Research Article

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Selenium biofortification of pea (*Pisum sativum* L.) microgreens through seed priming: Effects on agronomic performance and nutritional quality

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Abstract

BACKGROUND: Selenium (Se) deficiency is a widespread nutritional problem. Agronomic biofortification of microgreens through seed nutripriming is a promising approach to enhance Se intake. Microgreens, comprising nutrient-dense foods that can be produced year-round, are ideal targets for biofortification and may help alleviate malnutrition. This study evaluated the effects of Se nutripriming on germination, growth performance, chemical composition and antioxidant properties of pea (*Pisum sativum* L.) microgreens.

RESULTS: Nutripriming significantly increased seed Se content, with the 100 μM–12-h Se treatment resulting in a four-fold accumulation compared to controls, at the same time as preserving membrane integrity. Microgreens from primed seeds exhibited enhanced Se translocation to aerial tissues and an improved mineral profile. Short-term treatments (6 h) increased glucose and oxalic acid levels, whereas prolonged priming (12 h) favored sucrose accumulation. However, this 12-h duration significantly reduced the emergence rate and aerial biomass yield. The optimal antioxidant profile, characterized by the highest total phenolic content and strongest inhibition of lipid peroxidation, was achieved with the 50 μM–12-h Se treatment.

CONCLUSION: Se nutripriming enhanced the nutritional quality of pea microgreens but revealed a critical trade-off: although 12-h priming maximized Se uptake and antioxidant properties, it severely reduced agronomic production. Conversely, 6-h priming promoted better biomass yield, although germination remained lower than in the NP control. Therefore, optimizing priming duration is essential to balance the biofortification benefits with overall crop productivity.
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Supporting information may be found in the online version of this article.

Keywords: agronomic biofortification; antioxidant activity; free sugars/organic acids; green pea; nutrient uptake; seed nutripriming

INTRODUCTION

Modern agriculture is increasingly challenged by climate change, soil degradation, and the increasing scarcity of some essential nutrients, which collectively threaten global food security.[1,2] Among these concerns, selenium (Se) deficiency stands out as a critical issue, affecting almost one billion people worldwide due to insufficient dietary intake. Crops cultivated in Se-poor soils inevitably contain low levels of this vital micronutrient.[3] Projections under moderate climate change scenarios suggest soil Se losses of up to 8.7% in 66% of croplands,[4] which could further exacerbate Se deficiency. Moreover, even soils with adequate Se content may still yield Se-deficient crops if the element is not present in a phytoavailable form.[5] In humans, Se plays indispensable roles in antioxidant defense, immune regulation and thyroid hormone metabolism; its deficiency is associated with severe disorders such as cardiomyopathy, impaired fertility and elevated cancer risk.[6-8] To address these interconnected agronomic and nutritional challenges, agronomic biofortification emerges as a relatively simple and rapid approach to produce crops enriched

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with target minerals, offering faster results than breeding or transgenic crop development.[9,10] This strategy is regarded as one of the most viable and effective short-term solutions to combat malnutrition.

Seed priming is an emerging biofortification technique that enhances both the agronomic performance and mineral content of horticultural crops. It consists of controlled seed hydration to activate pre-germinative metabolic processes without radicle protrusion. This low-cost method accelerates and synchronizes germination, improves seedling vigor and strengthens resilience against abiotic stresses, thereby optimizing crop establishment.[11] A more advanced approach, nutripriming, utilizes nutrient-enriched solutions for hydration, providing dual benefits: improved germination performance and the nutritional value of the resulting plants.[9] In the context of Se biofortification, sodium selenate (Na_2SeO_4) has proven more efficient than sodium selenite (Na_2SeO_3) in translocating Se to edible tissues, making it a promising candidate for nutripriming.[12,13] However, the success of this method is highly species-dependent and requires careful adjustment of parameters, such as Se concentration and soaking duration, to maximize benefits at the same time as avoiding phytotoxic effects. The potential of nutripriming is particularly promising when applied to high-value, nutrient-dense crops such as microgreens.

In recent years, microgreens have gained significant recognition as nutrient-dense functional foods.[14] These young vegetable greens, typically harvested 7–21 days after germination once the first true leaves appear, require minimal space and inputs.[15] Among them, pea (*Pisum sativum* L.) microgreens are widely consumed because of their desirable sensory attributes, such as crunchiness and a slightly sweet taste, combined with their high content of essential micronutrients and protein, making them a valuable component of healthy diets.[16] Furthermore, their rapid growth cycle and high responsiveness to nutrient inputs make them ideal models for testing biofortification strategies.[17] Thus, Se-based seed nutripriming represents a promising strategy to enhance germination rate and synchrony at the same time as enriching pea microgreens with this element, producing biofortified foods capable of addressing both agronomic and nutritional challenges.

Accordingly, the preseny study aimed to evaluate the effects of nutripriming pea seeds with different Se concentrations and soaking durations on seed membrane integrity and Se content, emergence rate, and the chemical and biochemical composition of the resulting microgreens. By improving agronomic performance and promoting Se biofortification, this approach aimed to generate plant foods with enhanced nutritional value, thereby contributing to greater food security and human health.

MATERIALS AND METHODS

Chemicals, equipment and plant material

The chemicals and analytical standards used in the present study are listed in the Supporting information (Table S1). The laboratory equipment and chromatographic instruments, along with their respective manufacturers, are also provided in the Supporting Information (Table S2). Green pea (*Pisum sativum* L., cv. 'Telefone Anã') seeds were obtained from Flora Lusitana, Lda. (Cantanhede, Portugal).

Seed priming

Seed surface sterilization was carried out by immersing the seeds in 1% (*w/v*) sodium hypochlorite for 3 min, followed by thorough rinsing with deionized water.[18] The seeds were then dried in a forced-air oven at 35 °C for 24 h before priming.[19,20] Nutripriming was performed by soaking pea seeds (approximately 100 g per treatment) in freshly prepared sodium selenate solutions at Se concentrations of 25, 50, 75 and 100 μM (μmol L^{-1}), using a seed-to-solution ratio of 1:10 (*w/v*), for either 6 or 12 h in the dark. Hydropriming (HP), used as a reference treatment, consisted of soaking seeds in deionized water for the same durations. The Se concentrations and soaking times were selected based on previous reports.[18,21,22] A batch of non-primed seeds (NP) served as the untreated control. All treatments were performed in triplicate. After priming, seeds were rinsed with deionized water to remove surface-bound Se and then dried on absorbent paper at 35 °C until reaching their initial moisture content (approximately 3.74%). Seeds from each treatment were divided into three groups: one for electrolyte leakage assessment, one for Se quantification and another for pea microgreen production.

Analysis of selenium and other minerals in seeds

Seeds from each treatment group were ground, and subsamples of 250 mg were subjected to a microwave-assisted digestion with nitric acid. After diluting the digested samples with deionized water to a final volume of 50 mL, elemental analysis was performed using atomic absorption spectroscopy (AAS) with a PinAAcle 900T spectrometer (Perkin Elmer, Waltham, MA, USA).[23] Se was quantified by graphite furnace AAS using a matrix modifier of palladium and magnesium [0.1% Pd + 0.06% $Mg(NO_3)_2$]. Iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) were analyzed directly from the digest using flame AAS. For the determination of potassium (K) and sodium (Na), samples were diluted in a 1 g L^{-1} CsCl solution. Calcium (Ca) and magnesium (Mg) analyses required dilution in a 1 g L^{-1} $LaCl_3$ solution. Quantification of all elements was based on calibration curves prepared from standard solutions. The results were expressed as mg 100 g^{-1} of seeds.

Electrolyte leakage determination

Cell membrane integrity was assessed by measuring electrolyte leakage, following the method described by Rocha *et al.*[18] with minor modifications. Briefly, 15 seeds from each treatment group were placed in glass flasks containing approximately 45 mL of ultrapure water (volume adjusted based on seed mass) and incubated in a water bath at 25 °C, under constant shaking at 100 rpm, for 24 h. After incubation, the electrical conductivity of the solutions was measured (EC_1) using an EC59 PRO conductivity meter (Milwaukee Electric Tool Corp., Brookfield, WI, USA). Subsequently, the samples were autoclaved at 121 °C for 15 min to induce membrane rupture. After cooling to room temperature, a second conductivity measurement (EC_2) was taken. Electrolyte leakage (EL) was calculated using:

$$\text{Electrolytre leakage}\,(\%) = \frac{EC_1}{EC_2}\,x\,100 \qquad (1)$$

Production and monitoring of microgreens development

The experiment was conducted in a greenhouse during May and June 2024. Sowing was performed in 84-cell trays (3.5 × 3.5 cm, 5 cm deep), with two trays assigned to each treatment, totaling 22 trays (see Supporting information, Fig. S1). Each cell was filled with Siro® Germinação Bio (SIRO, Mira, Portugal), a fine-textured, low-salinity substrate (NPK 9–2-2; 2 kg m^{-3}), rich in organic

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matter (> 60%), with a pH of 5.5–6.5 (in $CaCl_2$) and electrical conductivity of 15 mS m^{-1}. Throughout the experimental period, irrigation was applied by nebulization at approximately 3.96 mm day^{-1}, and averaged greenhouse conditions were 23 ± 2 °C and 62 ± 7% relative humidity. Seedling emergence was recorded daily, and the cumulative emergence (%) at 7 and 25 days after sowing (DAS) was calculated according to:

$$\text{Emergence}(\%) = \frac{\text{Number of seeds emerged at 7 or 25 DAS}}{\text{Total number of sown seeds}} \times 100 \quad (2)$$

Microgreens were harvested 25 DAS by cutting the aerial parts at the substrate level. Roots were carefully removed, washed under running water to eliminate substrate residues and gently dried with absorbent paper. In the lab, the biomass (g) and length (cm) of the aerial and root parts were measured, along with the moisture content (%). Subsequently, the plant material was freeze-dried and ground for chemical analyses.

Determination of the chemical composition of microgreens

Mineral elements

The mineral composition of both the aerial and root parts of the microgreens was analyzed following the procedure described above. The results are presented as μg 100 g^{-1} for Se and as mg 100 g^{-1} for the other elements, expressed on a dry weight (DW) basis.

