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Interaction between selenium and sulfur promotes alteration in the internal quality traits in green and red lettuce

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Summary

The interaction between selenium (Se) and sulfur (S) was investigated in green and red lettuce grown in a hydroponic system containing a basal mineral complement at contrasting levels of Se and S. The experiment aimed to evaluate the impact of Se and S supplementation on the quality traits of the lettuce, including soluble sugars, organic acids, total protein and nitrate (NO_3^-) to result in better lettuce yield with improving quality. The Se and S concentrations determined in the lettuce leaves showed complex dependence on the various levels of selenate (SeO_4^{2-}) and sulfate (SO_4^{2-}) supplied via the foliar application and the nutrient solution, respectively.

The foliar application of Se resulted in a synergistic interaction between both elements. With elevated Se and S (Se2/S2), Se accumulation was enhanced drastically in red lettuce, and in green lettuce, the S concentration increased significantly. Regarding carbohydrate accumulation, including water-soluble sugars, a lower Se dose under adequate S conditions enhanced glucose levels significantly by 3.2 and 2.1-fold in green and red lettuce, respectively. A synergistic interaction between Se and S was found following higher Se and S treatment (Se2/S2), resulting in a significant ($p \leq 0.05$) reduction in glucose and fructose concentrations. However, higher S strikingly increased the accumulation of the reducing sugars (glucose and fructose) by 5.3 and 3.0-fold for glucose and 3.7 and 5.6-fold for fructose in green and red lettuce, respectively. Meanwhile, sucrose increased by 2.0-fold in red lettuce and remained unaltered in green lettuce. A low nitrate level (NO_3^-) was maintained in response to adequate or elevated S and Se levels. Se levels (Se1 and Se2) did not affect total protein concentration under contrasting sulfate/selenate. However, potential effects in terms of protein accumulation were associated with an adequate or increased S concentration. The data suggest that lower Se and sufficient S doses significantly increase glucose levels in both pigmented lettuce cultivars. Additionally, the synergistic interaction between Se and S could benefit the final nutritional value and quality of lettuce, especially for nitrate, where Se and S enrichment can ensure low nitrate levels.

Keywords: Se, S, foliar application, organic acids, soluble sugars, total protein, NO_3^-

Introduction

Along with oxygen, selenium (Se) belongs to a Group VIA (6a, which is called chalcogens) and it is an essential micronutrient element for maintaining homeostatic processes in humans and animals (Minich, 2022). An adequate amount of Se is important to improve human and animal health but, in excess, Se might create health complications (Wu et al., 2015). Se deficiency in humans is associated with several serious diseases, including cardiovascular disorders, hypothyroidism, viral severe diseases, male infertility, inflammation, increased incidence of various cancers, and an overall weakened immune system (Burk et al., 2015; Schiavon et al., 2020). Moreover, low Se levels in the serum elevate the risk of developing cancer, while excess Se levels may harm human health by causing liver cirrhosis, pulmonary oedema, and diabetes (Schiavon et al., 2020). According to the World Health Organization (WHO), the dietary requirements of Se are 60 and 70 μg Se day^{-1} for adult women and men, respectively (Kieliszek and Błażejak, 2013).

The range between Se dietary necessity and toxic Se doses is relatively narrow for humans and animals (Hadrup and Ravn-Haren, 2023). Therefore, maintaining a perfect balance between low Se and Se toxicity levels is critical. The Se concentration of food crops varies widely between geographical areas as the uptake of Se in plants directly depends on soil Se level (Natasha et al., 2018). According to estimates, insufficient Se intake affects up to 1 billion people globally; this can be attributed to nutrient-depleted soils, which are a real problem in some parts of the world, especially Europe (Kipp et al., 2015; Jones et al., 2017). For instance, Se-deficient soils exist in Germany, Denmark, Finland, Scotland, and certain Balkan countries (Jones et al., 2017). Most food crops demonstrate a much lower tolerance (<50 mg Se kg^{-1}), however Brassica vegetables, garlic, onion, and edible wild-grown mushrooms accumulate considerable amounts of Se due to their high content of sulfur (S)-containing compounds (Puccinelli et al., 2017). Se also has a beneficial impact on plants: at low concentrations, it has beneficial effects on plant growth and development (Hasanuzzaman et al., 2020). Moreover, the application of Se enhanced the content of nutrients including potassium, phosphorus, and S in the grains of soybean (Silva et al., 2023). It has been reported that under high-temperature stress, plant growth and productivity improved in response to Se application. This was associated with the induction of antioxidant activities and osmolyte concentrations and the maintenance of photosynthetic activity under stress conditions (Hawrylak-Nowak et al., 2018).

S is a macronutrient critical for plant growth and development. It is required for many essential pathways involved in human and animal metabolism besides the redox responsivity, which is stimulated by S bonds (Kopriva et al., 2015).

Se has analogous chemical properties to S. It is found in diverse oxidation states: selenide (Se^{2-}), elemental Se (Se^0), selenite (Se^{4+}), and selenate (Se^{6+}) (Barceloux, 1999). Thus, Se can substitute S in metal sulfide as the radii of S^{2-} and Se^{2-} are similar (0.174 and 0.191 nm, respectively) (White et al., 2004). Both sulfate and selenate, which are taken up into the chloroplast via SULTRS transporters, are reduced to cysteine (Cys) and selenocysteine (SeCys) in the S and Se assimilatory pathways and act as the crucial donors of reduced S and Se (Sors et al., 2005; White, 2018). Although both S and Se shared the same transporters during uptake by the plant root system, there is a complex interaction between both elements in plants. Therefore, the application of S can increase or reduce the Se content in food crops, depending on crop types, their S content, regime, and the timing of fertilizer application (Shinmachi et al., 2010). A previous report stated that S must be applied at a relatively moderate concentration to accumulate high levels of plant Se (Barak and

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GOLDMAN, 1997); hence, selenate and sulfate are known to compete for the influx to roots (SHENNAN et al., 1990). However, the selectivity for selenate and sulfate, which mediates the uptake of these anions into plants, varies depending on the plant species and the S nutritional status of the plant (WHITE, 2016).

