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Article

# Potassium Nitrate and Ascorbic Acid Priming Improved Tissue Chemical Composition and Antioxidant and Antimicrobial Activities of Linseed (*Linum usitatissimum* L.) Sprouts

Ahlem Zrig,* Salma Yousif Sidahmed Elsheikh, Foued Hamouda, Basma Najar, Emad A. Alsherif, Shereen Magdy Korany, Abdelrahim H. A. Hassan, and Hamada AbdElgawad

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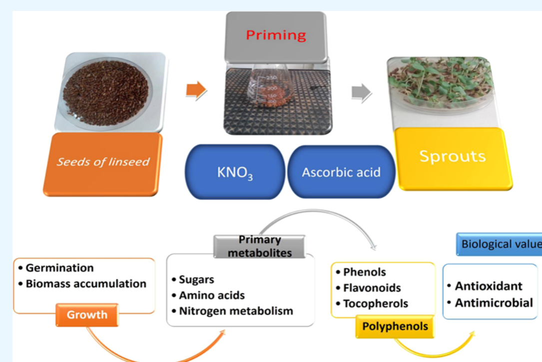
**ABSTRACT:** Potassium nitrate ($KNO_3$) and ascorbic acid (AsA) priming can effectively boost biomass accumulation and nutritional value of plants; nevertheless, few studies investigated their effects on seed sprouting. Thus, we aimed to explore the effects of $KNO_3$ and AsA priming on linseed (*Linum usitatissimum* L.) sprout growth and assess the changes in bioactive compound levels, which provide valuable insights into the potential benefits of these priming treatments on sprout quality and nutritional value. To this end, germination, biomass accumulation, photosynthetic pigments, primary and secondary metabolites, and mineral profiles in the primed sprouts were evaluated. Moreover, to assess the impact on biological value, we determined the antioxidant and antimicrobial activities of the treated sprout extract. A marked enhancement was observed in germination and pigment levels of $KNO_3$- and AsA-primed sprouts. These increases were in line with induced primary metabolites (e.g., carbohydrate and amino acid contents), particularly under $KNO_3$ treatment. There was also an increase in amino acid metabolism (e.g., increased GS, GDH, and GOGAT enzyme activities), nitrogen level, and nitrate reductase (NR) activity. The linseed sprouts primed with AsA exhibited strong antioxidant and antibacterial activities. Consistently, high levels of polyphenols, flavonoids, total AsA, and tocopherols, as well as improved activity of antioxidant enzymes [peroxidase (POX), catalase (CAT), and superoxide dismutase (SOD)], were recorded. This study proposes $KNO_3$ and AsA priming as an innovative approach to improving the nutritional and health-promoting properties of linseed sprouts. This knowledge will contribute to a better understanding of the biochemical processes involved in improving the nutritional quality and functional benefits of linseed sprouts.




## 1. INTRODUCTION

Linseed, also known as flaxseed, is a significant crop that serves multiple purposes. It is widely used in industries for oil and fiber production, and it is also consumed as a food source.[1] Additionally, it has medicinal properties due to its high oil content (41%), protein content (20%), and dietary fiber content (28%). With a moisture content of 7.7% and ashes content of 3.3%, linseed is rich in essential fatty acids, particularly polyunsaturated fatty acids (75%) including alphalinolenic acid (omega-3 fatty acid, 57%) and linoleic acid (omega-6 fatty acid, 16%).[2,3] Linseed is introduced into the daily diet to reduce body weight, cholesterol, and blood glucose levels as well as to prevent cardiovascular disease.[3,4] Furthermore, its significance lies in the domain of human nutrition, as it is gaining recognition as a beneficial food ingredient due to its active compounds that offer health benefits.[5] Linseed oil, a historically renowned commercial product, serves as both an edible oil and a pharmaceutical ingredient. It is widely recognized for its pharmaceutical applications, particularly as a drying agent when processed into solvents.[1] In addition to its health advantages, linseed is renowned for its high fiber content, which serves as a valuable resource in various textile sectors for crafting linen. Additionally, linseed is also cultivated for commercial paints or as an ingredient in wood-brooming,[2] as well as for ornamental purposes, thanks to its showy flowers.[1] In recent times, the growing concern among consumers regarding food quality and the importance of diversifying large-scale monoculture crops have led many countries to recognize the significance of cultivating linseed. This crop is consumed in substantial amounts as a functional food, which has sparked increased attention and interest. However, climate changes and lower

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yield of flax grains are the main reasons that limit the development of the industry of flax.

Seed priming is a recognized and advantageous technique utilized to mitigate the negative impacts of abiotic stresses in various crop species. Numerous methods of seed priming have been devised to enhance crop performance by improving the germination potential, seedling vigor, and overall yield. This technique, which involves treating seeds before sowing, induces physiological and biochemical modifications in the seeds, ultimately leading to positive outcomes in crop growth.[6] Nitrogen (N) fertilization as potassium nitrate ($KNO_3$), an osmo-priming method, is one of the chief agronomic practices of flaxseed production due to its positive response to nitrogen.[7] In fact, according to Wang and Xie, N fertilization boosts N usage effectiveness and grain output while increasing the N uptake and protein content in oilseed flax leaves. It also increases the N contribution of leaves and capsule peel to grain seeds.[8] Priming with nitrogen source compounds such as potassium nitrate ($KNO_3$) induces changes in the hormonal (abscisic acid and gibberellic acid) balance within the seeds, which play an important role in improving seed germination.[9] For instance, priming with $KNO_3$ reduces ABA levels promoting germination while increasing GA levels, which stimulates starch hydrolysis and induces high germination and seedling growth. Cucumber seedling growth, photosynthetic pigments, and nutrient content were induced by $KNO_3$ priming.[10] Priming with low $KNO_3$ levels also improved the antioxidant activity in pea seedling.[9] Besides, the utilization of $KNO_3$ as a priming agent has been found to yield the highest percentage of seed vigor and germination ratio in linseed species, suggesting that this technique is a promising approach for enhancing the overall quality and yield potential of *Linum usitatissimum* seeds.[11]

The utilization of plant growth regulators, such as phytohormones, enhances the productivity of different crops by employing priming mechanisms. In this regard, antioxidants play specific roles when incorporated into priming agents, with ascorbic acid (AsA) improving the emergence index (EI) and the final emergence percentage (FEP) of wheat while decreasing the mean of emergence time (MET).[12] The application of AsA as a seed priming agent induced the salt resistance of flax[13] and borage.[14] Canola seed priming with AsA enhanced the plant growth and seed oil content.[15] The role of AsA in enhancing photosynthesis, seed germination, leaf development, and inducing antioxidant levels is well known.[16] In addition, AsA treatment affects the selectivity of mineral uptake; for instance, it reduces the $Na^+/K^+$ ratio to protect plant cell membranes.[17] Xia's results indicate that priming with AsA could effectively alleviate aging damage in oat seeds.[14]

It is important to indicate that the effectiveness of priming treatments can vary depending on the plant species, plant developmental stage, and environmental conditions. Thus, conducting further research on the effect of $KNO_3$ and AsA priming on plants at different developmental stages, including the sprouting stage, is important for expanding our knowledge and practical applications in agriculture and horticulture. We hypothesize that $KNO_3$ and AsA treatments can be applied to enhance the bioactive phytochemicals, antioxidant activity, and antimicrobial activity of *L. usitatissimum* L. at the sprouting stage. Thus, the main objective of this study is to investigate the impact of seed priming with $KNO_3$ and AsA acid on the chemical composition and bioactivity of linseed sprouts. This will also contribute to a deeper understanding of the biochemical bases underlying the enhanced nutritional and functional properties induced by $KNO_3$ and AsA priming in linseed sprouts.

## 2. MATERIALS AND METHODS

**2.1. Experimental Design.** One hundred *L. usitatissimum* seeds were divided into three groups, i.e., a control group (hydro-primed seed), a $KNO_3$-primed group, and an AsA-primed group. The second group of seeds were immersed in 1% of $KNO_3$ and the third group were immersed in 200 mM AsA, at 25 °C for 24 h. All treated seeds were kept in the dark at 25 °C for germination. Under controlled growing circumstances (25 °C, 16 h of light and 8 h of night, 400 mol $m^{-2}$ $s^{-1}$ of PAR, and relative humidity (60%)), sprouting was carried out and monitored by cool white, fluorescent bulbs. After 9 days, for biochemical analysis, sprouts were weighed at 146 as fresh mass per tray and frozen and stored at −80 °C. The pretreated seedlings were rinsed in distilled water and laid on vermiculite-lined trays. Sprouts were watered with Milli-Q water every 2 days. At the beginning of the experiment, each tray received aquaponic water (150 mL). A total of 15 plants per tray were collected and used as biological replicates. All experiments were replicated at least three times.

