 2009; Paldi *et al.*, 2014). Its influences on several other physiological processes remain to be revealed by further investigations, in order to understand its role in living organisms.

Low temperature is a frequent environmental stress factor for crop plants grown in spring, such as lettuce, which is the most largely cultivated leafy vegetable, especially in temperate and cold regions (Kristkova *et al.*, 2008). It slows down growth, organogenesis and metabolic reactions.

Reduction of biomembrane permeability caused by chilling is compensated by overexpression of genes encoding for fatty acid desaturases, so cold tolerance results in lower levels of phosphatidylglycerol saturation and enhanced production of trienoic fatty acids. Quantity and protective activity of several antioxidants are also increased during hardening to cold stress, and because of cross-tolerance, cold-hardened plants can cope better with drought and high salinity (Koh, 2002; Macedo, 2012). Enhancement of cold tolerance of crop plants grown in early spring and late autumn is thought to be a key solution for optimization of their production and nutritive quality (Huang *et al.*, 2012).

The necessity to improve crop production under environmental stress conditions, without implementing the costly techniques of genetic engineering, rises at least two questions to be answered. 1. Do specific chemical agents, applied in small quantities before stress exposure, induce an enhanced hardening of given crop plants? 2. How pre-treatment with new priming agents influence plant physiological processes related to productivity and metabolic plasticity upon which stress tolerance relies? The emerging hypothesis is that S-methylmethionine induces or enhances metabolic changes which result in a better development and photosynthetic performance of cold-exposed lettuce plants, associated with an increased production of certain defence metabolites with a general health-promoting capacity. Our present understanding on how priming agents improve plant growth, development and metabolism is very limited, this is why more experimental support is needed to deepen our knowledge on physiological responses to various priming agents. This is why the aim of this work is to investigate the possibility of using vitamin U (S-methylmethionine) for priming lettuce plants for enhanced cold tolerance, and to demonstrate the positive effect of this treatment on seed germination, photosynthetic performance, carotenoid pigment and vitamin C content of leaves. The selected physiological and biochemical parameters are directly related to early development, to biomass production and to synthesis of health-promoting metabolites in the plant parts used for human consumption. In our knowledge there are no publications concerning the use of vitamin U for priming of the 'May King' cultivar of lettuce for chilling tolerance, and for the possible application of this treatment for quality-oriented production aiming to optimize early development, vitality and health-promoting substance content in leafy vegetables.

Materials and Methods

Biological material and experimental design

The 'May King' cultivar of lettuce (*Lactuca sativa* L.) was chosen for experiments, because it is one of the earliest producing and widely used cultivar from the butterhead type (*L. sativa* var. *capitata*), very frequently commercialized for fresh salad consumption (Kristkova *et al.*, 2008). The seeds were purchased from B&T World Seeds (Paguignan, France). 100 seeds for each experimental variant were pre-hydrated for 24 hours, and then put to germinate in Linhard vessels at 20 °C. For the experiments concerning germination dynamics in time, control seeds were hydrated

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with distilled water at 20 °C, others with aqueous solutions of 0.25 mM and 2 mM S-methylmethionine respectively, also at 20 °C. One variant was pre-hydrated in distilled water at 5 °C, and two others at 5 °C in 0.25 mM and 2 mM of S-methylmethionine solutions, respectively. Percentage of germinated seeds was determined on the second, the fourth and the sixth days. S-methylmethionine (vitamin U) concentrations were chosen based on previous series of experiments (Fodorpataki *et al.*, 2010).