Food crops are the primary source of Se for humans (NAVARRO-ALARCON and CABRERA-VIQUE, 2008), and although deficiency of Se in the diet is a huge problem worldwide (KIPP et al., 2015), introducing Se using appropriate methods can serve to increase Se content in foods of plant origin (SILVA et al., 2023). This will reduce the crisis of Se in countries with Se deficit and alleviate the adverse effects associated with soil Se limiting conditions (DENG et al., 2021; SILVA et al., 2023). Moreover, the toxicity of Se can be decreased under appropriate S fertilization as Se and S are analogs (SANTIAGO et al., 2019).

Lettuce is a leafy vegetable that provides considerable amounts of health-beneficial metabolites and has great potential for functional food production to improve human health and decrease the risk of disease when supplied with Se (PANNICO et al., 2019). It is classified as a Se non-accumulator, and it cannot tolerate high Se doses in tissues over 100 µg g^{-1} dry weight (TERRY et al., 2000). Hence, lettuce is a Se non-accumulator plant with a low ability to tolerate Se, and more competition between Se and S is expected under Se and S enrichment (WIESNER-REINHOLD et al., 2017; RAMOS et al., 2011). Therefore, studying the regulatory effects of S on the uptake of Se in food crops, mainly lettuce, has a meaningful impact on plant growth, yield contributing attributes, Se content, and human health. Moreover, Se enrichment has been reported to exert changes in carbohydrate metabolism, including reducing sugars (e.g. glucose and fructose), and organic acids in food crops (SHIRIAEV et al., 2023). In the current study, the authors would like to test the following hypotheses: (I) Se and S enrichment can be used as a potential fertilization strategy, contributing substantially to enhancing the crispness and sweetness of lettuce. (II) Se and S enrichment in the lettuce model allows beneficial effects on human health after consumption, depending on the Se-treatment dose.

Materials and methods

Experimental design

The experiment was conducted at the Institute of Plant Nutrition and Soil Science, Kiel University, Kiel, Germany. Two lettuce cultivars - Hawking RZ (green multi-leaf lettuce, V1) and Barlach RZ (red multi-leaf, V2) - were cultivated in a hydroponic system under standard greenhouse conditions as described by ABDALLA et al. (2022). Subsequently, the seedlings were transplanted individually in 10 L black containers. The basal nutrient solution containing a complete mineral complement was supplied as described by ABDALLA et al. (2022). Four replicates were arranged in a completely randomized design. To discover the effect of Se and S enrichment on the primary metabolism in lettuce plants, they were subjected to three different Se treatments using a foliar application (Se0: 0 µM; Se1: 0.2 µM; and Se2: 2.6 µM (Na_2SeO_4)) under three varied S levels which were supplied via the nutrient solution (S0: 0 mM; S1: 1 mM; and S2: 1.5 mM (K_2SO_4)) (ABDALLA et al., 2022). Se application was performed one month after transplanting (at 28 days after transplanting), and the dose was repeated once for three weeks. Se concentrations were calculated as 0.038 g L^{-1} and 0.205 g L^{-1} as Na_2SeO_4 (stock solutions) for both doses of 0.2 µM and 2.6 µM, respectively. Tow plants per pot were arranged, subsequently, at each time of application, 5 mL pot^{-1} (2.5 mL $plant^{-1}$) of Na_2SeO_4 (from each stock solution) was applied to the leaves of each plant that received 0.2 µM or 2.6 µM Se treatment. To improve wetting properties, 0.04% Silwet was used as a wetting agent. The lettuce plants were harvested at around 55 days of the experiment. The heads of the lettuce were washed with deionized water, and the fresh matter (FM) was recorded. Subsequently, the lettuce heads were dried at –53 °C in a freeze dryer (Gamma1-20, Christ, Osterode am Harz, Germany). The freeze-dried lettuce heads were ground to a fine powder and stored for further analysis.

Total Se quantification using ICP-MS

The concentrations of Se were determined by using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies 7700 Series, Boebelingen, Germany) as described by JEZEK et al. (2015).

Total S quantification using an elemental analyzer

S concentration was determined using an elemental analyzer (Flash EA1112, Thermo Fisher Scientific, Milano, Italy) as described by ZÖRB et al. (2013).

Quantification of inorganic anions, organic acids, and water-soluble sugars

Around 0.02 g of finely ground lettuce samples were extracted in 1 mL of double deionized water at 100 °C for 5 min to determine inorganic anions, organic acids, and soluble sugars following a modified protocol described by CATALDI et al. (2000). Briefly, the extracts were kept on ice for 30 min, and after that, the mixture was centrifuged at 12,000 rpm for 10 min. Afterwards, the supernatants obtained from centrifugation were diluted 10-fold with ultrapure and vortexed to perform protein precipitation. Subsequently, a few drops of chloroform were added, and the mixture was centrifuged at 4 °C for 5 min at 12,000 rpm. The collected supernatants were purified by C18 columns (Strata® 8B-S001-DAK, Phenomenex, Torrance, CA, USA). Soluble sugars (glucose, fructose, and sucrose) were separated using Dionex Carbo Pac PA 100 IC column (Thermo Scientific). The eluents used were deionized water (H_2O) : sodium hydroxide (NaOH), (A : B). 200 mM NaOH (B) was used at a flow rate of 1.0 mL/min. The gradient of eluent B was as follows: 25%, 50%, and 100% at 25 °C for 28 min. The injection volume was 10 µL. Quantitative and qualitative analysis was done using pulsed amperometric detection (PAD). Inorganic anions (SO_4^{2-} and NO_3^-) and organic acids (citrate, malate, and oxalate) were separated by Dionex IonPac AS11- Hc - 4 µm column (Thermo Scientific). The eluents used were H_2O : NaOH (A : B). 50 mM NaOH (B) was used at a 1.5 mL/min flow rate, and eluent A was rinsed at the same flow rate. The injection volume was 10 µL. The gradient of eluent B was as follows: 4%, 10%, 48%, 75%, and 100% (including washing and stabilization of the column) at 25 °C, and the gradient cycle was 30 min. Detection was performed by measuring conductivity. The concentrations of inorganic anions and organic acids were quantified using isocratic ion chromatography (Dionex ICS-5000 ICS-5000, Thermo Scientific).