**2.2. Pigment Analysis.** Two grams of fresh sprouts were mixed in 2 mL of acetone (80%) and then centrifuged at 4 °C at 2000 rpm for 10 min.[18] The supernatant was collected and then filtered (0.2 μm microfilter). Chlorophyll a (Chla), chlorophyll b (Chlb), and carotenoid were measured by the spectrophotometry method at 663, 645, and 475 nm, respectively. The concentrations of Chla, Chlb, and carotenoid pigments were determined.[18]

**2.3. Mineral Content Analysis.** Two hundred milligrams of linseed sprout group was mineralized ($HNO_3/H_2O$ solution) to measure mineral contents (Na, K, Mg, Ca, and Zn).[19] For the calibration solution, a multielement standard solution was employed at concentrations ranging from 0 to 50 ppm in 2% $HNO_3$. The control sample was nitric acid. The detection limits for detection and quantification were 0.00035 and 0.013 g/kg and 0.0042 and 0.3 g/kg, respectively.

**2.4. Sugar Content Analysis.** Fresh sprouts were mixed in 2.5 mL of methanol and then centrifuged at 2000 revolutions for 10 min at 4 °C.[20] After heating for 12 min in a water bath at 60 °C, the injection volume and column temperature were both set to 25 μL and 35 °C, respectively. Acetonitrile and high-performance liquid chromatography (HPLC)-grade water were combined in the mobile phase at a ratio of 75:25 (v/v) at a flow rate of 1 mL per minute. With the use of a calibration curve (1 to 12 mg/100 mL of acetonitrile/water (1:1, v/v)), identified sugar concentrations were measured.

**2.5. Determination of Amino Acids.** To measure the amino acids, 200 mg of fresh-weight sprouts were homogenized (ethanol 80%) and centrifuged at 22,000 g for 25 min.[21] Thereafter, the extract was redissolved and then filtered through a Millipore filter with a 0.2 μm pore size. The residue was re-extracted and centrifuged again. Millipore micro filters (0.2 μM pore size) were used to separate and filter the aqueous phase. The Waters Acquity UPLC-tqd system was used to measure amino acid levels by utilizing a measurement at 250 nm, low pressure, and a mobile phase (acetonitrile/water). The outcome was given in milligrams per gram of dry sample weight.

**2.6. Individual and Total Polyphenol and Flavonoid Contents.** To determinate the total polyphenols and


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flavonoids, 100 mg of sprouts were extracted with ethanol (80%).[22] The extract was centrifuged at 4 °C for 20 min. Clear supernatants were either directly used for calculating the total flavonoids or acid-hydrolyzed before calculating the total phenolics. The modified $AlCl_3$ colorimetric method was performed. Gallic acid was used to create the standard curve, and the phenolic content was measured by using the Folin−Ciocalteu method. To identify the polyphenols and flavonoids, the HPLC grade MeOH method was used as previously described by Gomaa and AbdElgawad.[23]

**2.7. Total Antioxidant Capacity Determination.** Thirty milligrams of sprouts were ground in liquid N for determining the total amount of antioxidants by FRAP analysis, and the resulting extract was suspended (2 mL, 80% ethanol). The extract was combined for 30 min with the FRAP reagent (0.3 M acetate buffer, pH 3.6), TPTZ (0.01 M), in HCl (0.04 mM), and $FeCl_3$ $6H_2O$ (0.02 M), and detected at 600 nm.[23]

**2.8. Investigation of Enzymatic Antioxidants.** We assessed the activities of ascorbate peroxidase (APX), POX, glutathione peroxidase (GPX), SOD, and CAT following the methods reported by Hassan et al. (2012)[24]. Sprouts were homogenized (MagNA Lyser, Germany) in 1 mL of $KPO_4$ buffer (50 mM, pH 7.0) containing 10% PVP, 0.25% Triton X-100, 0.001 PMSF, and 0.001 M AsA. The following units were used to denote POX, APX, GPX, and SOD: mol of oxidized pyrogallol, mol of AsA, mol of nicotinamide adenine dinucleotide phosphate (NADPH), and unit SOD (inhibition of nitro blue tetrazolium oxidation by 50%) per mg (protein) min, respectively. Pyrogallol was oxidized to measure the POX activity. AsA absorbance at 240 nm was decreased to represent the APX activity. The decrease in the NADPH concentration at 340 nm was used to quantify the GPX activity. While SOD activity was determined at 560 nm. CAT activity was calculated (2015).[25]

**2.9. Determination of Vitamins.** The quantities of vitamin C (AsA), vitamin A (retinol), and vitamin E (tocopherols) in sprouts were determined using HPLC methods.[19] Fresh leaves were homogenized in 0.1 N HCl to extract thiamine and riboflavin. The extraction of AsA was done using the meta-phosphoric acid. AsA was separated using a Polaris C18-A column (110 mm × 4.7 mm, 2.5 $\mu$m particle size; 42 °C). AsA was detected by using a diode array detector (DAD) (SPD-M10AVP, Shimadzu). Total AsA was determined after reduction with DTT (0.04 M). For the extraction of tocopherols, sprouts were homogenized in hexane. Using a Particil Pac 5 m column, vitamin E was separated and measured using HPLC. 5 ppm of 5,7-dimethyltocol was used (internal standard). Retinol was analyzed using reversed-phase HPLC on a silica-based C18 column. The mobile phase (81:9:10 of acetonitrile/methanol/water; and 68:32 of methanol/ethyl acetate). The solvent flow rate is 1.2 mL/min. The detection by DAD at 420, 440, 462, and 660 nm was performed, and the concentration was determined using a calibration curve.

**2.10. N, Ammonium, and Nitrate Content Analysis.** Nitrate levels were measured according to Cataldo et al.[26] The filtrate was mixed with salicylic acid and a $H_2SO_4$ solution. NaOH (2 M) was added to adjust the pH at 12. The absorbance was measured at 410 nm. The ammonium content was measured at 630 nm. The total N content was determined by mineralizing the dry leaves in $H_2SO_4$−$H_2O_2$.

**2.11. Antibacterial Activity.** Twenty grams of dried sprouts were blended, and the blended samples were extracted for 3 h (70 °C, 20 mL of 70% ethanol). The extracts were concentrated at 50 °C after being filtered (Whatman No. 2 filter paper). The antibacterial activity of linseed sprout extract was assessed against various microbial strains through the disc diffusion method, and the extent of inhibition was gauged by means of a Vernier caliper, in order to determine the antibacterial activity.

**2.12. Statistical Analyses.** Statistical analyses were carried out (R, Gplot, Agricola). A one-way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) were employed as post hoc tests. Principal component and hierarchical clustering analyses were carried out. The chemical components and their proportions (%) for each treatment were plotted. The parameters were clustered using the unweighted pair group method (UPGMA). A dendrogram that described the clusters was used to present the results. The JMP software package 13.0.0 was used.

## 3. RESULTS

**3.1. $KNO_3$ or AsA Priming Differentially Improved Seed Germination and Biomass Accumulation of Linseed Sprouts.** To investigate the potential role of $KNO_3$ and AsA in enhancing seed germination and biomass accumulation, fresh weight (FW) was measured (Figure 1).

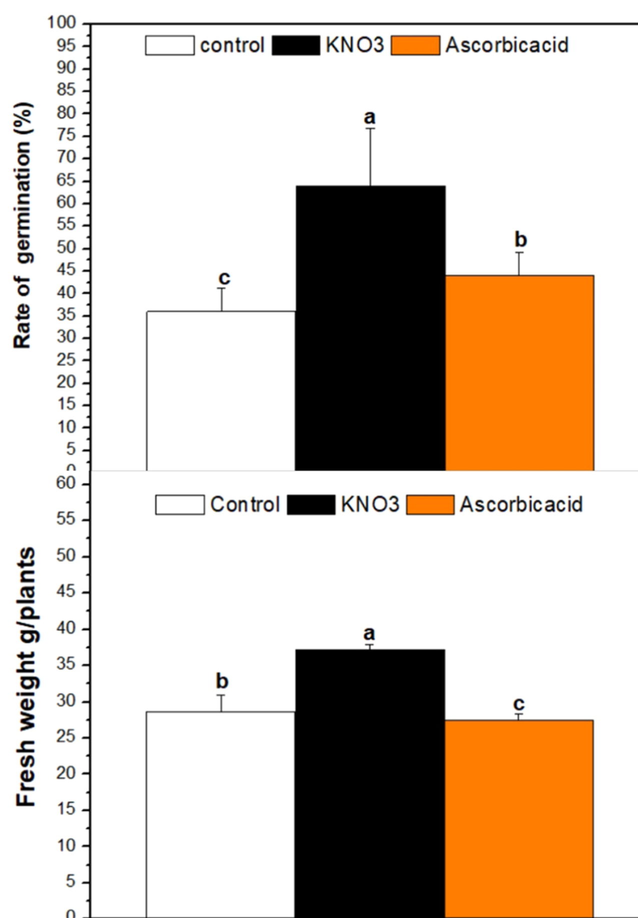


**Figure 1.** Effects of $KNO_3$ and ascorbic acid (AsA) priming at 1% on biometric characteristics (germination rate and fresh weight) of linseed (*Linum usitatissimum*) sprouts. Different letters indicate significant differences between the treatments. The statistical significance of the relative abundances was determined by Tukey's post hoc test, with $p \leq 0.05$.