Chlorophylls

The chlorophyll content in plant aerial parts was determined following the methodology developed by Nagata and Yamashita.[24] Ten milligrams of each sample were homogenized in 10 mL of an acetone/hexane solution (4:6, v/v) using a vortex mixer. The homogenate was filtered through Whatman No. 4 filter paper (Cytiva, Marlborough, MA, USA) and an aliquot of the filtrate was transferred to a quartz cuvette for absorbance (*A*) measurement at 645 and 663 nm. The concentrations (mg 100 g^{-1} DW) of chlorophyll *a* and chlorophyll *b* were calculated using:

$$\text{Chlorophyll } a = A_{663} \times 0.999 - A_{645} \times 0.0989 \quad (3)$$

$$\text{Chlorophyll } b = A_{663} \times (-0.328) + A_{645} \times 1,77 \quad (4)$$

Free sugars

Free sugars were extracted from 500 mg of plant aerial parts using 40 mL of 80% ethanol, with 1 mL of melezitose (25 mg mL^{-1}) added as an internal standard. The mixture was incubated in a water bath at 80 °C for 90 min with manual agitation every 15 min. After extraction, samples were centrifuged at 15 000 × *g* for 10 min, and the supernatants were filtered through Whatman No. 4 paper. Solvents were removed under reduced pressure. The residues were washed with diethyl ether to eliminate lipophilic compounds and dried. The final extracts were reconstituted in distilled water to a final volume of 5 mL, filtered through 0.2-μm nylon syringe filters, and analyzed by HPLC with refractive index detection under the conditions described by Barros *et al.*[25] Results were expressed as g 100 g^{-1} DW.

Organic acids

Organic acids were extracted from 250 mg of plant aerial parts using 25 mL of metaphosphoric acid by stirring at 150 rpm for 25 min. The mixture was then sequentially filtered through Whatman No. 4 filter paper and 0.2-μm nylon syringe filters into amber HPLC vials. The analysis was performed using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array detector, following the method described by Pereira *et al.*[26] The results were expressed as g 100 g^{-1} DW.

Evaluation of antioxidant properties

Extract preparation

Approximately 1 g of the aerial parts of microgreens was extracted by stirring (150 rpm) with 30 mL of 80% ethanol for 1 h. The mixture was then filtered through Whatman No. 4 filter paper, and the solid residue was re-extracted under identical conditions. The resulting extracts were combined and concentrated under reduced pressure, and the aqueous phase was then lyophilized. The dried extracts were reconstituted in 80% ethanol for the determination of total phenolic content (TPC) and evaluation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity. For the thiobarbituric acid reactive substances (TBARS) formation inhibition assay, extracts were reconstituted in 20 mmol L^{-1} Tris–HCl buffer (pH 7.4).

TPC

TPC was determined using the Folin–Ciocalteu colorimetric method, as described by Pinela *et al.*[27] Briefly, 500 μL of extract (at 2.5 mg mL^{-1}) or solvent (blank) was mixed with 2.5 mL of Folin–Ciocalteu reagent and 2 mL of sodium carbonate solution (75 g L^{-1}). The mixture was incubated at 40 °C for 30 min, and absorbance was measured at 765 nm using a SPECTROstar Nano spectrophotometer (BMG LABTECH, Ortenberg, Germany). Quantification was based on the calibration curve $y = 2.0372x + 0.043$ ($r^2 = 0.9981$) prepared with gallic acid (0.05–0.8 mg mL^{-1}). The results were expressed as mg gallic acid equivalents (GAE) g^{-1} extract.

DPPH radical-scavenging activity

DPPH solution was prepared by dissolving 12.0 mg of DPPH in 500 mL of methanol, protected from light, followed by sonication in an ultrasonic bath for 15 min. In a 96-well plate, 30 μL of each extract (0.234–15 mg mL^{-1}) was mixed with 270 μL of the DPPH solution. Trolox was used as a positive control, and the extraction solvent served as the blank control. After 60 min of incubation in the dark, the absorbance was measured at 515 nm. The EC_{50} values were calculated as previously described.[27]

TBARS formation inhibition capacity

An aliquot of freshly prepared porcine brain tissue homogenate in Tris–HCl buffer was incubated with extracts (0.059–7.65 mg mL^{-1}), $FeSO_4$ and ascorbic acid at 37.5 °C for 1 h. After adding trichloroacetic acid (28%) and thiobarbituric acid (2%), the mixture was incubated at 80 °C for 20 min. Samples were centrifuged, and absorbance was measured at 532 nm. Trolox and Tris–HCl buffer were used as positive control and blank, respectively. EC_{50} values were calculated as previously described.[27]

Statistical analysis

Data are presented as the mean ± SD. The main effects of priming time (0, 6 and 12 h) and Se concentration (0, 25, 50, 75 and 100 μM), as well as their interaction, were evaluated using a two-way analysis of variance (ANOVA). For factorial analysis purposes, NP seeds were considered as time 0 h and Se concentration 0 μM. Prior to ANOVA, assumptions of normality and

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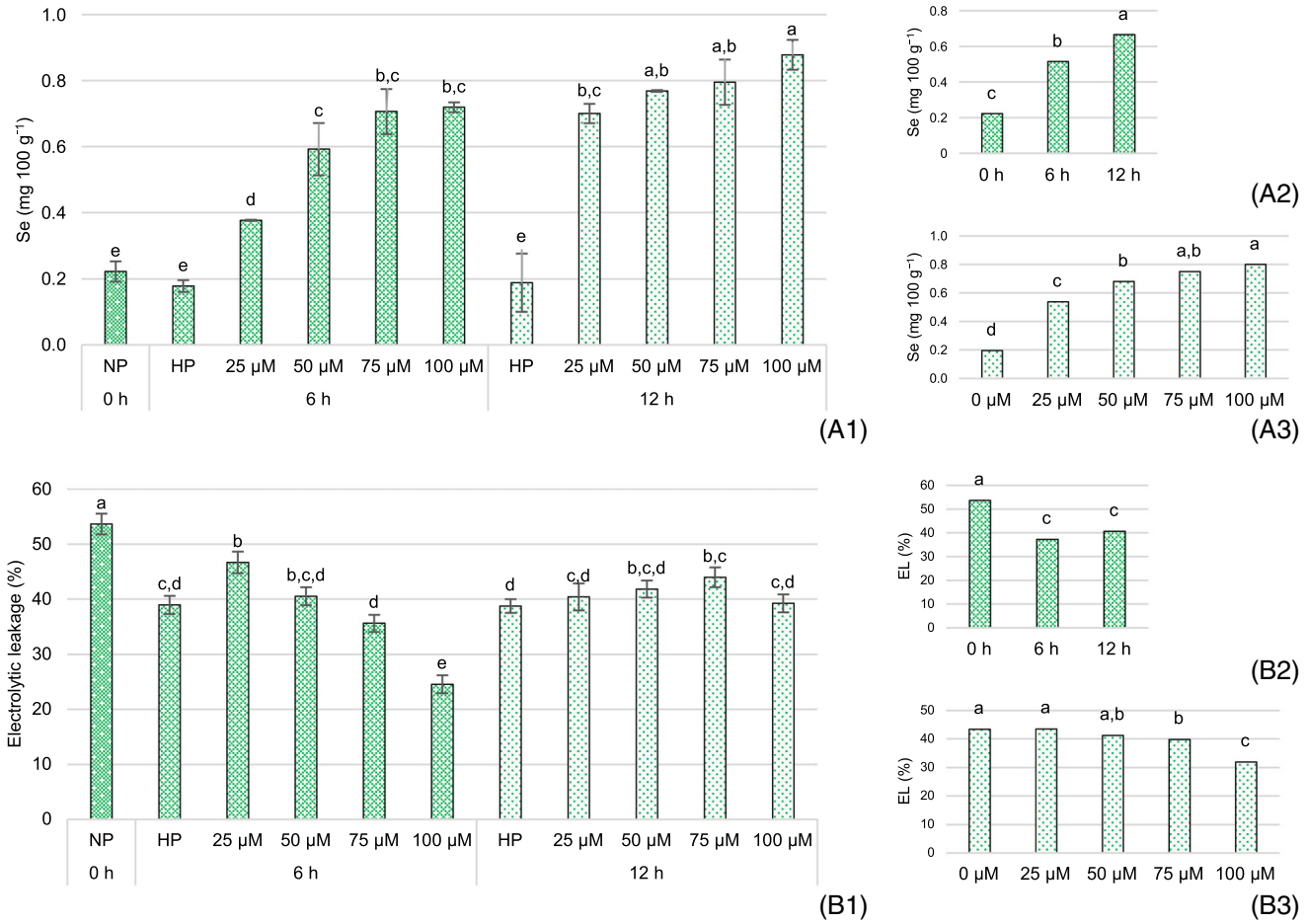


Figure 1. Effect of Se nutripriming and hydropriming (HP) for 6 and 12 h, as well as no priming (NP), on (A) Se contents and (B) electrolyte leakage (EL) in pea seeds. (A1) and (B1) Comparisons among all treatment combinations (priming time × Se concentration). (A2) and (B2) The main effect of priming time (0, 6 and 12 h) and (A3) and (B3) the main effect of Se concentration (0, 25, 50, 75 and 100 µM), based on two-way ANOVA. A significant time × Se concentration interaction was detected for both independent variables ($P < 0.001$). Bars represent the mean ± SD in (A1) and (B1), and estimated marginal means in (A2), (A3), (B2) and (B3). Different letters above bars indicate significant differences ($P < 0.05$, Tukey's test).

homogeneity of variances were assessed. When a significant time × Se concentration interaction was detected ($P < 0.05$), comparisons among individual treatment combinations were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test. In the absence of significant interaction, *post hoc* comparisons of estimated marginal means were conducted using Tukey's HSD test. Multivariate patterns associated with priming time and Se concentration were explored by linear discriminant analysis (LDA). Variable selection was performed using a stepwise procedure based on Wilks' lambda, with *F*-to-enter = 3.84 and *F*-to-remove = 2.71. All analyses were conducted at a 5% significance level using SPSS, version 22.0 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of priming on seed selenium content and membrane integrity

Changes in seed cell membrane integrity during imbibition can lead to electrolyte leakage, which is associated with reduced germination capacity.[28] Priming treatment can enhance seed vigor by restoring antioxidant systems, mobilizing reserve compounds and activating key enzymes, ultimately improving seedling development.