Total protein quantification

The proteins were extracted from the lettuce heads using the method described by DAMERVAL et al. (1986) and modified according to Zörb et al. (2004). The 100 mg aliquot of the fine powder was suspended in ice-cold 1.6 ml solution 1 (10% TCA/acetone and 80 µl 1M DDT). The mixture was placed in an ice-cold sonication bath for 15 min, then kept at –20 °C overnight, centrifuged at 13,000 rpm for 15 min at 4 °C and the supernatant discarded. The protein precipitate was washed with ice-cold 1.5 ml solution 2 (1.5 ml Acetone and 75 µl 1 M DDT, 6 µl 0.5 M EDTA), followed by sonication, then kept at -20 °C overnight and centrifuged as mentioned above. The washing step was repeated once without EDTA followed by centrifugation as above, and the pellet was dried using a vacuum centrifuge. The pellet was dissolved in 1 ml lysis buffer containing 8 M Urea, 2 M Thiourea, 4% CHAPS, and 30 mM Tris pH 8,8 with a 5 µl proteinase inhibitor cocktail. To enhance the dissolving membrane-bound protein the sample was shaken at 33 °C for 2 h using an Eppendorf shaker and centrifuged. The supernatant was stored at –20 °C for subsequent quantification. The total protein concentration in the lettuce head extracts was determined using the Bradford method using BSA as a protein standard (Bradford, 1976).

Statistical Analysis

The data of the Se and S accumulation, the plant biomass, in addition to water-soluble sugars, organic acids, inorganic anions, and total protein concentrations, were statistically analyzed using a three-way (Se and S treatment × 2 cultivars) analysis of variance (ANOVA). This was followed by multiple comparisons based on the adjustment method using Tukey's multivariate distribution.

Results

Selenium and sulfur accumulation in lettuce leaves

The Se and S bioaccumulation level in lettuce was significantly affected by applied Se and S fertilization. Both elements' concentrations in lettuce also depended on the fertilizer application technique used, especially the Se foliar treatment in the presence of S supplied in the nutrient solution.

Concerning Se concentration, it was enhanced significantly by increasing Se foliar application in both cultivars (V1 and V2). However, Se accumulation decreased significantly under higher Se and S conditions (Se2/S2) compared to the high Se under adequate S-supply (Se2/S1) in the green lettuce. Elevated Se and S application (Se2/S2) provoked significant Se accumulation in red lettuce in comparison to higher Se under adequate S treatment (Se2/S1) (Figure 1). Apart from Se accumulation, the S level increased significantly with increasing S treatments (S1: 1 mM; and S2: 1.5 mM). Although S accumulation improved drastically and synergistically under higher S and Se treatments (Se2/S2) in green lettuce, it remained unaltered in red lettuce under the same conditions (Fig. 1).

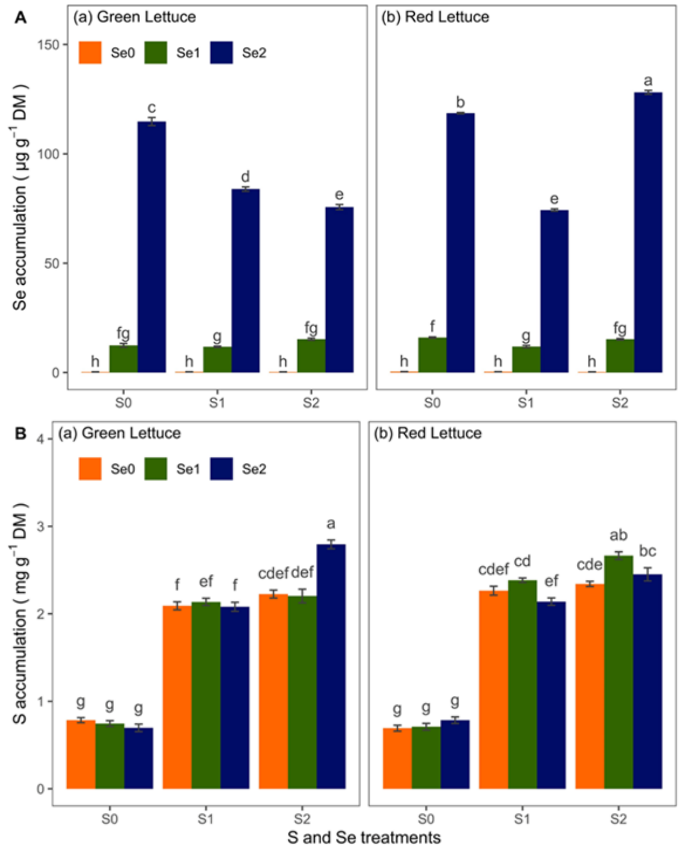


Fig. 1: Se and S accumulation in the leaves of green and red multi-leaf lettuce (cultivar: Hawking RZ (a), and Barlach RZ (b), respectively) grown in a hydroponic system treated with three S levels (S0: 0 mM; S1: 1 mM; and S2: 1.5 mM K_2SO_4) and three Se levels (Se0: 0 µM; Se1: 0.2 µM; and Se2: 2.6 µM Na_2SeO_4).

Plant biomass

The application of Se in the presence of S caused several variations in plant growth. Fig. 2 shows a top view comparing the green and red multi-leaf lettuce heads grown under varied Se and S supplies.

The FM in both cultivars increased significantly ($p \leq 0.05$) under S sufficiency (S1 = 1 mM) and without Se application (Se = 0 µM) in comparison to the S-deprived condition (control: S0 = 0 mM) (Tab. 1, Fig. 2A). A greater change (2.6 and 2.3-fold, respectively) in FM was observed in green and red lettuce. Although the FM decreased significantly under higher S and moderate Se levels in both cultivars, it increased significantly ($p \leq 0.05$) in response to elevated S and Se application (S2 and Se2) (Tab. 1, Fig. 2B).