For experiments performed in a later developmental phase, all lots of seeds were pre-hydrated with distilled water and germinated at 20 °C. On the fifth day similar germinated plantlets were transferred in hydroponic cultures, five plants for each vessel of 5 L, in half-strength Hoagland nutrient solution aerated with an aquarium pump (Hoagland and Arnon, 1950; Shavrukov *et al.*, 2012). In this solution plants were grown for two weeks in growth chambers (Sanyo Versatile Environmental Test Chamber), where the photosynthetically active photon flux density (provided by fluorescent tubes) was 330 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on leaf upper surfaces, the daily photoperiod was 14 h light and 10 h darkness, the temperature was kept at 20 °C, and the relative air humidity was 70%. The nutrient solution was replaced every four days (Fodorpataki *et al.*, 2008; Bartha *et al.*, 2010). Experiments were started after two weeks of growth in the Hoagland solution. Pre-treatment with vitamin U was performed for one day before cold treatment, by adding 0.25 mM or 2 mM S-methylmethionine to the nutrient solution in which the roots were immersed. Three vessels, each with five plants, were transferred for one week in a growth chamber with a constant temperature of 5 °C, all other growth conditions being identical with the above mentioned ones. One of these three vessels contained half-strength Hoagland solution with no vitamin U, another was supplemented with a final concentration of 0.25 mM vitamin U, while the third contained 2 mM vitamin U in the nutrient solution. The youngest fully-expanded leaves of lettuce plants were used for measurements of photosynthetic parameters and for extractions. Biochemical determinations were performed with extracts obtained from freshly harvested leaves.

Measurement of induced chlorophyll fluorescence parameters

Conventional and pulse amplification modulated parameters of induced chlorophyll fluorescence were determined *in situ* on intact leaves with an FMS-2 type fluorometer (Hansatech), in order to evaluate energetic efficiency of light use in photochemical reactions of photosystem II. From among the various measured and computed parameters, obtained upon continuous illumination for 5 minutes with white light of 3000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ intensity, the relative fluorescence decrease (R_{fd}), which is also known as the general vitality index of the photosynthetic apparatus, and is directly correlated (when measured at saturation irradiance) with the net carbon assimilation rate of leaves, was selected as a sensitive and relevant parameter. R_{fd} is calculated from the measured dark-adapted maximal fluorescence (F_m) and steady state chlorophyll fluorescence (F_s) in illuminated leaves, using the formula $R_{fd} = (F_m - F_s) / F_s$ (Lichtenthaler *et al.*, 2005).

Measurement of leaf gas exchange parameters

Gas exchange parameters of intact lettuce leaves were determined *in situ* with a Ciras-2 type leaf gas exchange meter (PP Systems) on the abaxial side epidermis, in the middle of the daily photoperiod, under constant conditions of illumination, temperature and air humidity (Bartha *et al.*, 2015). Stomatal conductance, intensity of transpiration and net photosynthetic carbon dioxide assimilation were the main physiological parameters registered on the youngest fully-expanded leaves.

Determination of carotenoid pigment content

Carotenoid pigment content was determined photometrically in leaf blade extracts with 80% (v/v) acetone, and calculated on a fresh weight basis according to Lichtenthaler and Wellburn (1983). Extracts were obtained from 0.25 g of lettuce leaves, finely homogenized in a final volume of 5 mL 80% acetone, and the supernatant resulting from centrifugation for 10 min at 4000 g was used for absorbance measurement.

Extraction and determination of vitamin C (total ascorbate content)

Extraction and determination of ascorbic acid (vitamin C) was performed according to Kampfenkel *et al.* (2005), based on reduction of ferric ion and photometric detection of iron(2+) complexed with 2,2'-dipyridyl. 0.5 g of lettuce leaves were homogenized in a prechilled mortar with 4 mL of 6% trichloroacetic acid, the mixture was centrifuged for 15 min at 15600 g and 4 °C, then 0.2 mL of supernatant was put into sodium phosphate buffer (pH 7.4) containing trichloroacetic acid, orthophosphoric acid, dithiothreitol, ferric chloride, N-ethylmaleimide and ethanolic solution of 2,2'-dipyridyl. After one hour of incubation at 42 °C, absorbance was measured at 525 nm, and molar concentration of ascorbic acid was determined by use of the standard curve obtained with known concentrations of pure ascorbic acid dissolved in 6% trichloroacetic acid.

The same leaves on which chlorophyll fluorescence and gas exchange parameters were measured *in vivo* were also used for determination of carotenoid and vitamin C content.