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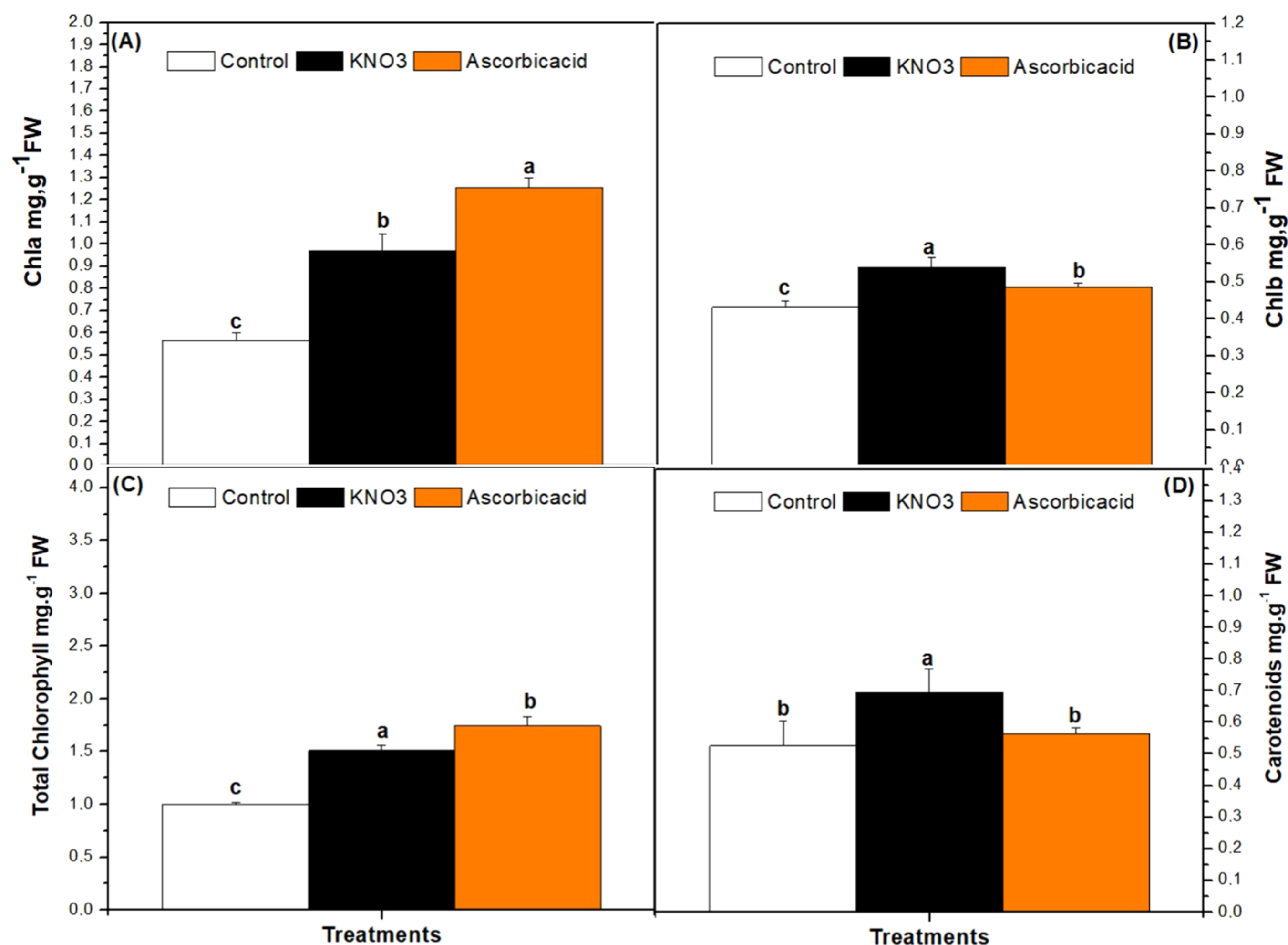


**Figure 2.** Effects of $KNO_3$ and ascorbic acid (AsA) priming at 1% on leaf pigments (A: Chla, B: Chlb, C: total Chl, C: carotenoids) of linseed (*Linum usitatissimum*) sprouts. Different letters indicate significant differences between the treatments. The statistical significance of the relative abundances was determined by Tukey's post hoc test, with $p \leq 0.05$.

The impact of different priming treatments on the germination rate was determined by germinated seeds %. It was found that seed priming with 1% $KNO_3$ resulted in a high germination percentage (up to 65%) compared to that of untreated seeds. This increase was also observed in AsA-treated seed at 1% but to a lesser extent (45%) compared to $KNO_3$ priming at 1%. In the same trend, priming with 1% $KNO_3$ enhanced the FW by 90% as compared with control seeds primed with pure water. However, the growth of seeds treated with AsA was not significantly improved in terms of FW compared to the control.

**3.2. $KNO_3$ or AsA Priming Increased Photosynthetic Pigment Contents in Linseed Sprouts.** In addition to their important role in photosynthesis, pigments such as carotenoids possess high potential activity as antioxidants. In line with the observed increase in growth, $KNO_3$ and AsA priming differentially increased photosynthetic pigments in linseed sprouts. $KNO_3$ priming increased the levels of total chlorophyll (Chla+Chlb), Chlb, and carotenoids (Figure 2). These increases were 86, 82, and 85%, higher than those in untreated sprouts, respectively. Furthermore, AsA priming enhanced the content of Chla in the sprouts by 71% more than priming with 1% $KNO_3$.

**3.6. $KNO_3$ or AsA Priming Improved Primary Metabolite Accumulation in Primed Linseed Sprouts.** Seed germination, seedling growth, and function rely on proper functioning of the primary metabolites. The primary metabolite accumulation also affects the nutritive and biological values of the seed sprouts. Thus, we determined the effects of $KNO_3$ and AsA at 1% on sugar levels, amino acids, and nitrogen metabolites and the activity of key related metabolic enzymes.

In the current study, the soluble sugar profile (glucose, fructose, sucrose, and platinose) was investigated in linseed (Table 1). $KNO_3$ at 1% priming improved the sugar content in linseed sprouts. For instance, the fructose in control sprouts was low, accounting for only 0.2 $mg \cdot g^{-1}$ FW. A great increase was observed in $KNO_3$-primed sprouts. Likewise, priming with $KNO_3$ led to a 3-fold increase in glucose and sucrose. However, no significant alteration was observed in the amount of platinose in response to $KNO_3$ priming at 1% (Table 1). The concentration in sprouted primed linseed seeds amounted to 0.6 $mg \cdot g^{-1}$ FW fructose, 1.58 $mg \cdot g^{-1}$ FW glucose, 0.7 $mg \cdot g^{-1}$ FW sucrose, and 0.98 $mg \cdot g^{-1}$ FW platinose after priming with ascorbic acids (Table 1).