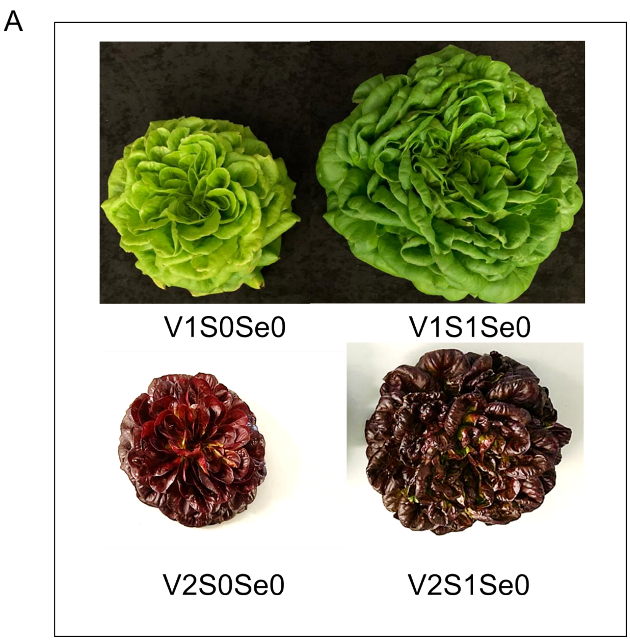
Quantification of water-soluble sugars, organic acids, and inorganic anions

Concerning the water-soluble sugars, S fertilization increased the concentration of glucose and fructose in Se non-treated green and red lettuce compared to the control (S0). In contrast, glucose and fructose concentrations were enhanced with greater changes (2.9 and 1.9-fold) and (1.9 and 2.0-fold) in green and red lettuce, respectively. Under higher S (S2) and without Se application in the green and red multi-leaf lettuce, the levels of glucose and fructose increased drastically to 125.0 ± 0.36 and 76.3 ± 1.1 mg g^{-1} DM, respectively, whereas in the red lettuce, concentrations were 106.5 ± 0.5 and 94.8 ± 1.6 for glucose and fructose, respectively. In red lettuce subjected to adequate S and elevated Se (Se2) fertilization, the glucose and fructose levels increased significantly to 72.2 ± 1.5 and 45.8 ± 1.5 mg g^{-1} DM, respectively. However, the increase in green lettuce was insignificant (Tab. 1). In green and red lettuce grown under higher S and Se conditions (Se2/S2) glucose and fructose levels declined dramatically (80.8 ± 0.25 and 53.9 ± 1.5) and (75.4 ± 1.4 and 66.1 ± 0.6) mg g^{-1} DM, respectively, in comparison to the control (S0/Se0).

The elevated Se and S treatments (Se2/S2) accumulated more fructose in red lettuce (66.1 ± 0.6 mg g^{-1} DM) than in the green cultivar (53.9 ± 1.5 mg g^{-1} DM).

Adequate and higher S fertilization significantly ($p \leq 0.05$) increased sucrose accumulation in the red lettuce (by 33.6% and 102.8%, respectively) compared to the control (S0Se0). However, sucrose accumulation decreased in green lettuce under the same conditions. In both pigmented lettuce cultivars, sucrose levels remained unaltered in plants grown under adequate S supply and moderate Se (S1Se1) compared to the control (S0Se0).

Apart from water-soluble sugars, organic acids are affected differently in green and red lettuce. For instance, malic acid decreased significantly in Se non-treated green lettuce under both S1 and S2 levels, compared to the control Se0S0. However, malic acid concentration was enhanced significantly ($p \leq 0.05$) under adequate S (S1) and lower Se supply to (30%) compared to the control (S1Se0). On the other hand, in red lettuce grown under S limitation, malic acid accumulation increased to 78.7%, and 71.9%, respectively, following Se1 and Se2 treatments, compared to the control (S0Se0) (Tab. 1), whereas, under adequate S and Se-limiting conditions, it enhanced up to 74.2% more than the control (S0Se0). Although Se1 under adequate S decreased malic acid significantly (by 33%), higher Se (Se2) under adequate S enhanced the malic acid level (by 26%). Regarding, citric acid, in green and red lettuce, its concentration remained unaltered under S starvation and Se0 and Se1 conditions. However, it decreased significantly under S1 and Se0 (by 45.4% and 47.6%, respectively) in green and red lettuce, compared to the control (S0Se0). Furthermore, it decreased drastically under S2 and Se0 conditions (by 70.8% and 142% respectively) in green and red lettuce, compared to (S0Se0) and declined non-significantly under S2 and (Se1 and Se2) compared to (S1Se0). Besides malic acid and citric acid, oxalic acid remained unaltered in green lettuce under adequate and higher S and all Se treatments compared to S0Se0. In red lettuce, oxalic acid concentration decreased significantly under adequate S (S1) and Se0 (by 37%) compared to the control (S0Se0). However, oxalic acid remained unaltered in plants treated with adequate S and higher Se (S1Se2) compared to the control (S0Se0). Furthermore, differential changes were observed in green and red leaf lettuce concerning the accumulation of anions such as NO_3^- and SO_4^{2-}. In both pigmented lettuce cultivars, NO_3^- levels remained unaltered under S deprivation and all Se treatments (Se0, Se1, and Se2) compared to the control (S0Se0). In green lettuce NO_3^- level significantly decreased (by 41.6%) under adequate S and moderate Se treatments. A drastic reduction in NO_3^- accumulation was observed in both green and red cultivars under higher S (S2) and Se varied treatments (by 51.3%, 42.9%, and 37.7% in V1 and 90.5%, 87.7%, and 89.2% in V2, respectively) compared to the control (S0Se0). In both cultivars, SO_4^{2-} enhanced with increasing S treatments; moreover, it was not affected antagonistically under S1 and S2 in response to lower or higher Se application.