Statistical analysis of experimental data

All experimental variants were set in 5 replicates, and measurements were repeated three times. Data were statistically processed in R environment, using ANOVA and the Tukey HSD test for finding the significance of differences between treatments. The results were expressed as mean \pm standard error (SE), and $p < 0.05$ was considered statistically significant.

Results and Discussion

Seed germination under cold treatment and priming

Germination of seeds of the 'May King' lettuce cultivar was significantly delayed by exposure to cold treatment (5 °C), but low temperature did not disturb the final percentage of germinated seeds. The presence of 0.25 mM or 2 mM vitamin U in the watering solution of seeds had no

significant influence on the dynamics of germination, but when vitamin U was present in the water used for one-day pre-hydration, it significantly compensated for the delay in germination caused by low temperature. This compensatory effect was not proportional with the concentration of vitamin U (2 mM did not cause a more rapid germination than 0.25 mM) and did not result in a total cessation of cold stress effect, because seeds exposed to low temperature and primed with vitamin U still germinated slower than the control kept at 20 °C (Fig. 1). The results suggest that priming with vitamin U reduces the inhibitory effect of low temperature on the first developmental steps of lettuce plants, and primed seeds will germinate quicker when sown in the cold soil of early spring or autumn. Similar results were reported for lettuce seeds by Kalhor *et al.* (2018), when the environmental stress factor was the increased salinity, and another naturally occurring, non-proteinogenic amino acid: the γ -amino butyric acid was used to reduce mean germination time in salt-exposed seeds of the Partavousi lettuce cultivar. For seeds of several crop species, a mild stress exerted by various unfavourable abiotic factors (high salinity, moderate drought, hypoxia, high temperature) leads to delayed germination, whereas severe stress results in a decreased germination percentage (Koh, 2002; Bartha *et al.*, 2015). Even though the low temperature treatment applied in the present experiments did not inhibit germination, the delay in formation of new plantlets may extend the vegetation period of lettuce plants. This is especially undesirable when lettuce is cultivated in the autumn period. The fact that priming with vitamin U results in a reduced germination time of cold-exposed seeds, ensures a shorter period of development until new plantlets will be able to begin an independent nutrition. This is the first report on the fact that pre-treatment with vitamin U shortened the germination time of lettuce seeds exposed to low temperature. The compensatory mechanism is not known, but may be related to osmoregulatory processes which enable a better hydration of seeds, as well as to activation of metabolic processes in the embryo which accelerate growth under low temperature (Macedo, 2012).

Variations in carotenoid pigment content of leaves

The carotenoid pigment content of the freshly harvested, fully developed young lettuce leaves was highly

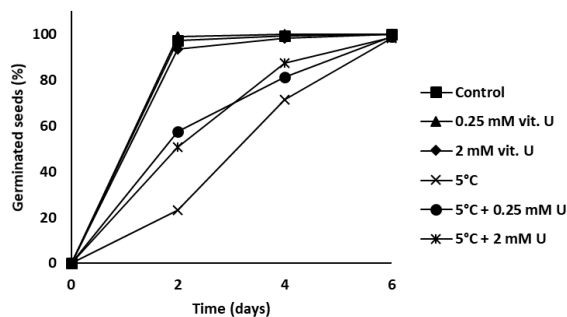


Fig. 1. Germination dynamics of lettuce seeds exposed to cold stress and to priming with two different concentrations of vitamin U (abbreviated in the graph as vit. U or U). (n = 5, vertical bars represent \pm SE from the means)