Eighteen amino acids were determined in linseed sprouts. The highest levels recorded were in the case of glutamine, glutamic acid followed by asparagine (Table 1). The $KNO_3$ priming caused an increase in almost all examined amino acids. Approximately 2-fold increase in glycine, alanine, cysteine, and


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**Table 1. Effects of $KNO_3$ and AsA Priming at 1% on Sugars and Amino Acid Content of Linseed (*Linum usitatissimum*) Sprouts[a]**

| treatments | control | $KNO_3$ | AsA |
|---|---|---|---|
| sugar (mg·g$^{-1}$FW) | | | |
| fructose | 0.23 ± 0.03c | 1.6 ± 0.70a | 0.68 ± 0.37b |
| glucose | 1.28 ± 0.10c | 3.68 ± 0.67a | 1.58 ± 0.24b |
| sucrose | 0.45 ± 0.11c | 1.8 ± 0.34a | 0.79 ± 0.07b |
| platinose | 0.45 ± 0.04b | 0.47 ± 0.10b | 0.96 ± 0.16a |
| amino acids (mg·g$^{-1}$FW) | | | |
| lysine | 0.49 ± 0.02b | 0.84 ± 0.04a | 0.53 ± 0.00b |
| histidine | 0.51 ± 0.03ab | 0.66 ± 0.00a | 0.35 ± 0.00b |
| alanine | 0.42 ± 0.01b | 0.76 ± 0.05a | 0.41 ± 0.02b |
| arginine | 0.22 ± 0.01b | 0.27 ± 0.01a | 0.28 ± 0.03a |
| isoleucine | 0.39 ± 0.01c | 0.90 ± 0.03a | 0.53 ± 0.07b |
| asparagine | 1.11 ± 0.02b | 1.27 ± 0.04a | 0.73 ± 0.02c |
| glycine | 0.40 ± 0.02b | 0.87 ± 0.11a | 0.46 ± 0.01b |
| serine | 0.69 ± 0.03b | 1.23 ± 0.13a | 0.73 ± 0.0ab |
| proline | 0.24 ± 0.00b | 0.28 ± 0.02a | 0.22 ± 0.02c |
| valine | 0.45 ± 0.02ab | 0.60 ± 0.02a | 0.25 ± 0.0b |
| cysteine | 0.31 ± 0.01c | 0.60 ± 0.01a | 0.45 ± 0.01b |
| leucine | 0.85 ± 0.03c | 1.43 ± 0.07a | 1.08 ± 0.03b |
| methionine | 0.38 ± 0.01b | 0.47 ± 0.01a | 0.38 ± 0.03b |
| threonine | 0.45 ± 0.03b | 0.74 ± 0.03a | 0.48 ± 0.02b |
| tyrosine | 0.25 ± 0.01b | 0.32 ± 0.01a | 0.26 ± 0.01b |
| glutamine | 2.87 ± 0.06b | 3.34 ± 0.07a | 2.3 ± 0.0b |
| glutamic acid | 2.77 ± 0.01a | 2.13 ± 0.00b | 1.66 ± 0.3b |
| tryptophan | 0.18 ± 0.00c | 0.29 ± 0.02a | 0.21 ± 0.01b |

[a]Data are represented by means ± standard errors. Different letters indicate significant differences between the treatments, with $p \leq 0.05$.

isoleucine than in control was observed. For instance, a higher increase was recorded for glycine and isoleucine levels by 54 and 56%, respectively, compared to hydro-primed seeds. However, there were no significant increases in glutamic acid, glutamine, and arginine levels when compared with their respective controls. Meanwhile, AsA priming increased the arginine, cystine, isoleucine, and leucine by about 20, 30, 27, and 21%, respectively, as compared to those in control sprouts.

To understand the observed changes in some amino acid levels, we measured some key metabolic enzymes (Table 2). The activities of glutamine dehydrogenase (GDH), glutamine synthase (GS), glutamate synthase (GOGAT), and dihydrodipicolinate synthase (HDPS) were observed. The results showed an increase in GDH, GOGAT, and GS by 16, 34, and 30%, respectively, after priming with $KNO_3$. The highest increase by about 45% was observed in HDPS. By contrast, GS, GDH, and GOGAT decreased in AsA-primed sprouts, but a slight increase in HDPS was recorded in response to further treatment of those in control sprouts (Table 2).

**Table 2. Effects of $KNO_3$ and AsA Priming at 1% on Mineral Content of Linseed (*Linum usitatissimum*) Sprouts[a]**

| treatments | control | $KNO_3$ | AsA |
|---|---|---|---|
| mineral content (mg·g$^{-1}$DW) | | | |
| calcium | 67.9 ± 16.07b | 91.7 ± 6a | 64.35 ± 6.41b |
| potassium | 109 ± 7.51b | 196.7 ± 19a | 95.11 ± 3.10c |
| magnesium | 56.52 ± 0.33b | 56.6 ± 7.37b | 66.5 ± 8.1a |
| sodium | 83.91 ± 11b | 120.2 ± 0.91a | 103.1 ± 2.8a |
| zinc | 1.80 ± 0.43a | 3.07 ± 0.90a | 3.32 ± 0.86a |

[a]Data are represented by means ± standard errors. Different letters indicate significant differences between the treatments, with $p \leq 0.05$.

The outcomes of $KNO_3$ priming and ascorbic acid priming on the nitrogen (N) content were studied in linseed sprouts (Table 2). Moreover, N reductase (NR) activity was significantly increased by 25% in response to $KNO_3$ priming at 1%. Meanwhile, an increase in protein content by 19% was recorded in sprouts treated with 1% of $KNO_3$. Further, the impact of 1% of AsA priming was explored. Overall, a decrease in N content and protein was detected in linseed sprouts, while a slight and nonsignificant increase was recorded in NR activity as compared to those in control sprouts.

**3.7. $KNO_3$ or AsA Priming Improved Secondary Metabolites in Primed Linseed Sprouts.** In accordance with increased primary metabolites, secondary metabolites were also differently induced by $KNO_3$ and AsA priming. Both priming treatments during germination increased the concentration of phenolic compounds in linseed sprouts. Priming with $KNO_3$ resulted in a minor increase in phenol and flavonoid levels (Figure 3). On the other hand, priming with 10% of AsA led to a substantial enhancement of total phenolics and flavonoids, with increases of 14 and 45%, respectively, when compared to hydro-primed seeds. However, both $KNO_3$ and AsA priming resulted in a significant increase in total antioxidant capacity (TAC), with increases of 27 and 28%, respectively. The present study revealed a significant increase in the antioxidant AsA (Vitamin C) in linseed sprouts after treatment with 1% of $KNO_3$ and/or AsA (Figure 4). Obviously, priming with 1% AsA increased the AsA (vit C), retinol, and tocopherol contents by 35, 80, and 43%, respectively, as compared to hydro-primed sprouts (Figure 4). Similarly, the retinol content was quadrupled in $KNO_3$-primed sprouts compared to the control sprouts. Meanwhile, no significant changes were observed in $KNO_3$-treated sprouts on AsA as well as tocopherol (Vitamin E), when compared to the untreated controls (Figure 4).

The AsA at 10% treatment had the best effect on endogenous glutathione content (GHS) which was increased by 30% as compared to control (Figure 5), while no significant effect was recorded in sprouts treated with 1% $KNO_3$. Similarly, the reduced AsA exhibited a significant elevation in response to AsA priming, while priming of 1% $KNO_3$ did not affect the levels of reduced AsA and GSH (Figure 5).

Data regarding antioxidant enzyme activities revealed that different priming treatments had a considerable impact in linseed sprouts (Figure 6). The outcome revealed that AsA-primed sprouts exhibited higher POD activity as compared with those primed with $KNO_3$ and control sprouts. AsA priming at 1% had a prominent impact on POD enzyme activity. Furthermore, our results exhibited that priming treatments have a remarkable impact on CAT enzyme activity. In fact, AsA priming induced a 31% increase in CAT enzyme activity, whereas $KNO_3$ priming results in a decrease in enzyme activity. Furthermore, AsA priming exerted a notable impact on SOD activity, exhibiting a 34% increase compared to those in the control sprouts (Figure 6). Conversely, $KNO_3$ showed no significant alteration of the SOD activity. A similar trend was observed in POX enzyme activity, with a substantial 77% increase observed in response to AsA (1%) priming, whereas no significant increase was recorded with $KNO_3$ priming (1%).

**3.8. $KNO_3$ or AsA Priming Improved the Nutritive and Biological Values of Linseed Sprouts.** The concentrations



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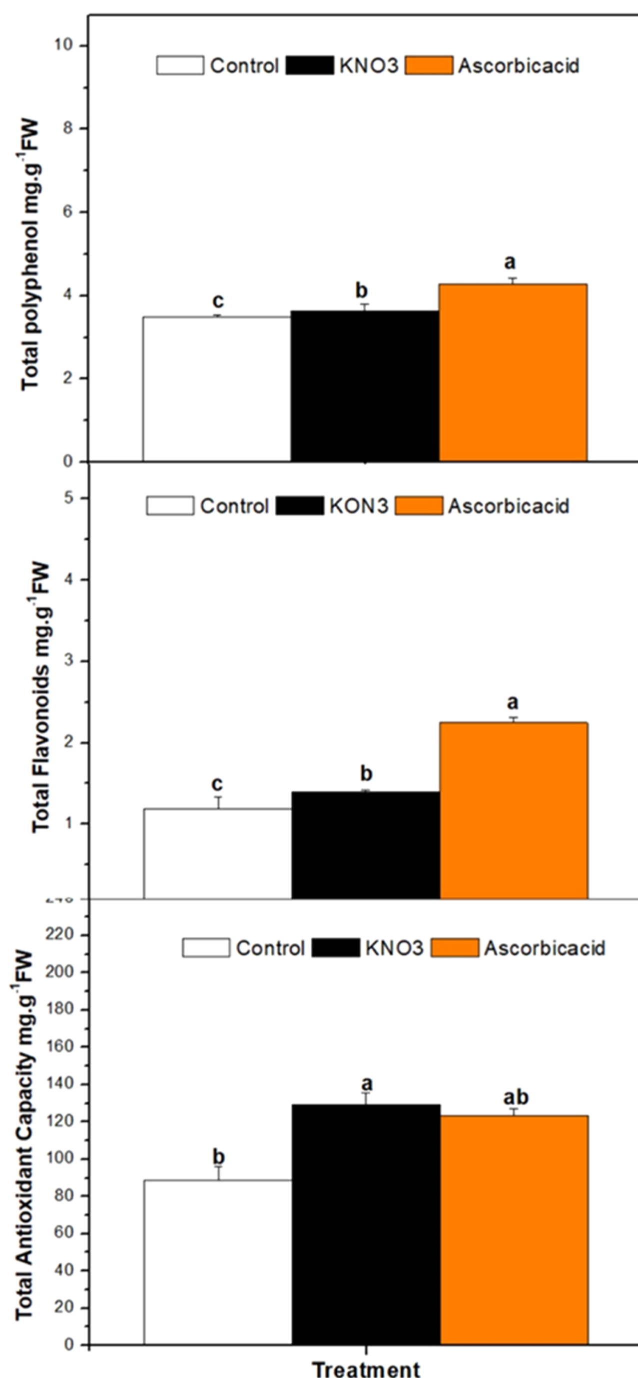