Total protein quantification

Statistical data analysis showed that S application drastically affects protein concentration (Fig. 3). With the increasing S level, the green lettuce protein concentration increased gradually. In red lettuce, protein levels increased under adequate S levels and remained unaltered under higher S levels (S2). The S treatment at 1.5 mM (S2) in the green cultivar had the highest protein concentration. Although in red lettuce, protein concentration increased compared to the control, in the case of the two S treatments at 1 mM and 1.5 mM, under S2 levels the protein concentrations remained unchanged. There were no

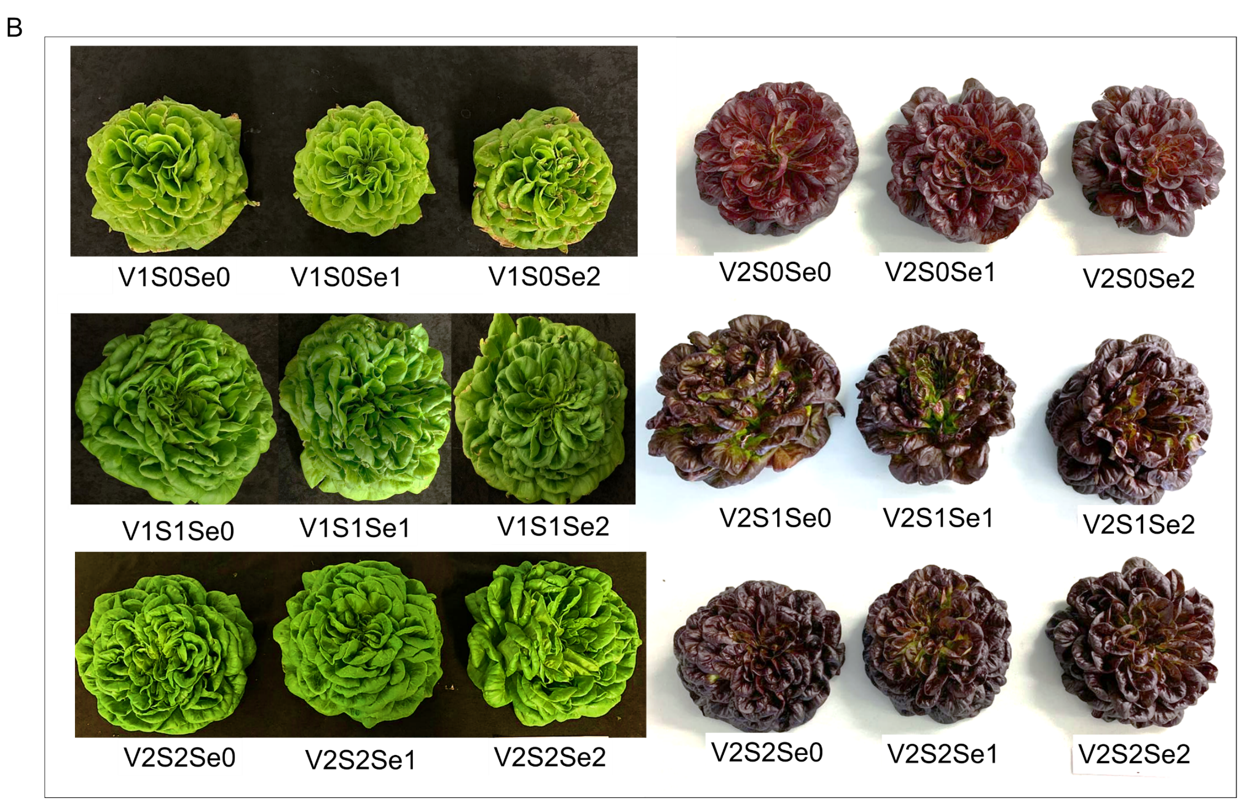


Fig. 2 A: Comparison of the green and red multi-leaf lettuce heads grown under S deficiency (S0) and S-sufficiency (S1). **B:** Comparison of the green and red multi-leaf lettuce heads (cultivar: Hawking RZ, V1 and Barlach RZ, V2, respectively) grown in a hydroponic system treated with three S levels (S0: 0 mM; S1: 1 mM; and S2: 1,5 mM (K_2SO_4)) and three Se levels (Se0: 0 μM; Se1: 0.2 μM; and Se2: 2.6 μM (Na_2SeO_4)).

Tab. 1: FM (g plant^{-1}), soluble sugars, organic acids, and anions (mg g^{-1} DM), of the green and red multi-leaf lettuce (cultivar: Hawking RZ, V1, and Barlach RZ, V2, respectively) grown in a hydroponic system treated with three S levels (S0: 0 mM; S1: 1 mM; and S2: 1.5 mM K_2SO_4) and three Se levels (Se0: 0 µM; Se1: 0.2 µM; and Se2: 2.6 µM Na_2SeO_4).