influenced by exposure to cold stress and by priming with vitamin U (S-methylmethionine). This amino acid derivative had no significant influence on total carotenoid content of leaves in the case of control plants grown hydroponically at room temperature (20 °C), but pre-treatment of lettuce plantlets for one day with 0.25 mM or 2 mM vitamin U (dissolved in the nutrient medium) succeeded to keep the carotenoid pigment content of cold-stressed leaves close to the level existing in control plants, while low temperature caused in non-primed plants a serious decrease of the carotenoid amount (roughly with 30%). The two different concentrations of vitamin U exhibited a similar degree of compensation for the carotenoid content decrease (Fig. 2). In analogy with the stimulating effect on phenylpropanoid pathway during alleviation of cold stress (Paldi *et al.*, 2014), vitamin U may also upregulate the mevalonic acid pathway which leads to carotenoid synthesis, and this upregulation seems to be effective when cold stress tends to inhibit production of these tetraterpenes. This priming effect may be important not only for the acclimation of lettuce plants to unfavourable temperatures, but also in the context of human consumption of lettuce leaves, because carotenoids have an antioxidative, protective role in scavenging such dangerous reactive oxygen species as singlet oxygen, hydroxyl radical or alkyl-peroxyl radicals, accumulation of which may cause serious oxidative damages to various biomolecules with vital functions in cells (Smirnoff, 2005; Altunkaya *et al.*, 2009; Oh *et al.*, 2009).

Performance of light reactions of photosynthesis

In vivo and *in situ* induced chlorophyll fluorescence parameters are known to be early and very sensitive physiological markers of stress tolerance of the light-converting photosynthetic apparatus of plants, on which the entire energetic charge and productive capacity finally depend (Lichtenthaler *et al.*, 2005). Different chlorophyll fluorescence parameters were determined, in order to monitor the photon-absorbing capacity of light-harvesting pigment antennae, the potential and effective quantum yield of photosystem II, the non-photochemical quenching related to protective mechanisms through dissipation of excess energy etc. (data not shown). It was found that for cold-stressed lettuce plants primed with vitamin U, the

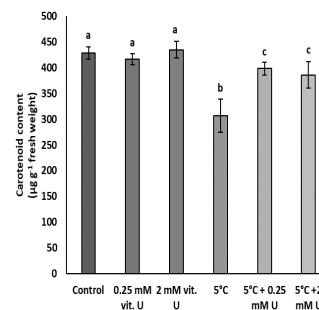


Fig. 2. Influence of cold treatment and of priming with vitamin U (U) on the carotenoid pigment content of fully developed lettuce leaves grown under similar light conditions (n = 5, vertical bars represent \pm SE from the means, different letters indicate statistically significant differences at p < 0.05, Tukey HSD test)

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relative fluorescence decrease (Rfd), also known as the overall vitality index of the photosynthetic apparatus performing the light reactions, is a suitably sensitive indicator for evaluation of damage experimented by leaf photosynthesis under the given growth conditions. Cold treatment reduced by more than 50% this vitality index as compared to the control plants grown at 20 °C, while priming with vitamin U led to a partial, but significant recovery of the vitality of photosynthetic apparatus (Fig. 3). Thus, priming with vitamin U helps lettuce plants to use more efficiently the incident light energy when photosynthesis is impaired by cold stress. Inhibitory effect of low temperature is mainly due to decreased fluidity of the thylakoid membranes leading to decelerated movement of electron transporter and of pigment antennae, as well as to slower reactions of carbon assimilation and feed-back down regulation of photochemical processes (Koh, 2002; Macedo, 2012). Similar positive influence on recovery of chlorophyll fluorescence parameters was reported by Kalhor *et al.* (2018) for lettuce plants exposed simultaneously to salt stress and treatment with γ -amino butyric acid, while Bartha *et al.* (2010) found that several parameters of induced chlorophyll fluorescence are suitable to distinguish among different degrees of salt tolerance in lettuce cultivars. Protection of the photosynthetic apparatus by S-methylmethionine against cold stress was also demonstrated in maize plantlets, the compound being applied during the entire period of cold treatment (Paldi *et al.*, 2014). The present study supports the concept of alleviation of abiotic stress to photosynthesis by externally supplied bioactive substances, and supplements the list of priming agents which are effective in sustaining a higher efficiency of photochemical processes under adverse growth conditions.