**Figure 3.** Effects of $KNO_3$ and ascorbic acid (AsA) priming at 1% on polyphenols, flavonoids, and total antioxidant activity (TAC) of linseed (*Linum usitatissimum*) sprouts. Different letters indicate significant differences between the treatments. The statistical significance of the relative abundances was determined by Tukey's post hoc test, with $p \leq 0.05$.

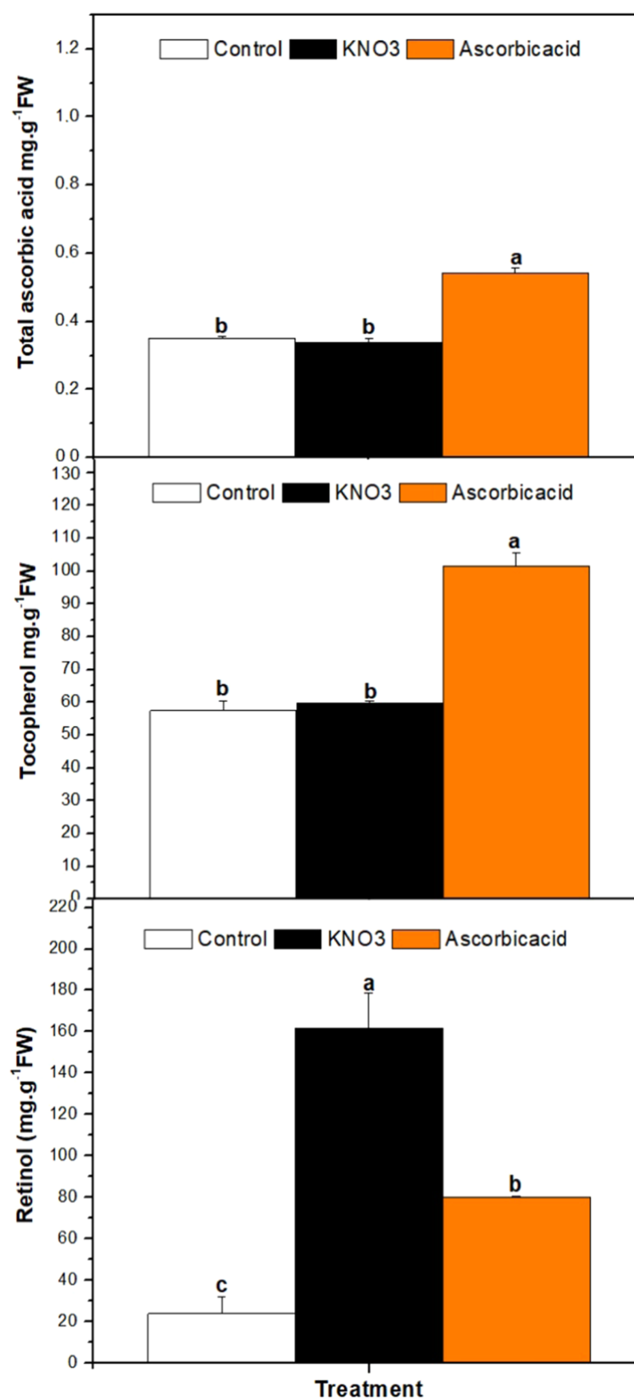


**Figure 4.** Effects of $KNO_3$ and ascorbic acid (AsA) priming on vitamins (ascorbic acid, tocopherol, and retinol) of linseed (*Linum usitatissimum*) sprouts. Different letters indicate significant differences between the treatments. The statistical significance of the relative abundances was determined by Tukey's post hoc test, with $p \leq 0.05$.

of Ca, K, Mg, and Zn were determined in dried linseed sprouts (Table 3).

The initial $Na^+$ content was almost 30% higher in primed sprouts with $KNO_3$ seeds compared to hydro-primed sprouts and, the Zn content was about 40% higher. Furthermore, $K^+$ accumulation in sprouts was notably enhanced by $KNO_3$ by 44%. Similarly, $Ca^{2+}$ content exhibits a notable increase by 22% in $KNO_3$-primed sprouts (Table 3). Likewise, priming with AsA increased the Zn, Mg, and Na contents by 45, 15, and 18%, respectively, as compared to hydro-primed seeds. However, no significant changes were observed in Ca and K contents in AsA-primed sprouts as compared to hydro-primed seeds.

**3.9. $KNO_3$ or AsA Priming Improved the Biological Value of Linseed Sprouts.** The antimicrobial activity of hydro-, $KNO_3$-, and AsA-primed linseed sprout extracts was evaluated by a disc diffusion assay (Table 4). The antibacterial activity was evaluated against *Bacillus subtilis*, *Streptococcus sp.*,

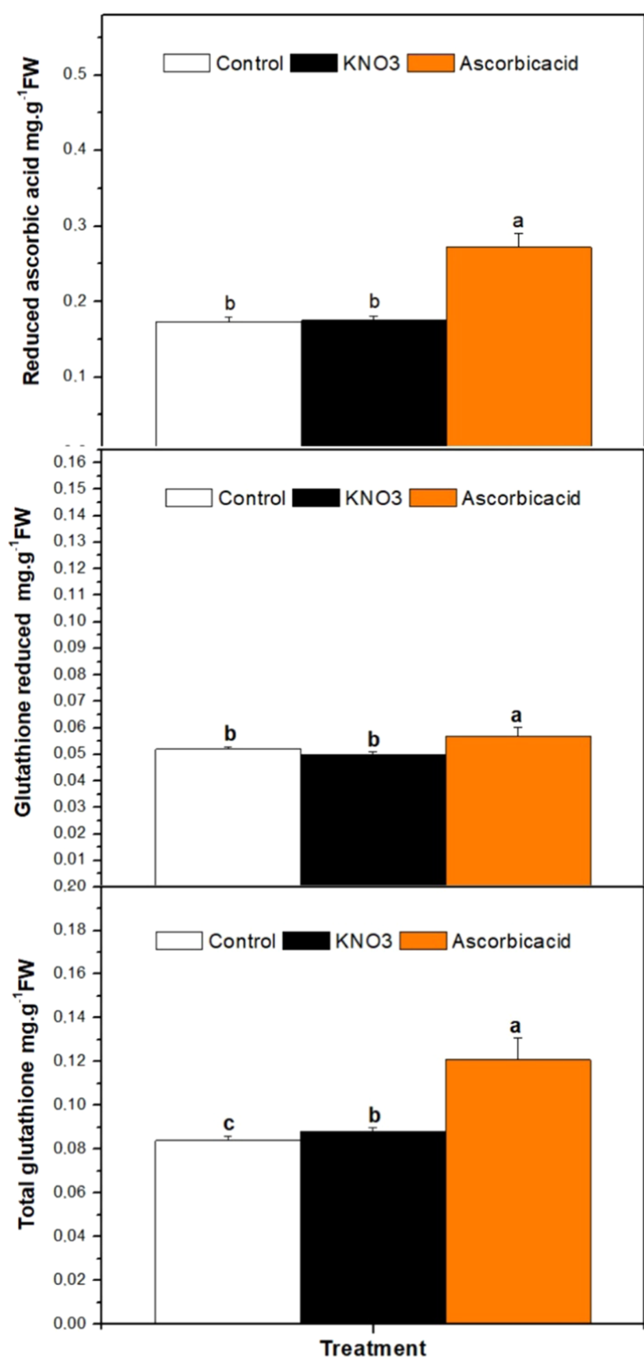


**Figure 5.** Effects of $KNO_3$ and ascorbic acid (AsA) priming on reduced ascorbate, reduced glutathione (RGSH), and total glutathione (GSH) of linseed (*Linum usitatissimum*) sprouts. Different letters indicate significant differences between the treatments. The statistical significance of the relative abundances was determined by Tukey's post hoc test, with $p \leq 0.05$.