Figure 1(A1) demonstrates that NP and HP seeds contained similarly low Se levels (≤ 0.22 mg 100 g^{-1}). By contrast, nutripriming significantly increased Se content ($P < 0.05$), with higher concentrations and longer treatment durations resulting in greater accumulation (Fig. 1(A2), (A3)). The 100 µM–12-h Se treatment resulted in approximately four times higher Se content compared to controls. A previous study using bread wheat seeds soaked for 12 h reported that Se concentrations increased with applied concentration, but not in a proportional manner.[18] Se accumulation in seeds can become saturated at higher concentrations or under prolonged soaking times, limiting further uptake.

As shown in Fig. 1(B1), NP seeds exhibited the highest electrolyte leakage, indicating membrane damage. Seed priming effectively protected membrane integrity (Fig. 1(B2)), with significantly lower leakage values ($P < 0.05$). Increasing Se concentrations, particularly in 6-h treatments, progressively reduced electrolyte leakage, correlating with the increase in Se content (Fig. 1(A)). The 100 µM Se treatment was most effective in maintaining membrane integrity (Fig. 1(B3)), demonstrating that Se enrichment can be achieved without compromising membrane integrity.

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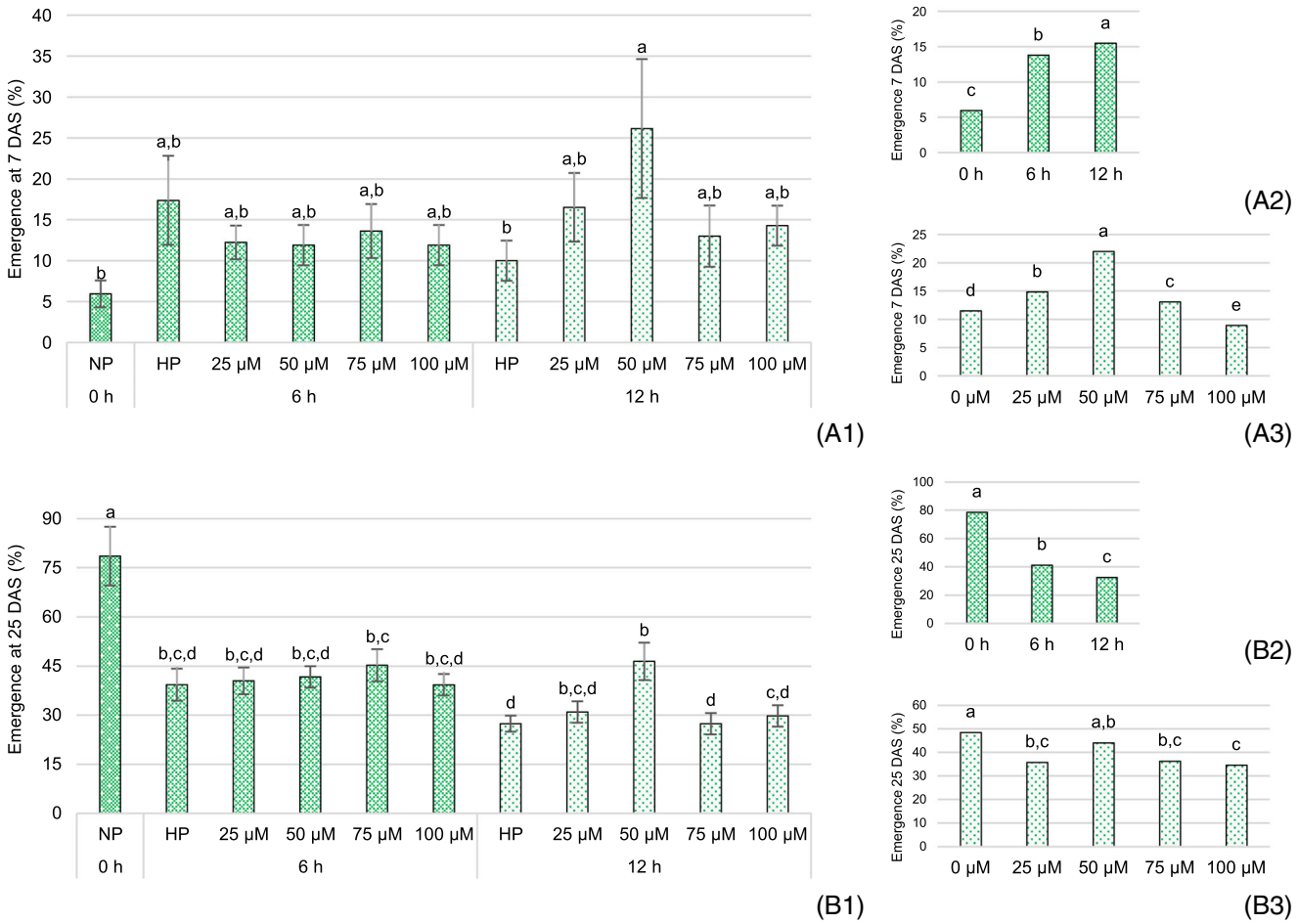


Figure 2. Effect of Se nutripriming and hydropriming (HP) for 6 and 12 h, as well as no priming (NP), on cumulative seedling emergence at 7 and 25 days after sowing (DAS). (A1) and (B1) Comparisons among all treatment combinations (priming time × Se concentration). (A2) and (B2) The main effect of priming time (0, 6, and 12 h) and (A3) and (B3) the main effect of Se concentration (0, 25, 50, 75 and 100 µM), based on two-way ANOVA. A significant time × Se concentration interaction was detected for emergence at 7 DAS ($P < 0.001$) and at 25 DAS ($P = 0.032$). Bars represent the mean ± SD in (A1) and (B1) and estimated marginal means in (A2), (A3), (B2) and (B3). Different letters above bars indicate significant differences ($P < 0.05$, Tukey's test).

Priming treatments induced some modifications in the mineral composition of pea seeds (see Supporting information, Table S3). In particular, priming substantially reduced Na levels compared to the NP control, likely due to a leaching effect during seed soaking. This reduction may benefit seed viability because excess Na can be detrimental.[29] In turn, the treatment did not reduce the levels of other elements, which is crucial for maintaining seed nutritional status and physiological vigor because micronutrients are known to strengthen antioxidant defenses and enzymatic activities critical for germination.

Effect of priming on emergence rate, microgreen size, and plant biomass

The emergence rate of pea microgreens at 7 and 25 DAS is presented in Fig. 2. At 7 DAS, corresponding to the second day after epicotyl emergence, seeds treated with 50 µM Se for 12 h exhibited a higher emergence rate (approximately 32%) than the NP and HP-12 h controls; however, this difference was not statistically significant ($P > 0.05$) compared to the other treatments (except NP and HP–12 h) (Fig. 2(A1)). Priming had a positive effect on seedling emergence at this early developmental stage, with higher estimated marginal means for primed seeds compared to the NP control (Fig. 2(A2)). By contrast, the effect of Se concentration followed a non-linear trend, with emergence increasing up to 50 µM Se and decreasing at higher concentrations (Fig. 2(A3)). However, this pattern was different at 25 DAS. Seeds from the NP group exhibited the highest emergence rate (>78%), whereas all other treatments remained below 50% (Fig. 2(B1)). Furthermore, seeds soaked for 6 h tended to show higher cumulative emergence rates than those soaked for 12 h, although this difference was not statistically significant ($P > 0.05$) in most cases. Among the 12-h treatments, seeds primed with 50 µM Se displayed comparatively higher emergence rates, similar to those observed in the 6-h treatments. These results indicate that priming duration influences pea microgreen emergence at later developmental stages, with shorter soaking times generally favoring higher cumulative emergence rates.

Figure 3 shows the height of pea seedlings at 7 and 25 DAS. At 7 DAS, seedlings derived from seeds nutriprimed for 12 h with 50 µM Se exhibited the greatest average height, followed by those from the 12-h HP treatment (Fig. 3(A1)). By contrast, seedlings from the NP and 75 µM–12-h treatments tended to be shorter than 1 cm. At 25 DAS, when harvest was performed, microgreens obtained from seeds nutriprimed for 12 h with 50 µM Se also showed the greatest average shoot length (Fig. 3(B1)). Moreover,