Green lettuce V1		**S0**			**S1**			**S2**	
	Se0	**Se1**	**Se2**	**Se0**	**Se1**	**Se2**	**Se0**	**Se1**	**Se2**
Plant biomass									
FM	142.4 ± 0.35 j	133.6 ± 0.33 l	93.7 ± 0.36 o	372.2 ± 0.6 a	338.0 ± 0.62 b	328.8 ± 0.31 c	284.4 ± 0.37 d	229.0 ± 0.47 g	255.6 ± 0.43 e
Soluble sugars									
Glucose	23.4± 0.36 ij	20.7 ± 0.40 j	14.5 ± 2.6 k	67.6 ± 1.1 f	74.0 ± 1.5 e	71.3 ± 1.8 ef	125.0 ±0.36 a	104.5±1.3 b	80.8±0.25 d
Fructose	25.4 ± 0.43 g	21.3 ± 1.0 gh	22.1 ± 0.4 gh	48.8 ± 0.3 de	49.2 ± 0.5 de	48.3 ± 0.8 de	76.3 ± 1.1 b	65.6 ± 0.9 c	53.9 ± 1.5 d
Sucrose	22.9 ± 0.77 cd	20.5 ± 0.6 cdef	23.1 ± 1.04 cd	18.4 ± 1.2 efgh	21.0 ± 0.6 cde	17.6 ± 0.5 efgh	17.8 ± 0.9 efgh	16.1 ± 0.4 gh	11.4 ± 0.4 i
Organic acids									
Malic acid	14.1 ± 0.32 abc	12.6 ± 0.38 cd	11.9 ± 0.16 cde	9.0 ± 0.55 fg	11.7 ± 0.39 cde	8.9 ± 0.04 fg	9.4 ± 0.13 ef	6.5 ± 0.41 g	6.6 ± 0.43 g
Citric acid	18.5 ± 0.41 a	20.7 ± 0.78 a	21.0 ± 0.78 a	10.1 ± 0.47 cd	12.8 ± 0.69 b	11.8 ± 0.78 bc	5.4 ± 0.29 fg	6.6 ± 0.48 ef	7.3 ± 0.11 bef
Oxalic acid	0.94 ± 1.0 bcd	1.3 ± 0.1 abc	1.4 ± 0.1 a	0.94 ± 0.1 bcd	0.74 ± 0.1 d	0.63 ± 0.1 d	0.77 ± 0.1 d	0.81 ± 0.03 d	0.9 ± 0.03 bcd
Anions									
NO_3	15.4 ± 0.6 ab	16.7 ± 0.4 a	12.7 ± 0.7 bcd	13.7 ± 0.12 abc	9.0 ± 1.3 ef	10.9 ± 0.8 cde	7.5 ± 0.3 f	8. 8 ± 0.5 ef	9.6 ± 0.5 def
SO_4	0.02 ± 0.01 d	0.01 ± 0 d	0.01 ± 0 d	1.19 ± 0.1 abc	1.03 ± 0.1 c	1.2 ± 0.13 abc	1.13 ± 0.11 bc	1.33 ± 0.1 abc	1.43 ± 0.1 abc
Red lettuce V2		**S0**			**S1**			**S2**	
	Se0	**Se1**	**Se2**	**Se0**	**Se1**	**Se2**	**Se0**	**Se1**	**Se2**
Plant biomass									
FM	136.7 ± 0.7 k	122.5 ± 0.8 m	78.6 ± 0.5 q	263.7 ± 0.75 f	211.7 ± 0.32 h	193. 6 ± 0.37 i	95.3 ± 0.55 o	90.7 ±0.21 p	98.3 ± 0.21 n
Soluble sugars									
Glucose	28.7 ± 0.46 i	26.8 ± 1.6 ij	29.3 ± 0.46 i	51.2 ± 0.62 h	60.7 ± 1.5 g	72.2 ± 1.5 ef	106.5 ± 0.5 b	89.3 ± 0.37 c	75.4 ± 1.4 de
Fructose	16.8 ± 1.0 h	17.1 ± 0.48 h	18.1 ± 0.79 h	34.1 ± 1.6 f	36.3 ± 1.3 f	45.8 ± 1.5 e	94.8 ± 1.6 a	78.6 ± 0.66 b	66.1 ± 0.6 c
Sucrose	14.3 ± 0.6 hi	14.6 ± 0.22 hi	34.3 ± 0.8 a	19.1 ± 0.8 defg	16.3 ± 0.7 fgh	16.8 ± 0.5 efgh	29.0 ± 0.6 b	30.7 ± 1.0 ab	24.5 ± 0.8 c
Organic acids									
Malic acid	8.9 ± 0.3 fg	15.9 ± 0.28 a	15.3 ± 0.22 abc	15.5 ± 0.51 ab	10.4 ± 0.19 def	13.1 ± 0.07 bc	15.4 ± 0.83 ab	13.0 ± 0.6 bcd	14.2 ± 1.0 abc
Citric acid	12.6 ± 0.23 bc	10.8 ± 0.29 bc	8.1 ± 0.26 de	6.6 ± 0.33 ef	4.9 ± 0.39 fg	3.6 ± 0.46 gh	1.3 ± 0.1 h	1.0 ± 0.01 h	1.3 ± 0.08 h
Oxalic acid	1.4 ± 0.03 a	1.6 ± 0.1 a	1.4 ± 0.04 a	0.88 ± 0.03 cd	0.89 ± 0.03 cd	1.3 ± 0.1 ab	0.77 ± 0.03 d	0.78 ± 0.1 d	0.76 ± 0.02 d
Anions									
NO_3	13.0 ± 0.3 bc	12.8 ± 0.2 bc	10.8 ± 0.4 cde	10.2 ± 0.5 cd	13.5 ± 0.4 bc	12.7 ± 0.02 bcd	1.23 ± 0.06 g	1.6 ± 0.12 g	1.4 ± 0.13 g
SO_4	0.04 ± 0 d	0.04 ± 0.01 d	0.03 ± 0.01 d	1.31 ± 0.1 abc	1.32 ± 0.2 abc	1.37 ± 0.04 abc	1.59 ± 0.11 a	1.53 ± 0.06 ab	1.60 ± 0.1 a The

data presented are the means ± SEs of four replicates. Different letters show statistically significant differences among all the treatments ($p \leq 0.05$; Tukey's test).

significant differences in protein accumulation under varied Se levels in both cultivars. However, in red lettuce Se levels at 2.6 µM significantly enhanced protein concentration under S-deprived conditions.

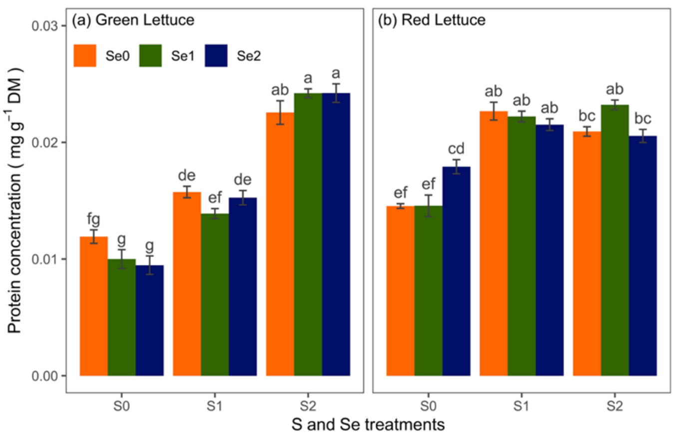


Fig. 3: Protein concentration in the leaves of green and red multi-leaf lettuce (cultivar: Hawking RZ (a), and Barlach RZ (b), respectively) grown in a hydroponic system treated with three S levels (S0: 0 mM; S1: 1 mM; and S2: 1.5 mM K_2SO_4) and three Se levels (Se0: 0 µM; Se1: 0.2 µM; and Se2: 2.6 µM Na_2SeO_4).

Discussion

Se and S interaction under Se foliar application and S supply via root

Previous reports indicated that S-deficient plants had higher Se levels than S-sufficient ones. This can be attributed to the lack of competition for selenite (SCHIAVON et al., 2012). Interestingly, a higher Se concentration in the red lettuce that grew under a higher S level might be attributed to Se foliar application. This finding contradicts the previous studies that reported on applying Se and S together via the root, where the elements competed for the influx to roots (WHITE et al., 2004).