*Escherichia coli*, *Pseudomonas aeruginosa*, *Sarcina lutea*, and *Staphylococcus aureus*. The antibacterial activity was tested against a group of Gram-positive and Gram-negative microorganisms. Interestingly, $KNO_3$ and AsA priming at 1% resulted in the enhancement of the antibacterial effect of the linseed sprout extract. In fact, $KNO_3$ priming at 1% improved the antibacterial activity against *B. subtilis* and *P. aeruginosa* by 28 and 22%, respectively. Overall, priming with 10% AsA induced an increase (30%). Moreover, the highest increase in antibacterial activity was recorded for *P. aeruginosa* (66%) treated with AsA-primed sprout extracts.

**3.10. Principal Component and Hierarchical Clustering Analyses.** The heat map of the HCA (Figure 7) performed on the medium value of triplicate analysis of all studied parameters showed two main groups: A and B, where this later was further divided into two clusters (B1 and B2). Group A was homogeneous because it is only composed of the plants treated with $KNO_3$ at 1%, while group B clusters included both control and ascorbic acid-primed groups. This was confirmed by the principal component analysis (PCA) results where the first axis, which is responsible for 64% of this dissimilarity, segregated samples treated with $KNO_3$ at 1% from the others, and it was allocated in positive loading along both PC1 and PC2 (Figure 8). This latter axis in this part was responsible for distinguishing plants treated with AsA (located in the upper left quadrant) from the control (located in the right down quadrant), where it is differentiated from the other treatments by its high content in glutamic acid.

## 4. DISCUSSION

**4.1. How Priming with $KNO_3$ or AsA Improved Biomass Accumulation?** One of the objectives of agriculture is to produce bioactive food. Sprouts, consumed by humans, provide an effective means of enhancing the intake of vital nutrients, such as minerals, vitamins, and other bioactive compounds. The utilization of elicitors can amplify the nutritional value of these products. We intended to establish the viability of priming as a method for fortifying flax sprouts. The process of priming is to enhance seed germination and seedling growth. Controlled seed hydration is used in this process during which a seed's metabolic activity is increased and then suspended prior to primary root extrusion. Our results showed that the priming improved the germination of linseed seeds treated with $KNO_3$ and AsA. In order to start the pre-germination process, priming promotes a number of biochemical changes in seeds, including the storage of metabolites, hydrolysis or metabolism, and enzyme activation.[27] Therefore, seed priming usually causes a rapid absorption and restoration of seed metabolism, resulting in a maximal germination % and faster germination rates.[28] Interestingly, $KNO_3$ priming had the greatest effect on the seed germination when compared to AsA priming. This finding is consistent with other studies of soybean[29] and wheat seeds.[28] It was hypothesized that presoaking of seeds in $KNO_3$ would reactivate aquaporins and trigger the amylase activity that is essential for embryo development.[30] Furthermore, $KNO_3$ is a known chemical treatment that enhances germination.[31] It, for instance, reduces the germination time when only physical environment management is a concern.[31] $KNO_3$ was observed by Demir and Mavi[32] to be effective in improving germination of the seeds of watermelon. In this regard, $KNO_3$ induced gibberellic acid levels which stimulated starch hydrolysis by increasing amylase activity and induced high germination and seedling growth.[9] Some enzymes involved in cell division, nucleic acid metabolism, and protein synthesis use potassium as a cofactor. Besides, nitrate concentrations are another factor contributing to $KNO_3$ beneficial effects.[33] Nitrates acting as a signaling molecule can improve seed germination.[34] Similarly, which reported that $KNO_3$ priming enhanced three Medicago seed growth.[35] Our findings were in line with many other studies.[30,36] Here, we notice that AsA priming improved the germination and fresh

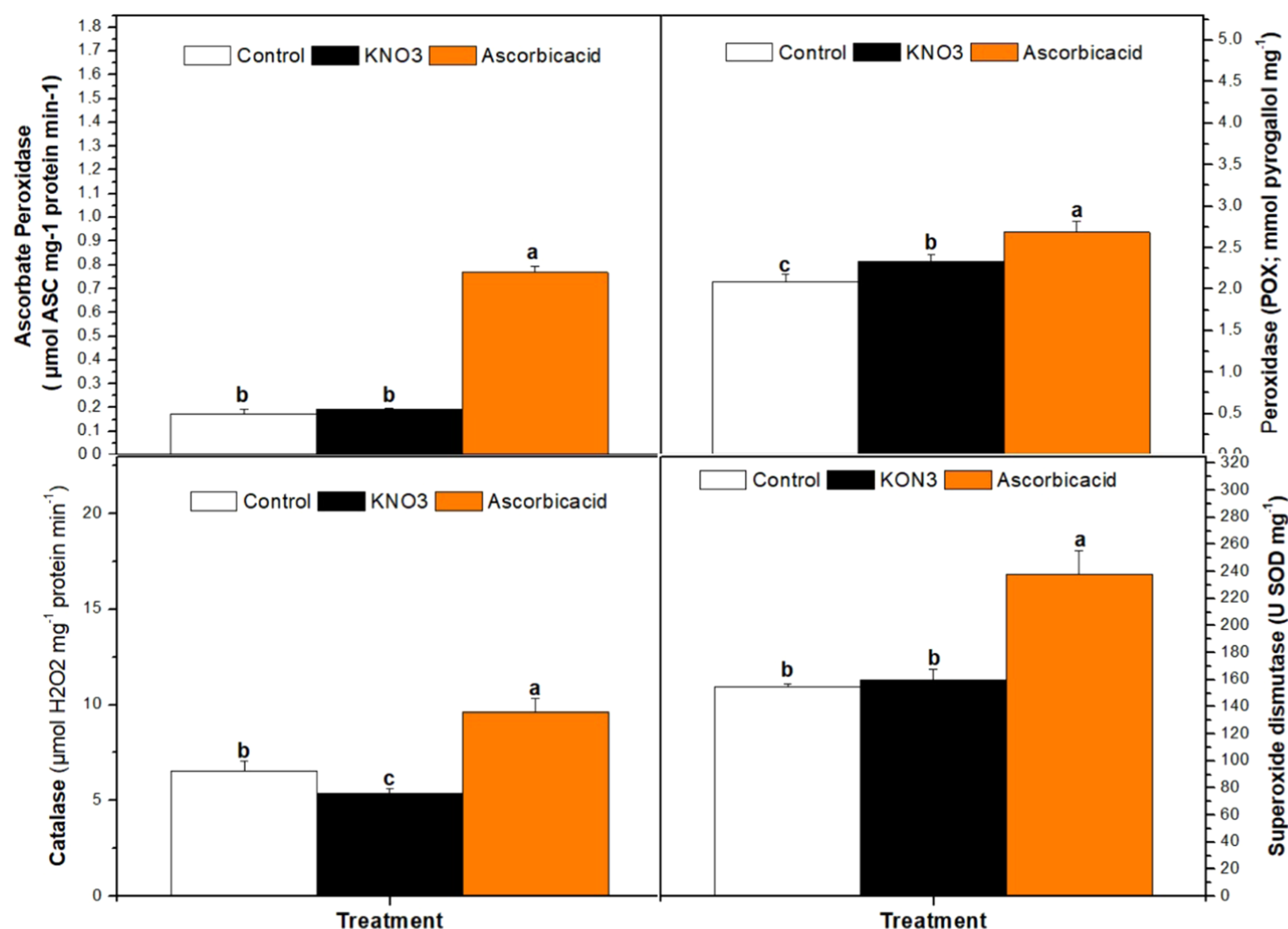


**Figure 6.** Effects of $KNO_3$ and ascorbic acid (AsA) priming at 1% on antioxidant enzymes of linseed (*Linum usitatissimum*) sprouts. Different letters indicate significant differences between the treatments. The statistical significance of the relative abundances was determined by Tukey's post hoc test, with $p \leq 0.05$.