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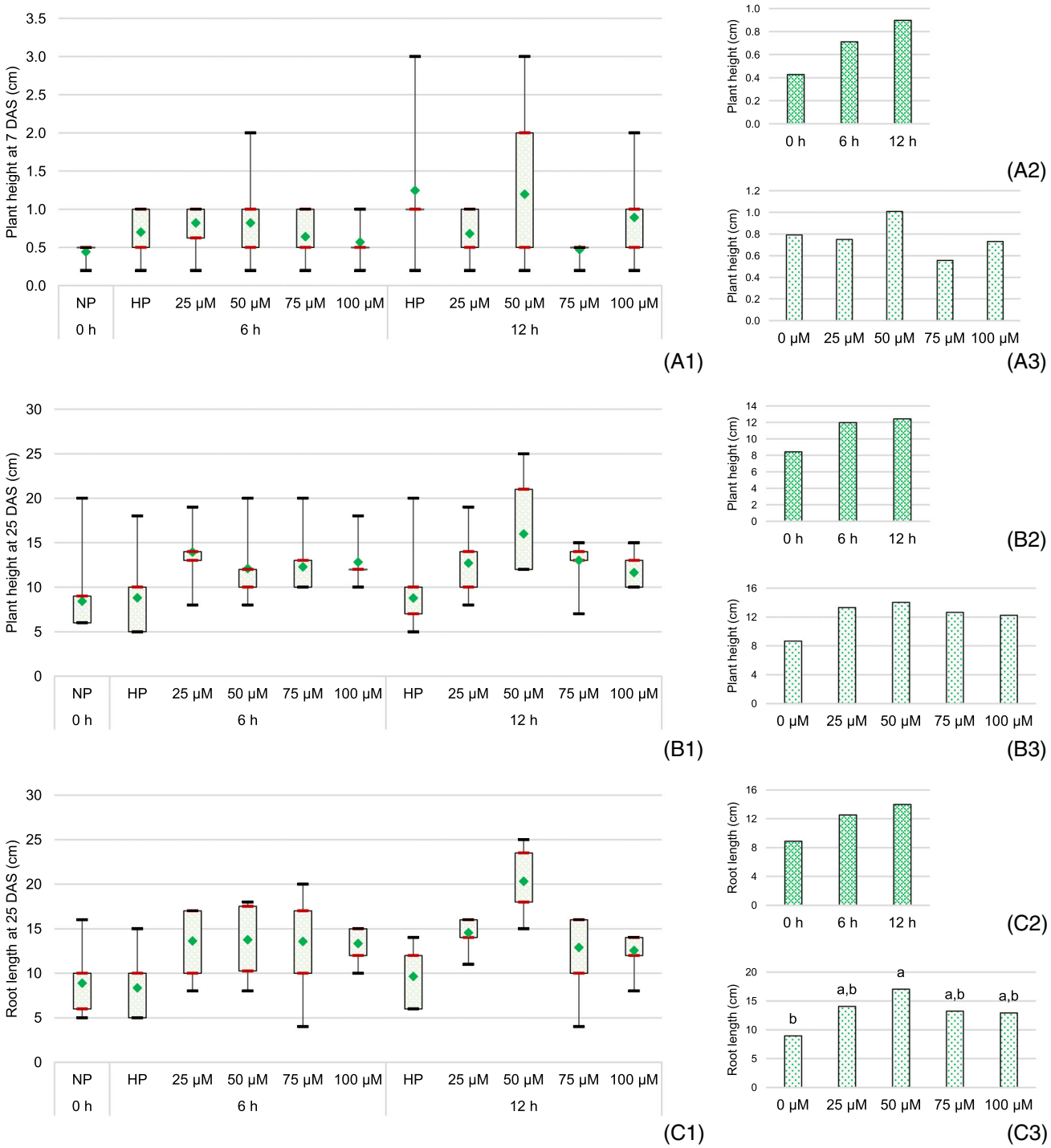
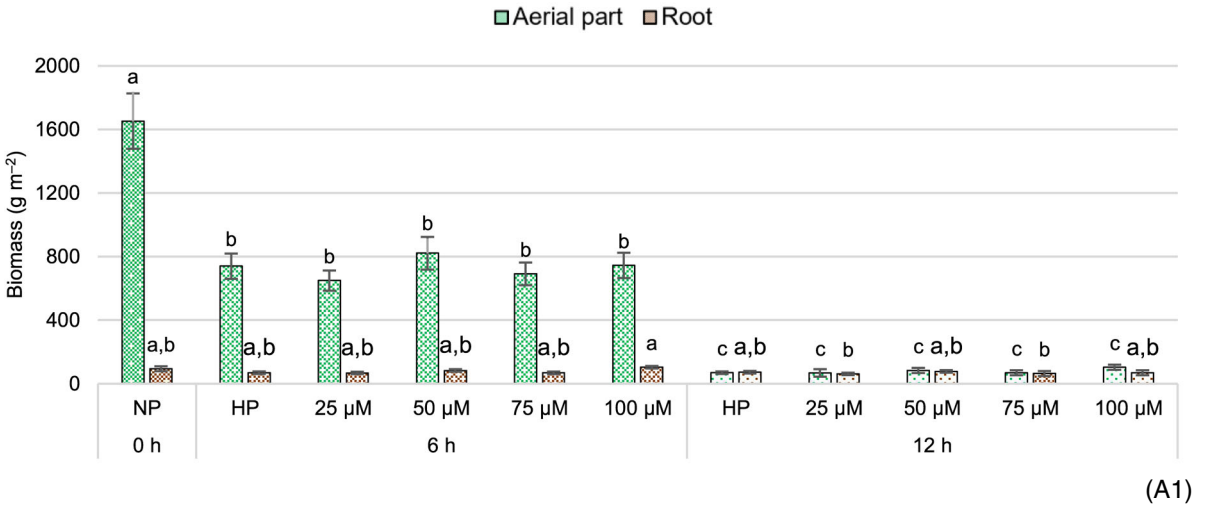


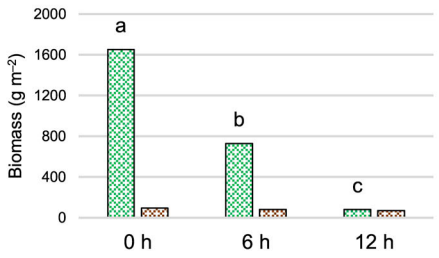
Figure 3. Effect of Se nutripriming and hydropriming (HP) for 6 and 12 h, as well as no priming (NP), on plant height at (A) 7 days after sowing (DAS) and (B) 25 DAS, and on (C) root length at 25 DAS. Boxplots in (A1), (B1) and (C1) show the distribution of data for all treatment combinations (priming time × Se concentration): boxes indicate the interquartile range (Q1–Q3), green diamond symbols indicate the median, and vertical whiskers represent the minimum and maximum values. (A2) and (B2) The effect of priming time (0, 6, and 12 h) and (A3) and (B3) the main effect of Se concentration (0, 25, 50, 75 and 100 µM), based on two-way ANOVA. No significant time × Se concentration interaction was detected for plant height at 7 DAS ($P = 0.427$), plant height at 25 DAS ($P = 0.071$) or root length at 25 DAS ($P = 0.548$). Bars in (A2), (A3), (B2), (B3), (C2) and (C3) represent estimated marginal means. Different letters above bars indicate significant differences ($P < 0.05$, Tukey's test).

even the shortest plants within this treatment were longer than the average shoot length observed in the remaining treatments. Greater variability in shoot height was observed in the NP and HP groups, with values ranging approximately from 13 to 15 cm. Although Fig. 3(A2), (B2) suggests a tendency toward increased plant height with longer priming duration at both evaluation times, these differences were not statistically significant ($P > 0.05$). Moreover, no significant effects of Se concentration

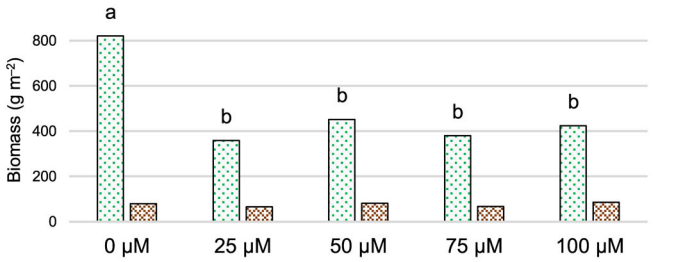
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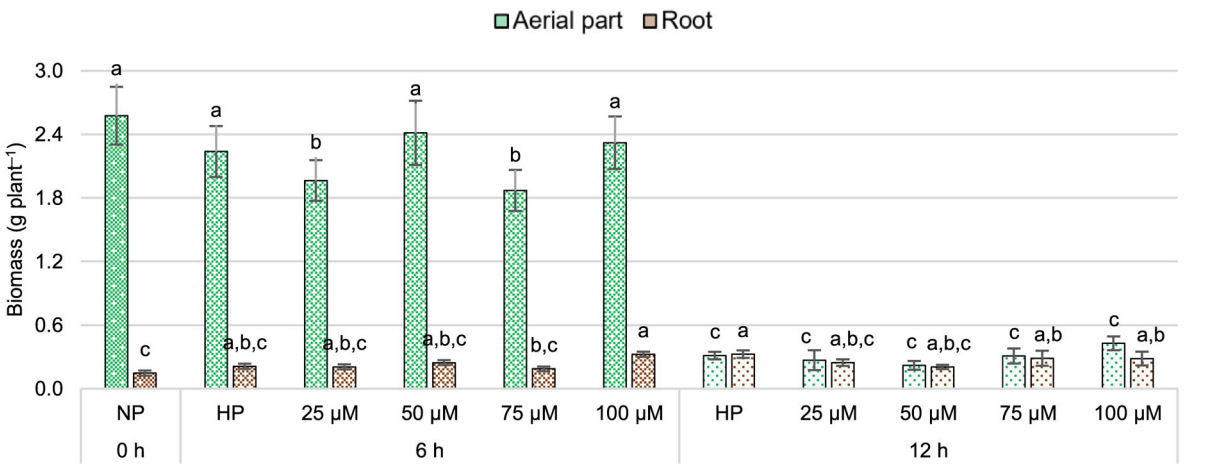
(A1)



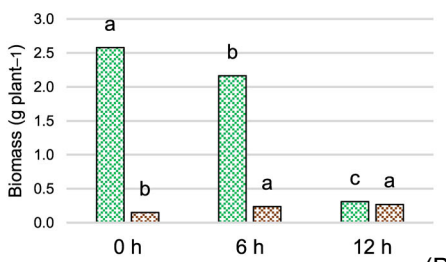
(A2)



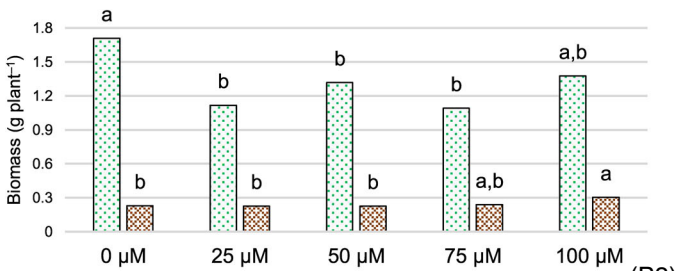
(A3)



(B1)



(B2)



(B3)