Alleviation of cold stress influence on carbon dioxide assimilation

The fact that relative chlorophyll fluorescence decrease was correlated with net carbon dioxide assimilation is supported by gas exchange measurements on leaf surfaces. Stomatal conductivity for atmospheric carbon dioxide, depending on diameter of the opened stomata pores, reflected the capacity of plants to supply the inorganic carbon source for synthesis of new organic products on which crop yield relies. The dynamics of carbon dioxide uptake is influenced by several abiotic factors of the environment, among which low air temperature is a major determinant (along with drought) of stomatal closing, being opposed to the opening influence of light and low carbon dioxide concentration. In the present experiments, exposure to cold induced a serious decrease in net carbon dioxide assimilation rate of lettuce leaves, while administration of vitamin U prior to cold treatment resulted in a much less diminished uptake and utilization of atmospheric carbon dioxide supply (Fig. 4). Combined with the previously presented results concerning chlorophyll fluorescence, one can conclude that in cold-stressed plants priming with vitamin U improved with similar degrees both light energy utilization in the light reactions of photosynthesis and carbon dioxide assimilation in the biosynthetic metabolism of photosynthetic carbon reduction cycle. When studying differentiated salinity tolerance of several lettuce cultivars, Bartha *et al.* (2015) found that stomatal conductivity is less

affected by salt stress in more tolerant plants, enabling a better assimilation under adverse growth conditions. Our findings support the results obtained by Sgheri *et al.* (2017), who demonstrated that improved carbon dioxide supply alleviates environmental stress effects to photosynthesis. They also reported that enhanced carbon dioxide assimilation increases the amount of antioxidant metabolites (e.g. luteolin) in leaves, which improves health benefits of lettuce consumption.

Vitamin C content of leaves primed for cold stress

While carotenoid pigments are the most common lipophilic antioxidants produced by plants, vitamin C or ascorbic acid is the most abundant hydrophilic antioxidant, synthesized mainly in leaves. Human organisms cannot produce neither carotenoids nor ascorbic acid, but these substances exert the same antioxidative, protective role in human cells as in plant cells (Smirnoff, 2005).

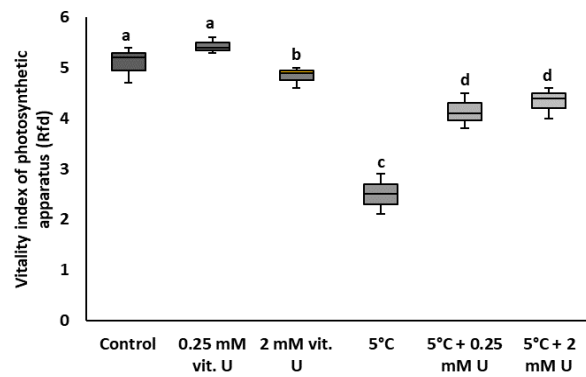


Fig. 3. The vitality index of the photosynthetic apparatus in lettuce leaves, determined from relative chlorophyll fluorescence decrease (Rfd), in plants exposed to cold stress and primed with vitamin U (U) (n = 5, vertical bars represent \pm SE from means, different letters indicate statistically significant differences at p < 0.05, Tukey HSD test)

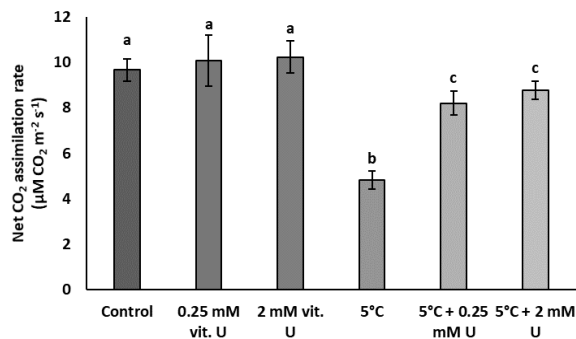


Fig. 4. Variations of the rate of net carbon dioxide uptake through stomata of lettuce leaves exposed to low temperature and primed with different concentrations of vitamin U (U) (n = 5, vertical bars represent \pm SE from means, different letters indicate statistically significant differences at p < 0.05 with Tukey HSD test)