**Table 3. Effects of $KNO_3$ and AsA Priming at 1% on N, Protein Content Metabolic Enzymes, and Mineral Content of Linseed (*Linum usitatissimum*) Sprouts[a]**

| treatments | control | $KNO_3$ | AsA |
|---|---|---|---|
| N, protein, and amino acid metabolism | | | |
| N (g/100 g) | 26.7 ± 1.7b | 27.4 ± 1.0a | 24.6 ± 0.92c |
| total protein (mg·g$^{-1}$ FW) | 165 ± 0.092b | 206.2 ± 5.6a | 156.8 ± 6.5b |
| NR ($\mu$mol/mg protein·min) | 55.9 ± 2.9b | 75.5 ± 2.7a | 56.6 ± 2.1b |
| GDH ($\mu$mol/mg protein·min) | 5.6 ± 0.36ab | 6.7 ± 0.326a | 4.9 ± 0.31b |
| GOGAT ($\mu$mol/mg protein·min) | 8.5 ± 0.54b | 12.9 ± 0.838a | 7.7 ± 0.43b |
| GS ($\mu$mol/mg protein·min) | 16.1 ± 1.043 | 23.3 ± 1.5ab | 5.5 ± 0.18c |
| DHDPS ($\mu$mol/mg protein·min) | 1.4 ± 0.07b | 2.4 ± 0.21a | 1.5 ± 0.06b |
| CGS ($\mu$mol/mg protein·min) | 0.06 ± 0.004b | 0.08 ± 0.005a | 0.06 ± 0.002b |

[a]Glutamine synthase (GS), glutamine dehydrogenase (GDH), glutamate synthase (GOGAT), and dihydrodipicolinate synthase (HDPS). Data are represented by means ± standard errors. Different letters indicate significant differences between the treatments, with $p \leq 0.05$.

biomass accumulation in linseed sprouts, as mentioned earlier. According to our findings, this improvement of the emergence and seedling viability was conducted by Dolatabadian and Modarressanavy[37] on wheat seeds. By altering the plant physiological structure, enhancing nutrient uptake, and improving plant redox status, AsA has the potential to enhance plant vigor and yield characteristics. In this regard, priming with AsA induced a sequence of biochemical transformations in seeds that initiate the pre-germination process. These transformations include inhibiting hydrolysis or metabolism, promoting imbibition, and activating enzymes.[38] As a result, primed seeds typically exhibit accelerated absorption and restoration of seed metabolism, resulting in higher germination percentages and faster germination rates.[38]

In the present investigation, the observed increment of growth parameters in linseed sprouts primed with $KNO_3$ and AsA was thought to be connected to the increase in leaf pigments. Additionally, the high level of $Mg^{2+}$ in linseed sprouts resulted in Chl production by $KNO_3$.[2828] $Mg^{2+}$ ions in Chl are covalently linked with four N atoms, as a reason for

**Table 4. Effects of $KNO_3$ and AsA Priming at 1% on Antimicrobial Activity of Linseed (*Linum usitatissimum*) Sprouts[a]**

| treatments | control | $KNO_3$ | AsA |
|---|---|---|---|
| antibacterial activity (zone inhibition (cm)) | | | |
| *Bacillus subtilis* | 16.4 ± 0.83c | 22.9 ± 0.9b | 25.2 ± 0.71a |
| *Streptococcus sp* | 16.4 ± 0.2c | 18.8 ± 0.8b | 23.8 ± 0.75a |
| *Escherichia coli* | 11.6 ± 0.6c | 13.4 ± 0.6b | 20.0 ± 1.4a |
| *Pseudomonas aeruginosa* | 8.6 ± 0.7c | 11.2 ± 0.38b | 26.0 ± 0.65a |
| *Sarcina lutea* | 11.3 ± 0.52c | 14.5 ± 0.6b | 21.6 ± 1.0a |
| *Staphylococcus aureus* | 6.4 ± 0.38b | 7.9 ± 0.75b | 13.8 ± 0.98a |
| antifungal activity (zone inhibition (cm)) | | | |
| *Candida albicans* | 17.79 ± 0.2c | 21.1 ± 0.3b | 26.8 ± 0.19a |

[a]Data are represented by means ± standard errors. Different letters indicate significant differences between the treatments, with $p \leq 0.05$.

$KNO_3$ priming which improve the uptake of nutrients. According to these findings, the greater physiological function of the photosynthetic pigment may account for the increased impact of seed priming techniques on this pigment.[30]

### 4.2. Priming with $KNO_3$ or AsA Differentially Improved the Nutritive Value by Accumulating Minerals and Bioactive Primary and Secondary Metabolites.

Additional negatives include the acceptability and sustainability of seed priming, which will be the subject of future research of bioactive and secondary metabolites, the efficiency of this strategy, how it is used in the marketplace, and how priming affects the nutritional value of flax seeds and sprouts. Both $KNO_3$ and AsA priming had a positive effect on the sugar content, where the highest increase was observed in $KNO_3$-treated sprouts. Increased sugar levels can provide high sugar availability for amino acid production. Consequentially, high levels of essential amino acids in plants are commonly accepted to have a significant impact on their nutritional value and health-promoting qualities. The increase in amino acid levels could be attributed to the increase in the activity of key enzymes (GS, GDH, and GOGAT). In this study, the priming with $KNO_3$ increased almost all amino acid contents than the ascorbic acid priming. We suggest that $KNO_3$ priming had a

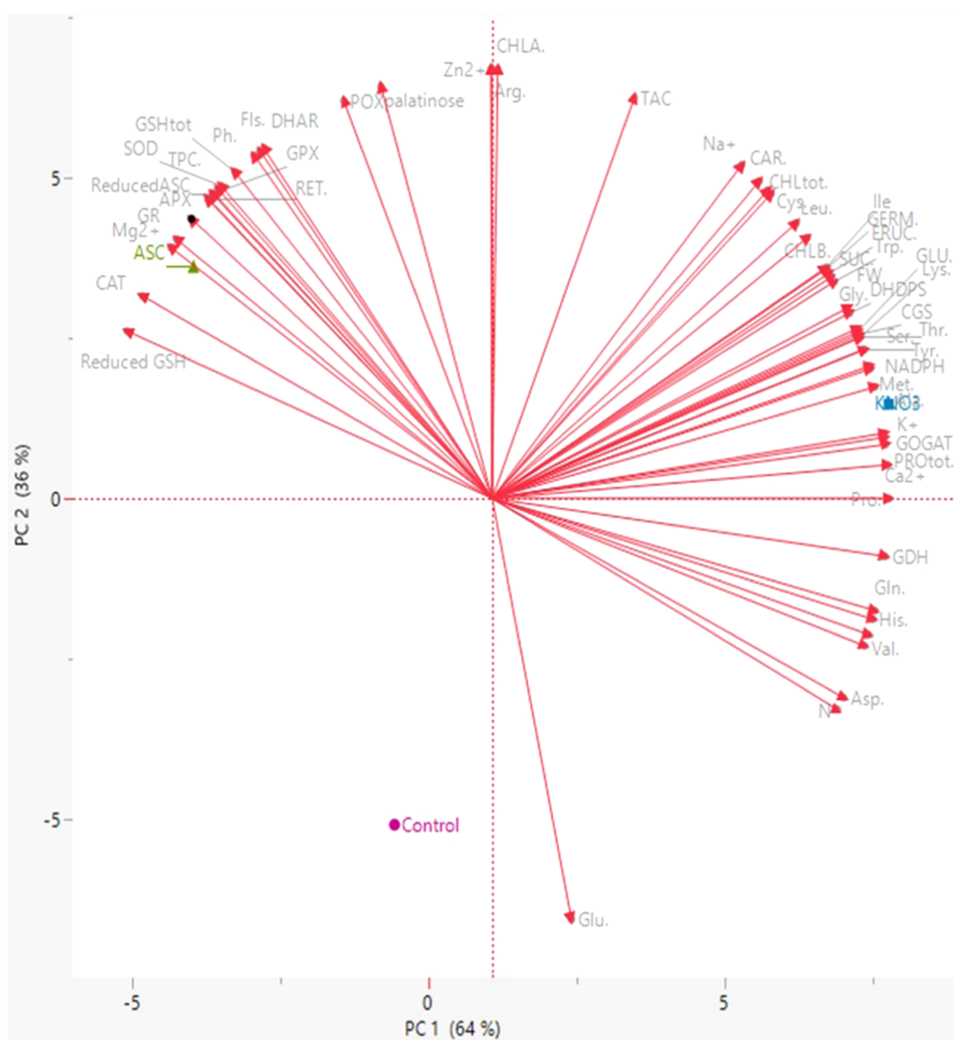


Figure 7. Principal component analysis (PCA) plot depicting primary and secondary metabolite proximities between hydro, $KNO_3$, and ascorbic acid (AsA) priming treatments.