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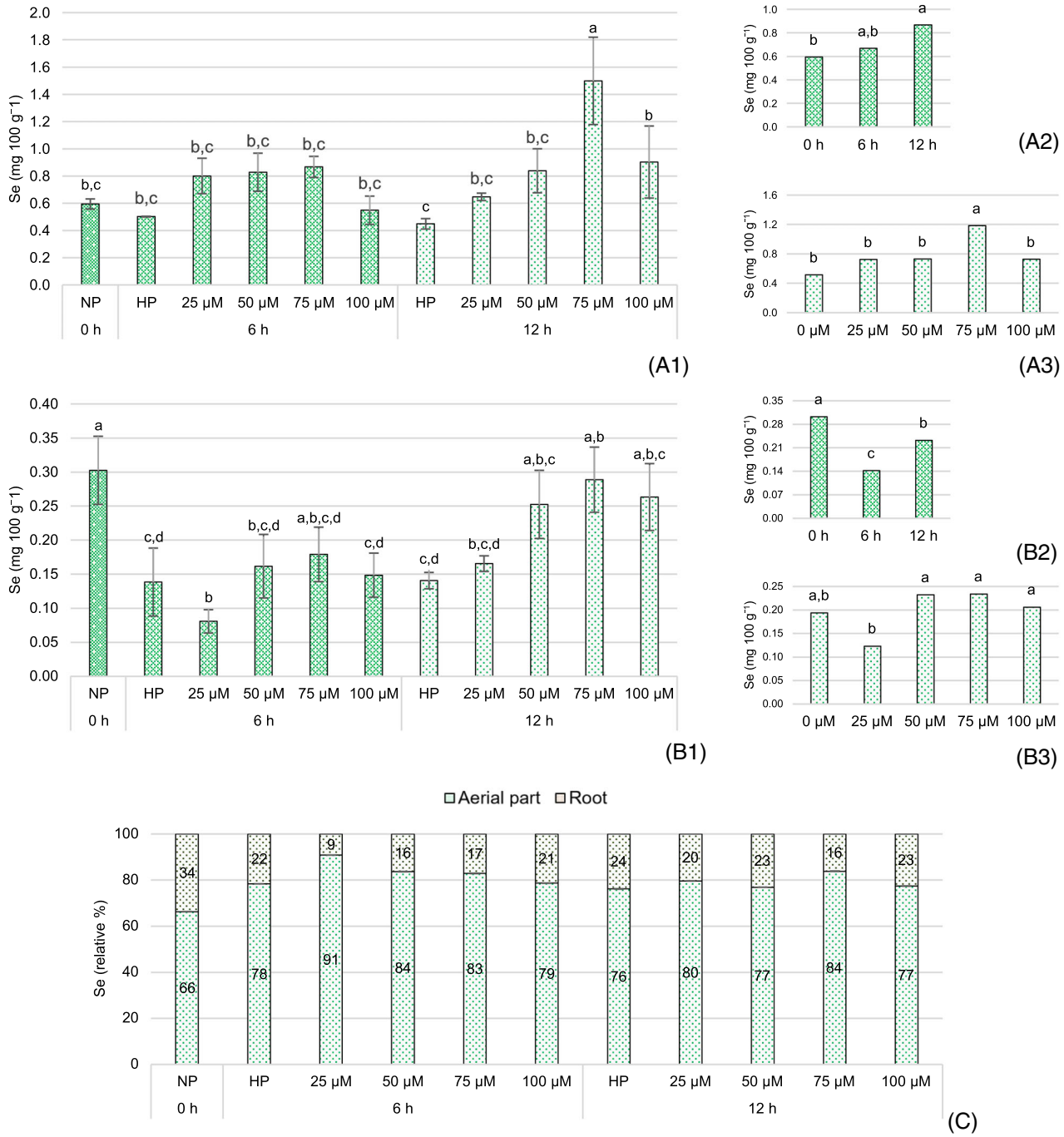


Figure 5. Effect of Se nutripriming and hydropriming (HP) for 6 and 12 h, as well as no priming (NP), on Se concentrations in the (A) aerial part and (B) root part of pea microgreens. (A1) and (B1) Comparisons among all treatment combinations (time × Se). (A2) and (B2) The main effect of priming time (0, 6 and 12 h) and (A3) and (B3) the main effect of Se concentration (0, 25, 50, 75 and 100 µM), based on two-way ANOVA. A significant time × Se concentration interaction was detected for Se concentration in the aerial part ($P = 0.001$), whereas no significant interaction was found for the root part ($P = 0.152$). Bars represent the mean ± SD in (A1) and (B1) and estimated marginal means in (A2), (A3), (B2) and (B3). Different letters above bars indicate significant differences ($P < 0.05$, Tukey's test). (C) The relative distribution (%) of total Se between aerial and root tissues for each treatment.

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Figure 4. Effect of Se nutripriming and hydropriming (HP) for 6 and 12 h, as well as no priming (NP), on fresh biomass of pea microgreens, expressed (A) per unit area (m^2) and (B) per plant unit, for the aerial part and roots. (A1) and (B1) Comparisons among all treatment combinations (time × Se). (A2) and (B2) The main effect of priming time (0, 6 and 12 h) and (A3) and (B3) the main effect of Se concentration (0, 25, 50, 75 and 100 µM), based on two-way ANOVA. No significant time × Se concentration interaction was detected for aerial ($P = 0.676$) or root ($P = 0.153$) biomass per unit area, whereas the interaction was not significant for aerial biomass per plant ($P = 0.191$) but was significant for root biomass per plant ($P = 0.015$). Bars represent the mean ± SD in (A1) and (B1) and estimated marginal means in (A2), (A3), (B2) and (B3). For each plant part, different letters above bars indicate significant differences ($P < 0.05$, Tukey's test).

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Table 1. Effects of priming time and Se concentration on the mineral composition of pea microgreens

Independent variables		Macrominerals (mg 100 g^{-1} DW)				Microminerals (mg 100 g^{-1} DW)			
		K	Na	Ca	Mg	Fe	Mn	Cu	Zn
Treatment combinations									
0 h	NP	5673 ± 359 c	266 ± 2 d	3144 ± 150 b,c,d	401 ± 7 b,c	10.17 ± 0.64 a	1.61 ± 0.01 c,d,e,f	1.10 ± 0.01 a,b	6.38 ± 0.01 b,c,d
6 h	HP	7365 ± 403 b	294 ± 3 b,c	2827 ± 16 d	388 ± 6 c,d,e	7.17 ± 0.17 d	1.43 ± 0.07 f,g	0.98 ± 0.03 b,c,d	5.96 ± 0.13 e,f
	25 μM	7200 ± 823 b	274 ± 5 c,d	3162 ± 136 b,c,d	377 ± 15 c,d,e	7.56 ± 0.06 c,d	1.46 ± 0.02 d,e,f,g	1.09 ± 0.02 a,b,c	5.78 ± 0.09 f
	50 μM	6128 ± 95 b,c	266 ± 3 d	3403 ± 88 a,b,c	388 ± 5 c,d,e	9.84 ± 0.80 a,b	1.66 ± 0.01 c,d	1.20 ± 0.01 a	5.98 ± 0.14 d,e,f
	75 μM	5561 ± 175 c	284 ± 3 b,c,d	2767 ± 209 d	393 ± 16 c,d	9.16 ± 0.27 a,b,c,d	1.39 ± 0.08 g	0.96 ± 0.01 b,c,d	6.60 ± 0.05 a,b,c
	100 μM	5826 ± 188 c	303 ± 2 b	2839 ± 338 d	358 ± 3 e	10.59 ± 1.84 a	1.43 ± 0.03 f,g	1.15 ± 0.16 a	6.25 ± 0.01 c,d,e
12 h	HP	9033 ± 679 a	292 ± 3 b,c	3481 ± 114 a,b	401 ± 10 b,c	9.06 ± 0.22 a,b,c,d	1.64 ± 0.01 c,d,e	0.97 ± 0.01 b,c,d	6.05 ± 0.06 d,e,f
	25 μM	9651 ± 606 a	302 ± 4 b	2978 ± 168 c,d	373 ± 21 c,d,e	8.07 ± 0.12 b,c,d	1.45 ± 0.11 e,f,g	0.93 ± 0.04 c,d	5.72 ± 0.05 f
	50 μM	6313 ± 340 b,c	358 ± 3 a	3851 ± 52 a	438 ± 2 a	10.13 ± 0.65 a,b	1.91 ± 0.04 a,b	0.95 ± 0.01 b,c,d	6.83 ± 0.14 a
	75 μM	3880 ± 132 d	289 ± 17 b,c	1082 ± 21 e	364 ± 13 d,e	9.28 ± 0.67 a,b,c	1.77 ± 0.16 b,c	1.18 ± 0.04 a	6.70 ± 0.31 a,b
	100 μM	5730 ± 37 c	339 ± 14 a	3436 ± 233 a,b,c	432 ± 7 a,b	9.57 ± 0.18 a,b,c	2.10 ± 0.02 a	0.90 ± 0.01 d	6.33 ± 0.26 b,c,d,e
Estimated marginal means									
Time	0 h	5673 b	266 c	3144	401 a	10.17 a	1.61 b	1.10 a	6.38 a
	6 h	6416 a	284 b	3000	381 b	8.86 b	1.47 c	1.08 a	6.11 b
	12 h	6921 a	316 a	2965	402 a	9.22 a,b	1.77 a	0.99 b	6.32 a
	P-value	< 0.001	< 0.001	0.261	< 0.001	0.025	< 0.001	< 0.001	0.001
[Se]	0 μM	7357 b	284 b	3150 b	397 a	8.80 b,c	1.56 b,c	1.02	6.13 c
	25 μM	8426 a	288 b	3070 b	375 c	7.81 c	1.46 c	1.01	5.75 d
	50 μM	6220 c	312 a	3627 a	413 a	9.98 a	1.78 a	1.07	6.41 b
	75 μM	4721 d	287 b	1924 b	378 b,c	9.22 a,b	1.58 b	1.07	6.65 a
	100 μM	5778 c	321 a	3138 b	395 a,b	10.08 a	1.77 a	1.02	6.29 b,c
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.106	< 0.001
Time × [Se]	*P*-value	< 0.001	< 0.001	< 0.001	< 0.001	0.031	< 0.001	< 0.001	< 0.001

Mean ± SD for each treatment combination (time × Se) are presented in the upper section of the table. Estimated marginal means for the main effects of priming time and Se concentration, and the *P*-values of the interaction (time × Se), obtained by two-way ANOVA, are shown in the lower section. For each dependent variable, post-hoc comparisons were performed using Tukey's HSD test, and different lowercase letters within treatment combinations or within each factor (time or Se concentration) indicate significant differences ($P < 0.05$). When a significant time × Se interaction was detected for a given variable ($P < 0.05$), biological interpretation should be based primarily on comparisons among treatment combinations.