The findings align with recent reports indicating a drastic reduction in lettuce FM under S deprivation, leading to delayed development and maturity (ABDALLA et al., 2021; CHUNG et al., 2022). Noticeably, the green lettuce under S deficiency had tiny and narrow leaves, which, in the end, resulted in inhibited development shown as very small lettuce heads compared to lettuce grown under S sufficiency. In contrast, the red lettuce exhibited better acclimation to S deprivation, as evidenced by relatively bigger leaves than green ones. However, the leaf biomass of red lettuce drastically enhanced under S sufficiency (Fig. 2). Noticeably, in green lettuce grown under higher Se and adequate or higher S, mild brown discoloration at the tips and margins of lettuce leaves was observed, which might be a sign of stress.

An inhibitory effect of Se in FM was reported previously when lettuce plants were supplied with high levels of selenite (RAMOS et al., 2010). Moreover, a significant increase in FM in lettuce plants when supplied with 10 µM Na_2SeO_4, accompanied by a reduction in FM was observed at an elevated Se level of 20 µM (SANTIAGO et al., 2020). Accordingly, a low Se dose can improve lettuce FM.

The impact of Se and S enrichment on lettuce internal quality traits

Sugars have a crucial role in crop quality, as they directly affect the flavor, storage length, and biosynthesis of other quality-related metabolites such as organic acids, amino acids, and antioxidant nutrients, which consequently influence consumer preference (DURÁN-SORIA et al., 2020; CHEN et al., 2020). The soluble sugars, including glucose, fructose, and sucrose, were quantified under all Se and S treatments. Hence, S plays a vital role in photosynthesis and chlorophyll formation. S deprivation will negatively affect carbon assimilation and, consequently, the concentration of soluble sugars (JOBE et al., 2019). The previous report indicated a strong relationship between the sulfide level, water-soluble sugar level, and TOR (the target of rapamycin) activity (DONG et al., 2017), where sulfide fumigation for 6 hours enhanced sugar concentrations including glucose, fructose, and sucrose (DONG et al., 2017). The effect of Se on carbohydrate metabolism and nutrient composition has been previously studied (TAN et al., 2022; BHADWAL and SHARMA, 2022; ABDALLA et al., 2022). For instance, several reports indicated that varied Se levels impact plant growth differently, suggesting that lower and not higher Se concentrations positively affect plant growth (DAS et al., 2019).

Moreover, previous results showed that, although Se application significantly enhanced the accumulation of soluble protein and water-soluble sugar (e.g. fructose, and glucose), it reduced sucrose levels in pea sprouts (TAN et al., 2022). Sucrose regulates many processes, such as carbohydrate metabolism, sucrose transport (Fig. 4), and anthocyanin accumulation (YOON et al., 2021). Sucrose level remained unchanged in both cultivars grown under lower Se and adequate S compared to the control (Se0S0). This can provide direct evidence that sucrose concentration did not increase, and that it was converted into reducing sugars, probably to support the cryoprotection of the cells (ZÖRB et al., 2014). A previous report investigating factors affecting the sugar content of potatoes indicated that the sucrose concentration reveals tuber maturity, which is associated with a lower sucrose level (KUMAR et al., 2004). Additionally, a recent study that evaluated the effect of K_2SO_4 and KCl fertilization on quality

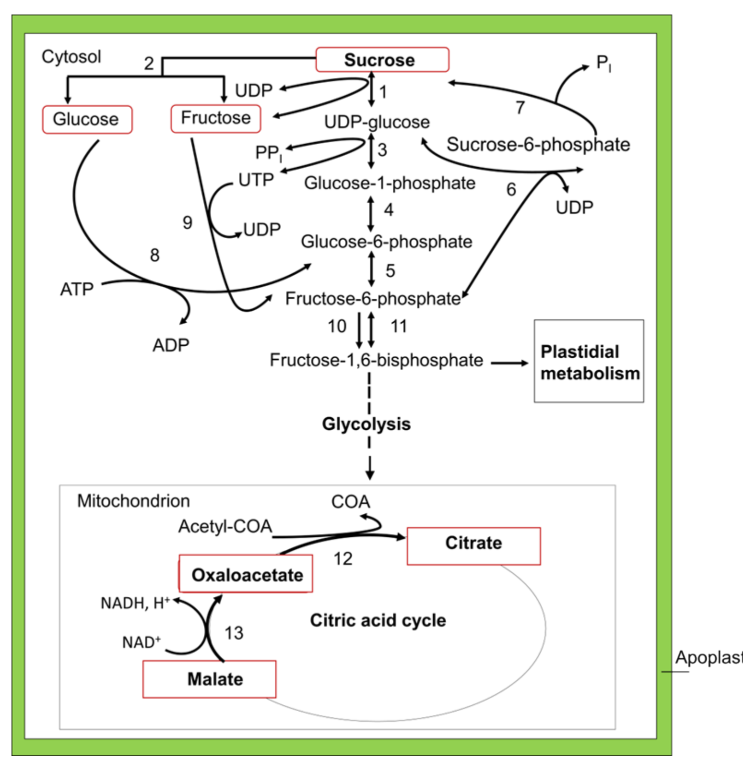


Fig. 4: Cytosolic and mitochondrial carbohydrate metabolism. Enzymes: 1: sucrose synthase; 2: invertase; 3: UDP-glucose pyrophosphorylase; 4: phosphoglucomutase; 5: phosphoglucose isomerase; 6: sucrose phosphate synthase; 7: sucrose phosphate phosphatase; 8: hexokinase; 9: fructokinase; 10: phosphofructokinase; 11: pyrophosphate-fructose-6-phosphate 1-phosphotransferase; 12: citrate synthase; 13: malate dehydrogenase. Abbreviations: Pi: inorganic phosphate; PPi: pyrophosphate.

parameters, including soluble sugars in potato tubers, suggested that the fertilization impact on sugar content, including sucrose, was minor and associated with other factors such as cultivar, year, and storage period (Wilmer et al., 2022). Regarding carbohydrate metabolism (Fig. 4), previous research demonstrated that moderate Se (1.25 mg l^{-1}) fertilization in pea sprouts significantly exhibited the highest enzyme activity, especially for acid invertase and neutral invertase. In contrast, the sucrose synthase activity remained unaltered with the change in Se levels (Tan et al., 2022). The study suggested that since the difference in the concentration of the soluble sugar agreed with that of the activities of acid invertase and neutral invertase, it can be assumed that these enzymes have a critical role in soluble sugar accumulation. Apart from water-soluble sugars, organic acids such as malic acid, and citric acid accumulation were significantly affected under Se and S enrichment. Se treatment of 10 µM enhanced citric acid, while malic acid significantly increased under higher Se application (100 µM) in strawberry fruits (Mimmo et al., 2017).