This is why elevated amounts of carotenoids and vitamin C confer higher health benefit for human consumers. Our experiments revealed that vitamin U, administrated in the concentrations of 0.25 mM and 2 mM in the nutrient medium of lettuce plantlets, induced by itself a moderate, but statistically significant increase in the vitamin C content of fresh leaves, and when plants exposed to low temperature were primed with vitamin U, this pre-treatment for one day completely abolished the decreasing influence of cold stress on vitamin C content of leaves (Fig. 5). This demonstrates that priming with vitamin U enhanced cold tolerance of lettuce plants and helped them maintaining a higher pool of vitamin C which contributes to detoxification of excess hydrogen peroxide when it accumulates under abiotic stress conditions. Treatment with different bioactive substances was considered a special type of preharvest factor influencing vitamin C content of several crop plants (Lee and Kader, 2000), while in greenhouse grown broccoli the temperature increase under low light intensity represented a condition which resulted in higher ascorbic acid content (Schonhof *et al.*, 2007). Oh *et al.* (2009) demonstrated that several adverse growth conditions, such as heat shock, chilling, drought or excessive light intensity stimulate expression of the gene encoding for L-galactose dehydrogenase, which is a key enzyme of ascorbic acid synthesis in plants. Our results support the fact that priming of stress-exposed plants may enhance vitamin C content of lettuce leaves, which, if consumed freshly, has an improved health-promoting quality. The action mechanism of vitamin U in plants is unknown at present, but increased vitamin C content of cold-treated leaves primed with this amino acid derivative may be related to stimulation of ascorbic acid synthesis from simple carbohydrates (hexoses), as well as to activation of antioxidative defence mechanisms implying elevated ascorbate concentrations (Smirnoff, 2005; Roje, 2006). In our knowledge this is the first evidence on improvement of vitamin C content of lettuce leaves due to priming with vitamin U.

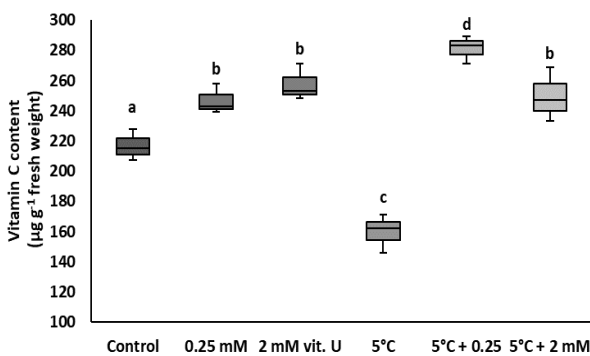


Fig. 5. Influence of cold stress and of priming with vitamin U (U) on the vitamin C content of freshly harvested, fully developed lettuce leaves ($n = 5$, vertical bars represent \pm SE from the means, different letters indicate statistically significant differences at $p < 0.05$ with Tukey HSD test)

Conclusions

During the early spring or late autumn cultivation periods, the delaying effect of low temperature on germination of lettuce seeds can be significantly compensated with a short period of priming with aqueous solution of vitamin U (S-methylmethionine). Vitality of the photosynthetic apparatus, which converts incident light energy into chemically stored energy, as well as uptake, and assimilation of carbon dioxide by leaves are less inhibited by cold stress if hydroponically grown lettuce plants are primed with millimolar amounts of vitamin U dissolved in the nutrient medium. Leaves of lettuce plants primed with 0.25-2 mM vitamin U have higher vitamin C content and less reduced carotenoid content upon growth at low temperature, thus they have a higher quality as health-promoting food source. Our findings may improve cold tolerance of lettuce in its early developmental stages, resulting in more efficient cultivation and better quality for human consumption. Our results are the first reports on the beneficial influence of priming with vitamin U on photochemical efficiency of photosynthesis, on carbon dioxide assimilation, on carotenoid pigment and ascorbic acid content of lettuce leaves exposed to cold stress. The fact that effects of priming are not proportional with concentration suggests that vitamin U may act as a signaling molecule to boost physiological performances through improvements in antistress pathways that involve sulfur-containing compounds. Further investigations are needed to elucidate the mechanism of action of S-methylmethionine in plants exposed to different environmental stresses (e.g. by comparing the effects of vitamin U with those of other sulfur-containing active substances), taking into account that scientific information regarding the role of this biologically active natural metabolite is very scarce.

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