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Table 2. Effects of priming time and Se concentration on mineral composition of pea microgreen roots

Independent variables		Macrominerals (mg 100 g^{-1} DW)				Microminerals (mg 100 g^{-1} DW)			
		K	Na	Ca	Mg	Fe	Mn	Cu	Zn
Treatment combinations									
0 h	NP	6675 ± 139 d,e	1509 ± 35 a	394 ± 18 a,b	562 ± 45 c,d,e	7.94 ± 0.23 b	1.62 ± 0.04 d	0.81 ± 0.09 b,c,d,e	7.88 ± 0.41 a,b,c
6 h	HP	7957 ± 76 a,b,c	1089 ± 508 a	373 ± 8 b,c,d	508 ± 18 d,e	6.76 ± 0.56 c,d	2.60 ± 0.06 a,b,c	0.78 ± 0.05 d,e	7.66 ± 0.25 a,b,c
	25 µM	8684 ± 311 a	1085 ± 385 a	384 ± 5 a,b,c	519 ± 8 d,e	6.61 ± 0.35 c,d,e	2.64 ± 0.22 a,b,c	0.82 ± 0.07 b,c,d,e	7.84 ± 0.03 a
	50 µM	7861 ± 55 a,b,c,d	1091 ± 291 a	368 ± 3 b,c,d	637 ± 55 a,b,c	7.19 ± 0.40 d,c	3.38 ± 0.14 a	0.89 ± 0.08 a,b,c,d	8.51 ± 0.20 a,b,c
	75 µM	6975 ± 649 c,d,e	1027 ± 204 a	362 ± 2 b,c,d	582 ± 25 b,c,d,e	6.67 ± 0.09 c,d,e	1.51 ± 0.02 d	0.99 ± 0.12 a,b	7.01 ± 0.09 c,d
	100 µM	7503 ± 43 a,b,c,d	1493 ± 93 a	353 ± 2 b,c,d	543 ± 47 d,e	5.71 ± 0.07 d,e,f	1.71 ± 0.07 d	0.80 ± 0.05 c,d,e	7.65 ± 0.28 a,b,c
12 h	HP	8360 ± 413 a,b	347 ± 2 b,c	346 ± 14 c,d	564 ± 10 c,d,e	5.61 ± 0.10 e,f	3.06 ± 0.11 a	0.76 ± 0.09 d,e	7.88 ± 0.48 a,b,c
	25 µM	7383 ± 364 b,c,d	297 ± 6 c	335 ± 8 d	436 ± 8 e	5.34 ± 0.39 f	1.83 ± 0.02 b,c,d	0.81 ± 0.04 b,c,d,e	7.47 ± 0.01 b,c
	50 µM	7209 ± 155 b,c,d	1056 ± 39 a	397 ± 7 a,b	669 ± 13 a,b	8.32 ± 0.22 b	2.69 ± 0.97 a,b	0.66 ± 0.01 e	7.94 ± 0.04 a,b,c
	75 µM	5895 ± 303 e	1072 ± 15 a	337 ± 11 d	619 ± 18 a,b,c,d	7.96 ± 0.90 b	1.87 ± 0.39 b,c,d	1.06 ± 0.05 a	6.40 ± 0.14 d
	100 µM	7106 ± 1008 c,d,e	1002 ± 194 a,b	428 ± 42 a	687 ± 44 a	9.86 ± 0.09 a	2.89 ± 0.05 a	0.98 ± 0.02 a,b,c	8.06 ± 0.73 a,b
Estimated marginal means									
Time	0 h	6675 b	1509 a	394 a	562 a,b	7.94 a	1.63 b	0.81	7.88
	6 h	7796 a	1157 b	368 b	558 b	6.59 b	2.37 a	0.86	7.74
	12 h	7191 b	755 c	368 b	595 a	7.42 a	2.47 a	0.86	7.55
	p-Value	< 0.001	< 0.001	0.034	0.031	< 0.001	0.002	0.553	0.165
[Se]	0 µM	7664 a,b	982 a,b	371 a,b,c	545 b	0.77 b	2.43 b	0.79 b,c	7.81 a,b
	25 µM	8034 a	691 b	359 b,c	477 c	5.97 c	2.23 b,c	0.81 b,c	7.65 b
	50 µM	7535 a,b	1074 a	383 a,b	653 a	7.76 a	3.04 a	0.77 c	8.23 a
	75 µM	6435 c	1050 a,b	349 c	601 a	7.31 a,b	1.69 c	1.03 a	6.71 c
	100 µM	7305 b	1248 a	390 a	615 a	7.79 a	2.30 b	0.89 b	7.86 a,b
	P-value	< 0.001	0.007	0.001	< 0.001	0.031	< 0.001	< 0.001	< 0.001
Time × [Se]	*P*-value	0.019	0.010	< 0.001	< 0.001	0.031	< 0.001	0.001	0.033

Mean ± SD for each treatment combination (time × Se) are presented in the upper section of the table. Estimated marginal means for the main effects of priming time and Se concentration, and the *p*-values of the interaction (time × Se), obtained by two-way ANOVA, are shown in the lower section. For each dependent variable, *post hoc* comparisons were performed using Tukey's HSD test, and different lowercase letters within treatment combinations or within each factor (time or Se concentration) indicate significant differences ($P < 0.05$). When a significant time × Se interaction was detected for a given variable ($P < 0.05$), biological interpretation should be based primarily on comparisons among treatment combinations.

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Table 3. Effects of priming time and Se concentration on chlorophyll, free sugar, and organic acid composition of pea microgreens

Independent variables		Chlorophylls (mg 100 g^{-1} DW)		Free sugars (g 100 g^{-1} DW)		Organic acids (g 100 g^{-1} DW)					
		Chlorophyll *a*	Chlorophyll *b*	Glucose	Sucrose	Oxalic acid	Malic acid	Ascorbic acid	Citric acid	Succinic acid	Fumaric acid
Treatment combinations											
0 h	NP	588 ± 20 b,c	208 ± 15 a,b	2.18 ± 0.09 b,c	nd	13.75 ± 0.91 c	nd	tr	0.51 ± 0.06 f	nd	0.017 ± 0.006 c,d
6 h	HP	418 ± 19 c,d	131 ± 9 d,e	1.41 ± 0.06 f	0.24 ± 0.03 c	13.20 ± 0.23 c,d	nd	tr	0.77 ± 0.09 d,e	nd	0.009 ± 0.001 d
	25 μM	354 ± 20 d,e	117 ± 8 e,f	1.68 ± 0.06 e	nd	16.17 ± 0.20 b	nd	tr	1.03 ± 0.01 b,c	0.51 ± 0.17 b,c	0.022 ± 0.003 b,c,d
	50 μM	624 ± 29 a	213 ± 9 a	2.16 ± 0.04 b,c	nd	17.33 ± 0.30 a	4.19 ± 0.43 a	tr	1.13 ± 0.01 a,b	0.36 ± 0.04 c,d	0.037 ± 0.002 a
	75 μM	309 ± 18 e	98 ± 7 f	2.22 ± 0.05 b	nd	15.70 ± 0.09 b	3.60 ± 0.40 a	tr	0.59 ± 0.11 e,f	nd	0.009 ± 0.003 d
	100 μM	648 ± 30 a	226 ± 12 a	2.46 ± 0.10 a	nd	17.46 ± 0.13 a	5.04 ± 0.53 a	0.011 ± 0.001	1.28 ± 0.10 a	0.38 ± 0.06 c,d	0.033 ± 0.009 a,b
12 h	HP	410 ± 27 c,d	140 ± 9 d,e	1.94 ± 0.03 d	0.37 ± 0.02 b	15.31 ± 0.17 b	nd	tr	0.91 ± 0.03 c,d	nd	0.012 ± 0.007 d
	25 μM	457 ± 28 c	153 ± 12 c,d	1.98 ± 0.06 c,d	0.25 ± 0.02 c	16.20 ± 0.35 b	nd	tr	1.31 ± 0.03 a	1.14 ± 0.06 a	0.041 ± 0.001 a
	50 μM	457 ± 27 c	147 ± 8 c,d	2.14 ± 0.11 b,c,d	0.24 ± 0.02 c	11.34 ± 0.01 e	3.45 ± 0.13 a	tr	0.98 ± 0.08 b,c	0.26 ± 0.01 d	0.022 ± 0.004 b,c,d
	75 μM	546 ± 30 c	183 ± 12 b,c	2.49 ± 0.09 a	nd	11.52 ± 0.14 e	nd	tr	1.02 ± 0.06 b,c	0.61 ± 0.03 b	0.029 ± 0.002 a,b,c
	100 μM	407 ± 28 c,d	127 ± 7 d,e,f	2.49 ± 0.02 a	0.56 ± 0.04 a	12.61 ± 0.09 d	3.44 ± 0.12 a	tr	0.91 ± 0.03 c,d	0.35 ± 0.01 c,d	0.018 ± 0.002 c,d
Estimated marginal means											
Time	0 h	588 a	208 a	2.18 a	nd	13.75 b	nd	tr	0.51 b	nd	0.017 b
	6 h	471 b	157 b	1.99 b	0.05 b	15.97 a	2.57 a	0.002	0.96 a	0.25 b	0.022 a,b
	12 h	455 b	150 b	2.21 a	0.28 a	13.40 b	1.36 b	tr	1.03 a	0.47 a	0.024 a
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.077
[Se]	0 μM	472 b	160 b	1.84 c	0.20 b	14.09 a	nd	tr	0.73 c	nd	0.013 c
	25 μM	406 c	135 c	1.83 c	0.12 c	16.19 c,d	nd	tr	1.17 a	0.83 a	0.031 a
	50 μM	541 a	180 a	2.15 b	0.12 c	14.34 c	3.77 a	tr	1.06 b	0.31 b	0.030 a
	75 μM	427 c	140 c	2.36 a	nd	13.61 d	1.80 b	tr	0.81 c	0.31 b	0.019 b,c
	100 μM	527 a	176 a,b	2.48 a	0.28 a	15.03 b	4.25 a	0.006	1.09 a,b	0.36 b	0.025 a,b
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Time × [Se]	*P*-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Mean ± SD for each treatment combination (time × Se) are presented in the upper section of the table. Estimated marginal means for the main effects of priming time and Se concentration, and the *P*-values of the interaction (time × Se), obtained by two-way ANOVA, are shown in the lower section. For each dependent variable, *post hoc* comparisons were performed using Tukey's HSD test, and different lowercase letters within treatment combinations or within each factor (time or Se concentration) indicate significant differences ($P < 0.05$). When a significant time × Se interaction was detected for a given variable ($P < 0.05$), biological interpretation should be based primarily on comparisons among treatment combinations. nd, not detected; tr, trace.