Collectively, soluble sugars and organic acids are essential components of the taste and overall acceptance and organoleptic quality of food crops (Famiani et al., 2015).

Regarding the anions such as NO_3^- and SO_4^{2-}, differential changes were detected in green and red leaf lettuce under Se and S treatments (Tab. 1). The quantification of NO_3^- in crops is essential to produce vegetables with better food quality. Moreover, high NO_3^- in leafy vegetables significantly threatens human health. In a recent study, exogenous selenate decreases NO_3^- accumulation more than selenite in lettuce plants in a concentration-dependent manner (Bian et al., 2020).

NO_3^- in leafy vegetables, including lettuce, is a source of dietary NO_3^-, and a low nitrate level in the edible parts of plants is significant for human health (Salehzadeh et al., 2020). Accordingly, it is very important to determine the optimum fertilization level to produce food crops with adequate NO_3^- levels. Se treatment likely induced NO_3^- assimilation by stimulating the activity of the enzymes involved in nitrogen metabolism including nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate synthase (GOGAT) (Lei et al., 2018). The authors indicated that the optimum Se concentration for reducing NO_3^- in hydroponic lettuce is 0.5 µmol L^{-1} (Lei et al., 2018). Moreover, sulfate fertilization can cause a significant decline in NO_3^- accumulation in lettuce plants (Karipidis et al., 2019).

Lettuce is not a source of protein, as it has a low protein level (Kim et al., 2016). S application significantly enhanced protein levels (by 33.3%) in red lettuce in response to adequate S, and by 91.7% under higher S in green lettuce. In red lettuce, a significant increase (53.3%) was found only under sufficient S levels compared with the control (S0Se0).

Previous studies demonstrated that although Se treatment impacts the protein concentration in food crops, the Se treatment dose is the limiting factor. For instance, a suitable Se level can enhance protein biosynthesis, but elevated Se doses increase Se toxicity and subsequently reduce protein synthesis (Wrobel et al., 2020; Deng et al., 2021; Huang et al., 2022). This might be a negative effect as, under S deficiency, the non-specific integration of seleno-amino acids into protein leads to Se-induced toxicity in plants (Tian et al., 2017); therefore, different defensive proteins can be activated.

Recommendation for the production of lettuce under Se foliar fertilization

In comparison to soil application, Se foliar application is usually more efficient in shortening the transport distance of Se from plant roots to shoots, and consequently increasing Se concentrations in food crops (Ros et al., 2016). Accordingly, Se foliar application might reduce the competitive effect when S is supplied via the roots. In principle, the efficiency of the foliar fertilization method depends on the speed of ion absorption via the leaves and stems, and its mobility (Ježek et al., 2012). As an S analog, the Se uptake mechanism and assimilation by plants can be compared to S. In an early investigation, the absorption rate of ^{35}S isotope (in SO_4^{2-} form) by the primary leaf of the common bean was studied. It has been reported that after 24 hours following S foliar application, 20% was absorbed by the leaves. Subsequently, the absorption increased by 30% after 48 hours and reached 50% after four days. In eight days, the absorption was enhanced by 70% of the applied S. S is mobile and has a medium absorption rate compared with other elements. Thus, based on the physicochemical similarity between Se and S, Se could follow a similar uptake mechanism upon foliar application (Bukovac and Wittwer 1957). It is crucial that following harvesting of Se-enriched lettuce, the leaves should be washed several times to remove surface-deposited Se residues.

In the current report, Se foliar application ensures successful Se accumulation in lettuce plants and can secure the recommended daily dose of 60 and 70 µg Se day^{-1} for adult women and men, respectively. Since lettuce contains around 95% water (Hawrylak-Nowak, 2013), the current findings indicate that consuming 100 g of fresh green and red lettuce leaves treated with moderate Se (Se1) and adequate S (S1) levels can contribute 59 µg Se day^{-1} to Se dietary requirements (Ram et al., 2016; Zou et al., 2019). Additionally, consuming 100 g fresh weight of both pigmented lettuce cultivars grown under moderate Se and higher S (Se1/S2) can provide 75 µg Se day^{-1} dietary requirements. Furthermore, under higher Se levels (Se2) and adequate or higher S (Se2S1) and (Se2S2), consuming 100 g of fresh weight green and red lettuce can contribute 420-370 and 380-640 µg Se day^{-1}, respectively, which is higher than the recommended dose. In this regard, we recommend that the intake of only 17-19 g or 18-11 g fresh weight green and red lettuce respectively under (Se2S1) and (Se2S2) can provide the dietary requirements with µg Se day^{-1}. Collectively, foliar application of low Se levels under adequate and higher S fertilization via the nutrient solution can benefit human health and provide the daily recommended dose. It was also observed that Se levels enhanced drastically under S deprivation, resulting in higher Se accumulation, which could be toxic to humans. Accordingly, the current study recommends that lettuce can be enriched with Se simultaneously with S application because S counteracts Se toxicity.

Based on scientific research on Se foliar fertilizer, which was applied in the form of sodium selenate directly onto apple plants in the field using a fertilizer rate of 0.075 kg Se/ha × m CH (kg Se per hectare meter canopy height) with the aid of a backpack sprayer (Groth et al., 2021), it is possible to apply this method on a larger scale, especially in those areas with Se-deficient soil.

Conclusion

The main conclusion of the current report supports the idea that Se and S application dramatically impacts the quality of lettuce. Hence, improving the nutritional constituents of lettuce to nourish human health remains highly important. The data presented here indicate that S application under lower Se can promote lettuce quality.

Further investigation is needed to determine the response of lettuce plants to the involvement of both S and Se and their impact on the major metabolic pathways, especially nitrogen, and S assimilation, for an in-depth understanding of the underlying regulatory mechanisms.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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