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Table 4. Effects of priming time and Se concentration on the total phenolic content and *in vitro* antioxidant activity of pea microgreens

Independent variables		Total phenolics (mg GAE g^{-1} extract)	DPPH radical-scavenging activity EC_{50} values (mg L^{-1})	TBARS formation inhibition capacity EC_{50} values (mg L^{-1})
Treatment combinations				
0 h	NP	63 ± 6 b,c	76 ± 6	148 ± 17 a,b
6 h	HP	44 ± 4 d,e	84 ± 9	191 ± 20 b
	25 μM	40 ± 4 e	97 ± 11	150 ± 17 a,b
	50 μM	50 ± 5 c,d,e	77 ± 19	247 ± 36 c
	75 μM	67 ± 7 b,c	73 ± 9	337 ± 24 c
	100 μM	61 ± 6 b,c,d	76 ± 11	171 ± 20 b
12 h	HP	53 ± 6 c,d,e	95 ± 9	130 ± 20 a,b
	25 μM	65 ± 8 b,c	77 ± 7	154 ± 20 b
	50 μM	73 ± 7 a,b	64 ± 8	89 ± 10 a
	75 μM	86 ± 7 a	74 ± 7	182 ± 10 b
	100 μM	50 ± 4 c,d,e	102 ± 21	147 ± 14 a,b
Estimated marginal means				
Time	0 h	63 a	76	148 a
	6 h	52 b	81	222 b
	12 h	65 a	82	140 a
	P-value	< 0.001	0.771	< 0.001
[Se]	0 μM	53 b	85	156 a
	25 μM	53 b	87	151 a
	50 μM	61 b	70	175 a
	75 μM	77 a	74	259 b
	100 μM	55 b	89	159 a
	P-value	< 0.001	0.108	< 0.001
Time × [Se]	*P*-value	< 0.001	0.06	< 0.001

Mean ± SD for each treatment combination (time × Se) are presented in the upper section of the table. Estimated marginal means for the main effects of priming time and Se concentration, and the *P*-values of the interaction (time × Se), obtained by two-way ANOVA, are shown in the lower section. For each dependent variable, *post hoc* comparisons were performed using Tukey's HSD test, and different lowercase letters within treatment combinations or within each factor (time or Se concentration) indicate significant differences ($P < 0.05$). When a significant time × Se interaction was detected for a given variable ($P < 0.05$), biological interpretation should be based primarily on comparisons among treatment combinations. GAE: gallic acid equivalent. Trolox EC_{50} values: 4.14 ± 0.13 mg L^{-1} in the DPPH assay and 5.39 ± 0.28 mg L^{-1} in the TBARS assay.

or interactions between priming time and Se concentration were detected for plant height at either measurement period.

After harvest, the root length was also measured, and the results are presented in Fig. 3(C). The longest roots were observed in plants from the 50 μM–12-h treatment, with values ranging from 15 to 25 cm and an average of approximately 20 cm, consistent with the greater shoot height observed for this treatment. By contrast, plants treated with 75 μM for 6 and 12 h showed a wide variation in root length, ranging from 4 to 20 cm and from 4 to 16 cm, respectively. The NP and HP–6 h plants exhibited shorter mean root lengths, averaging approximately 8–9 cm. According to the two-way ANOVA, Se concentration had a significant nonlinear effect on root length, with an increase up to 50 μM followed by a decrease at higher concentrations (Fig. 3(C3)).

Figure 4 shows the fresh biomass of pea microgreen aerial parts and roots, expressed per area unit and per plant. The NP group exhibited the highest aerial biomass yield, reaching 1.65 kg m^{-2} (Fig. 4(A1)), which is consistent with the higher emergence rate observed for this treatment. As confirmed by the two-way ANOVA, priming time significantly affected aerial biomass per unit area, with shorter soaking times (6 h) yielding higher biomass than prolonged soaking (12 h) (Fig. 4(A2)). Regarding the main effect of Se concentration, biomass yields were lower in Se-treated seeds than in the 0 μM group (Fig. 4(A3)), reflecting the markedly higher values observed for the NP control. Among the 12-h treatments, aerial and root biomass values were comparable. When considering fresh biomass per plant (Fig. 4(B)), the lower yields observed in the 12-h treatment group were evident, whereas the effect of Se concentration was less pronounced.

Regarding root biomass, the trends differed from those observed for the aerial parts. According to a one-way ANOVA, no significant differences ($P > 0.05$) were observed in root biomass per unit area between the NP group and the 6-h and 12-h treatment groups (Fig. 4(A1)), and no significant interaction between the independent variables was detected ($P = 0.153$). Similarly, root biomass per plant was comparable across most individual treatments (Fig. 4(B1). However, a two-way ANOVA indicated a tendency for increased root biomass per plant with priming, particularly at the highest Se concentration (Fig. 4 (B2), (B3)).

These results highlight the negative impact of prolonged seed priming on aerial biomass in pea microgreens, particularly after 12 h of soaking. As a legume, prolonged Se exposure during seed priming may have affected *Rhizobium* symbiosis and nitrogen fixation, contributing to growth variations. In line with this, Álvarez-Herrera *et al.*[30] and Tamindžic *et al.*[31] showed that optimized priming improved germination and early growth in pea, whereas excessive or suboptimal conditions reduced biomass yield. In other species, studies have shown that moderate Se priming enhances germination, seedling growth and stress tolerance in

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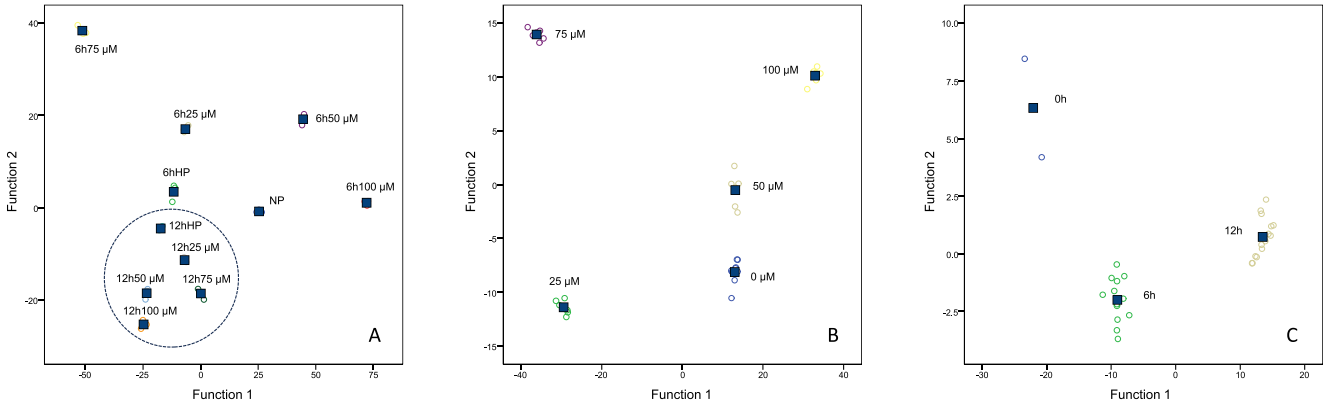


Figure 6. Linear discriminant analysis (LDA) performed to evaluate the overall effects of Se seed priming on the edible aerial parts of pea microgreens. Spatial distribution of the markers established by the coefficients of the canonical discriminant function considering the effect of (A) individual treatment combinations (priming time × Se concentration), (B) Se concentration irrespective of priming time, and (C) priming time irrespective of the Se concentration. The plots represent the scores of the first two canonical discriminant functions, which explain the highest proportion of variance in each model. Separation among groups reflects differences in the multivariate profiles of the evaluated parameters.

rice,[32] wheat,[18,21] *Brassica rapa*[33] and maize,[22] whereas prolonged or high-concentration treatments can impair biomass accumulation. Collectively, these findings emphasize that both the Se concentration and particularly the duration of seed priming are critical for maximizing germination and early growth, and that overexposure may compromise biomass.

Effect of priming on mineral and biochemical composition of microgreens

The Se levels quantified in pea microgreens are presented in Fig. 5. In the aerial parts, only microgreens derived from seed priming with 75 µM Se for 12 h showed significantly higher Se concentrations ($P < 0.05$) than the other treatments (Fig. 5(A1)). Furthermore, samples that underwent nutripriming tended to exhibit higher Se content than the NP and HP controls. In the roots, no significant time × Se concentration interactions were found ($P = 0.152$). As shown in Fig. 5(B1), both HP controls and nutripriming with 25, 50 and 100 µM Se for 6 h resulted in lower Se concentrations compared to NP plants. According to the two-way ANOVA, the 6 h and 25 µM Se treatments were associated with lower Se concentrations (Fig. 5(B2), (B3)). This pattern suggests that the cultivation substrate may have contained a baseline level of Se, which could have influenced Se accumulation in the roots. Interestingly, although NP control plants accumulated approximately 66% of the total Se in the aerial parts, microgreens derived from primed seeds accumulated between 77% and 91%, indicating that Se originating from primed seeds is more efficiently translocated from roots to shoots. In practical terms, a serving of 35 g of fresh Se biofortified microgreens provides, on average, approximately 100% of the adult Se daily reference intake (DRI) of 55 µg, as established by Regulation (EU) No 1169/2011,[34] highlighting the nutritional relevance of Se biofortification through seed priming. This observation further suggests that Se in the substrate may not have been in forms readily transported to aerial tissues, unlike sodium selenate (Na_2SeO_4), which is efficiently mobilized.[12,13]

The treatments affected the accumulation of other minerals in both shoots and roots. In the aerial parts (Table 1), 50 and 100 µM Se combined with 12 h priming enhanced the accumulation of macro- and micronutrients, particularly in the 50 µM–12-h treatment, which showed elevated levels of Ca, Mg, Fe, Mn and Zn. The sample with the highest Se content also displayed high levels of some micronutrients, but the lowest levels of K and Ca. From a nutritional perspective, a serving of 35 g of fresh microgreens provides approximately 24% of the recommended daily intake of K, 21% of the recommended daily intake of Ca, and 7% of the recommended daily intake of Mg and Cu, reinforcing the contribution of pea microgreens to dietary mineral intake. Root mineral differences were generally less pronounced than those observed in the aerial parts, and significant time × Se concentration interactions were detected for all elements (Table 2). The trends included higher K in 25 µM–6 h, reduced Na in 25 µM and HP–12 h samples, and elevated Fe, Mn and Mg in 100 µM–12 h. Overall, 12-h priming with 50, 75 and 100 µM Se enhanced mineral accumulation, with distinct element distribution patterns between pea microgreen shoots and roots, suggesting selective transport and compartmentalization.

Chlorophyll *a* and *b*, the primary pigments responsible for light capture during photosynthesis, were also affected by Se seed priming, corroborating previous reports on pigment biosynthesis.[33] The two-way ANOVA demonstrated a significant interaction between priming time and Se concentration. As shown in Table 3, chlorophyll *a* content ranged from 309 to 648 mg 100 g^{-1} DW and chlorophyll *b* from 98 to 226 mg 100 g^{-1} DW. The highest levels of both pigments were observed in microgreens treated for 6 h with 100 µM Se, which did not differ significantly ($P > 0.05$) from the 50 µM–6-h treatment. The lowest chlorophyll concentrations were observed in the 6-h treatments with 25 and 75 µM Se, particularly for chlorophyll b, which was significantly lower than that in NP and higher Se treatments. These results indicate that, under short-term priming (6 h), chlorophyll accumulation responded to Se concentration, whereas this pattern was not maintained under prolonged priming. This suggests a time-dependent modulation of pigment biosynthesis, potentially mediated by redox-related mechanisms or stress-regulated adjustments in chloroplast metabolism. Under 12-h priming, chlorophyll contents were generally lower than those observed in the most effective 6-h treatments, and no significant differences ($P > 0.05$) were detected among Se concentrations. Although the 75 µM–12-h Se treatment yielded moderately high chlorophyll levels, these remained lower than those recorded for the 50 and 100 µM–6-h treatments, indicating that prolonged priming was

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not associated with additional enhancement of pigment accumulation in pea microgreens.

Se seed priming significantly influenced sugar metabolism in pea microgreens, particularly glucose accumulation (Table 3). For both priming durations, glucose levels increased with Se concentration, reaching significant maxima in the 100 μM–6-h and 75 and 100 μM–12-h treatments. The significant time × Se concentration interaction indicated that glucose accumulation depends on the combined effects of both independent variables. These results corroborate previous findings by García-Tenesaca *et al.*,[16] who also reported enhanced glucose accumulation in Se-biofortified pea microgreens. Sucrose was generally undetectable in 6-h treatments, except for HP controls, but was present in most 12-h Se treatments (except 75 μM), peaking at 100 μM Se (0.56 g 100 g^{-1} DW). These findings suggest that prolonged seed soaking may modulate disaccharide metabolism, possibly as part of stress responses or energy storage regulation. The absence of detectable free sugars in commercially sourced pea microgreens was previously reported by Wojdyło *et al.*,[35] highlighting differences among species or variations in developmental stage.

Regarding organic acids, oxalic acid was the predominant compound in pea microgreens, with levels ranging from 11.34 to 17.46 g 100 g^{-1} DW (Table 3). The highest oxalic acid concentrations occurred in the 50 and 100 μM–6-h Se treatments, significantly exceeding those in NP controls, suggesting that short-term Se exposure stimulates oxalic acid accumulation, possibly linked to stress signaling or detoxification.[36] By contrast, 50–100 μM–12-h Se treatments significantly reduced oxalic acid levels. Citric acid was consistently detected across all samples, peaking at 100 μM–6-h and 25 μM–12-h treatments, suggesting potential modulation of energy metabolism through the tricarboxylic acid (TCA) cycle. By contrast, malic acid was detected only in Se-treated samples, particularly at higher concentrations (50–100 μM, except for 75 μM–12 h), with values statistically similar among treatments. Ascorbic acid was mostly found in trace amounts, except for the 100 μM–6-h treatment (11.14 mg 100 g^{-1} DW). Succinic acid, a key intermediate in the TCA cycle and stress signaling, appeared only in Se-primed samples, notably those exposed to 25–100 μM for 12 h. Its absence in some samples may reflect differential carbon allocation strategies or rapid utilization for defense or growth-related functions.[37] In turn, fumaric acid levels remained low across treatments, not exceeding 41 mg 100 g^{-1} DW (Table 3). The study by Wojdyło *et al.*[35] on commercial pea microgreens also identified oxalic, citric and succinic acids, with citric acid being the most abundant, followed by oxalic acid. Wojdyło *et al.*[35] further reported the presence of ascorbic acid.

Overall, Se seed priming enhanced the mineral and biochemical composition of pea microgreens, with effects dependent on both Se concentration and priming duration. Short-term priming (6 h) with moderate to high Se levels (50–100 μM) favored higher chlorophyll contents and promoted glucose and organic acid accumulation, whereas Se biofortification of the aerial parts was maximized at moderate Se concentration (75 μM) and was largely driven by prolonged priming (12 h). By contrast, extended priming generally reduced chlorophyll levels and some metabolic benefits. These results align with previous studies showing that seed priming with Se and Zn can improve the nutritional quality and bioactive compound content of microgreens and vegetables. For example, Mejía-Ramírez *et al.*[38] reported increased carotenoid and phenolic levels and reduced glutathione in tomato fruits from Se-primed seeds, whereas Poudel *et al.*[39] demonstrated enhanced Zn accumulation and antioxidant activity in Zn-primed pea microgreens. Collectively, these findings highlight seed priming as a simple and effective strategy to enhance plant nutraceutical value and mineral biofortification when appropriate combinations of priming duration and Se concentration are applied.

Effect of treatments on the antioxidant properties of microgreens

The antioxidant properties of pea microgreens were significantly influenced by Se seed priming, as reflected by changes in TPC and lipid peroxidation inhibition capacity (Table 4). The interaction between priming time and Se concentration was significant for TPC and TBARS formation inhibition. The highest TPC (86 mg GAE g^{-1} extract) was recorded in microgreens derived from seeds primed with 75 μM Se for 12 h, followed by those from the 50 μM–12-h Se treatment (which did not differ significantly). By contrast, the lowest phenolic levels were measured in samples subjected to HP and 25 μM–6-h Se treatments. It was interesting to note that increasing Se concentrations led to higher TPC in microgreens; this increase persisted up to 75 μM Se, but decreased at 100 μM Se, suggesting a possible negative impact. These results indicate a biphasic response to Se exposure: moderate Se levels may favor phenolic accumulation as part of the plant's antioxidant defense system, whereas excessive Se may impose metabolic constraints or induce oxidative stress, thereby limiting the synthesis of protective metabolites.

The DPPH radical-scavenging activity showed no statistically significant differences among treatments ($P > 0.05$), despite some variability in EC_{50} values (63.54–102.45 mg L^{-1}) (Table 4), suggesting that non-phenolic antioxidants may also contribute to radical-scavenging capacity. Nevertheless, the 50 μM–12-h Se sample exhibited the lowest EC_{50} value (63.54 mg L^{-1}), indicating superior radical-scavenging capacity, in agreement with its relatively high TPC. In contrast, more pronounced treatment effects were observed in the TBARS formation inhibition assay, which evaluates protection against lipid peroxidation. The 50 μM–12-h Se treatment exhibited the strongest effect (EC_{50} = 88.74 mg L^{-1}), consistent with its elevated TPC, whereas much higher concentrations were required for 50 μM–6-h and 75 μM–6-h samples (247.04 and 336.55 mg L^{-1}, respectively) to achieve 50% lipid peroxidation inhibition (Table 4). Overall, these results demonstrate that Se priming, particularly at 50 μM for 12 h, not only enhances phenolic accumulation, but also strengthens the ability of microgreens to protect lipids from oxidation, possibly through synergistic actions of phenolic compounds and other antioxidants, or through enhanced activity of antioxidant enzymes for which Se acts as a cofactor.

Overall, these findings indicate that Se nutripriming can enhance antioxidant-related traits in pea microgreens, particularly phenolic accumulation and protection against lipid peroxidation, although optimal outcomes depend on a careful balance between Se concentration and priming duration. Although moderate Se levels combined with prolonged priming improved antioxidant performance, these benefits must be weighed against potential trade-offs in germination and biomass yield when defining optimal production protocols.

Overall effects of seed priming

A LDA was performed to evaluate the effects of Se concentration and treatment duration on the edible aerial parts of pea microgreens, simultaneously considering biomass yield, chemical composition, and antioxidant activity. When samples were grouped

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by treatment (Fig. 6(A)), a clear separation and clustering of markers corresponding to the 12-h priming duration was observed, whereas samples from the 6-h treatment appeared more dispersed. Both HP markers clustered closely together and were located relatively near the NP control. Sucrose, oxalic acid, malic acid, ascorbic acid, succinic acid, Ca, K, chlorophylls, biomass yield, TPC and TBARS formation inhibition activity were the variables with discriminant capacity. Regarding the effect of Se concentration independently of soaking time, the model defined four discriminant functions. The first explained 80.3% of the variance, whereas the second explained 11.5% and effectively separated the markers according to Se content, which was higher in the 75 and 100 μM groups (Fig. 6(B)). The effect of seed soaking time, irrespective of Se concentration, was also evaluated. In this case, the model defined two discriminant functions (Fig. 6(C)). The first explained 96.7% of the variance, separating the samples based on sucrose content, which was higher in the 12 h group. The second explained 3.3% of the variance, showing positive correlations with biomass yield, chlorophylls and TPC, and a negative correlation with TBARS inhibition capacity.

CONCLUSIONS

Selenium seed nutripriming can be regarded as a dual-effect strategy for pea microgreen production, enhancing nutritional quality at the same time as imposing trade-offs in agronomic performance that depend on priming conditions. Nutripriming increased Se accumulation in seeds and promoted translocation to edible aerial tissues, at the same time as modulating mineral accumulation and antioxidant-related traits. Se biofortification was maximized at 75 μM Se and was largely driven by prolonged priming (12 h), whereas antioxidant performance, particularly lipid peroxidation inhibition, was most pronounced at 50 μM Se with a 12-h priming. However, this nutritional enrichment was accompanied by a clear agronomic penalty, reflected in reduced emergence rates and aerial biomass. By contrast, short-term priming (6 h) better preserved these agronomic traits, although still enhancing mineral composition relative to the NP control, which showed the highest emergence rate. These findings highlight the importance of tailoring priming parameters to specific production goals. Future research should focus on elucidating the molecular mechanisms underlying Se-induced nutrient transport and its interaction with legume–rhizobia symbiosis to develop strategies to mitigate the observed yield-quality trade-off.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

CM and MR were responsible for investigations. CM and MR were responsible for formal analysis.

CM, MR and JP were responsible for validation. CM and MR were responsible for writing the original draft. JP was responsible for conceptualization, methodology, supervision, resources, and reviewing and editing.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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