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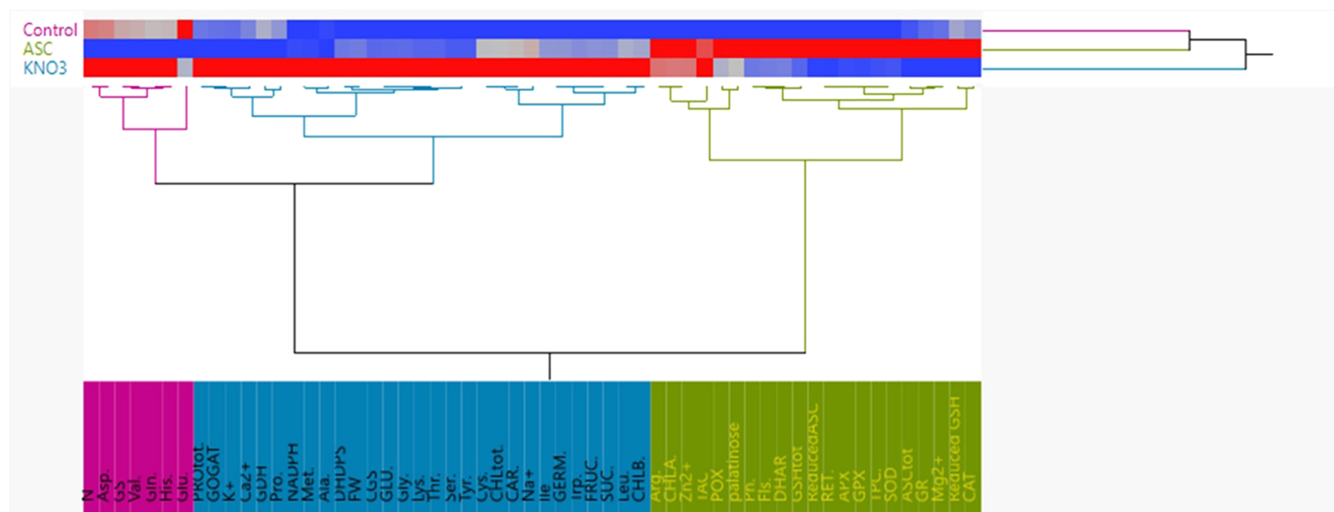


**Figure 8.** Dendrogram of cluster hierarchical analysis performed on primary and secondary metabolites between hydro, $KNO_3$, and ascorbic acid (AsA) priming treatments.

positive effect on NR. As NR activity increases, more nitrates could be reduced, which increase the potential for amino acid and protein biosynthesis and N assimilation. These results are similar to the ones obtained for *Medicago* sprouts.[35] However, the priming with AsA did not significantly affect the total N and protein and NR activity. Thus, $KNO_3$-primed sprouts can be used as a functional food to increase essential amino acids and protein synthesis in diets. These results are in accordance with Marques et al.[39]

Here, priming with $KNO_3$ and AsA at 1% showed a positive synergism with other chemical stimulants. In fact, this outcome revealed that the priming with AsA increases the vitamin content rather than the $KNO_3$ priming. It is believed that exogenous AsA as seed pretreatment can effectively regulate and improve the endogenous AsA as well as tocopherol levels.[16] Noteworthily, the positive effect of $KNO_3$ priming on vitamin contents indicates the potential use of linseed sprouts as a remarkable source of vitamins for supporting immunity.[35] Priming with $KNO_3$ improved the nutritive value in linseed sprouts by accumulating minerals. Indeed, these goods are excellent for customers who are lactose intolerant due to their high Ca content.[40] As a consequence, the linseed sprouts can contribute to the overall daily dietary intake of essential elements.[41]

Owing to its high concentration of physiologically active secondary metabolites, linseed has developed a reputation as a promising functional food, such as phenolic and flavonoid contents found in its seeds. Phenolic chemicals are significant food metabolites that can act as strong antioxidants.[42] Seed priming with $KNO_3$ and AsA enhances phenolic and flavonoid contents as well as the antioxidant mechanism of linseed sprouts as compared to untreated seedlings. In this study, linseed sprouts treated with ascorbic acid exhibit a high phenol and flavonoid content rather than sprouts treated with $KNO_3$. Compared to other food items, linseed possesses rich phenolic compound profiles with flavonoids constituting a major part, resulting in a higher antioxidant capacity.[43] In this study, AsA priming led to the increase of SOD, POD, CAT, and APX enzyme activities in linseed sprouts at higher rates than priming with $KNO_3$. There are not much data on how $KNO_3$ priming affects the antioxidant metabolism of plant seedlings. Only a small number of articles have described a relationship between the SOD, POX, CAT, and AOX enzymes.[44,45]

Indeed, the increase in SOD was related to the contribution of AsA to the production of this enzyme and may produce ascorbyl radicals.[46] Furthermore, the application of AsA increases the CAT activity, and our obtained results are in line with the results of El-Bassiouny and Sadak.[47] Besides, the APX activity is linked with the chloroplast AsA pool.[48] Our study is also in line with many other studies which reported that exogenous application of AsA could improve the antioxidant enzyme activity.[29,49] Interestingly, priming with $KNO_3$ improved the accumulation of the carbohydrate content in linseed sprouts. Increased carbohydrate contents in seed sprouts justified their increased emergence, growth, and seedling vigor. In line with our results, Alqahtani et al.[50] found that maize primed with $KNO_3$ had higher carbohydrate content. Noteworthily, priming with AsA increases the carbohydrate content. Similar findings were recorded in maize[51] and *Pisum sativum*.[52]

Proper maintenance of the human body relies on the essential minerals and trace elements, as they directly influence its physiological functions.[53] The primary essential minerals for humans include Ca, P, K, Na, and Mg. Sprouts, with their visually appealing appearance, have the potential to serve as alternative sources of essential elements. Utilizing sprouts as dietary supplements presents a novel and intriguing approach to enhance the nutritional content of various byproducts.[53] Linseed is generally rich in Mg and K,[54] and priming with $KNO_3$ and AsA improved the contents of sprout leaves of Ca, Na, and Zn levels. In the present study, the increased mineral contents by $KNO_3$ priming could improve the health-promoting effects of linseed sprouts more than those by AsA priming. In this regard, $KNO_3$ priming induced nutrient accumulation in seedling of *Medicago*.[35]

**4.3. AsA Priming Increased the Antibacterial Activity of Linseed Sprouts more than $KNO_3$ Priming.** Following exposure to $KNO_3$ and ascorbic acid priming, the antibacterial potential of linseed sprouts was evaluated using the disc diffusion method in terms of the widths of bacterial growth inhibition zones. The ability of linseed sprout extracts to fight against microorganisms that might cause food poisoning was examined, including *B. subtilis*, *Streptococcus sp.*, *E. coli*, *P.*


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aeruginosa, *S. lutea*, and *S. aureus*. Earlier reports indicated that linseed sprouts exhibited a high potential antimicrobial activity against different pathogens.[55] In this study, the pretreatment with $KNO_3$ and AsA separately enhanced the antimicrobial activity in linseed, but the highest increment was detected in sprouts primed with AsA. The improvement in the sprouts' antibacterial properties may be due to an increase in the amounts of bioactive metabolites, such as phenolic contents, in response to $KNO_3$ and AsA priming.[35] A previous study highlighted the significance of the phenolic chemicals of linseed in stimulating the degradation of bacterial DNA, as well as denying the gyrase activity.[56] High vitamin content and improved antibacterial activity were also well correlated.[56]

## 5. CONCLUSIONS

This study elucidated the modifications occurring in the chemical compositions of tissues, encompassing the accumulation of bioactive components in $KNO_3$- and AsA-primed sprouts. Furthermore, it successfully identified specific targets that can be utilized to enhance the production of compounds in linseed sprouts. In terms of enhancing the accumulation of primary metabolites in linseed sprouts, $KNO_3$ priming appeared to be more effective than AsA priming. However, with respect to antioxidant metabolites and enzymes and antibacterial activity, AsA showed a better effect. This knowledge will advance our understanding of the biochemical processes involved in improving nutritional quality and functional benefits of linseed sprouts.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

## ■ AUTHOR INFORMATION


### Corresponding Author

**Ahlem Zrig** − *Higher Institute of Preparatory Studies in Biology and Geology, University of Carthage, 1054 Tunis, Tunisia; Laboratory of Engineering Processes and Industrial Systems, Chemical Engineering Department, National School of Engineers of Gabes, University of Gabes, 6072 Gabes, Tunisia;* orcid.org/0000-0003-2919-6556; Phone: (+216) 97 901 249; Email: ahlem18zrig@yahoo.fr

### Authors

**Salma Yousif Sidahmed Elsheikh** − *College of Science, Arar, Department of Biological Science, Northern Border University, Arar 25698, Saudi Arabia*

**Foued Hamouda** − *Research Unit in Enterprise and Decisions, Higher Institute of Management, 6002 Gabes Tunis, Tunisia*

**Basma Najar** − *Department of Agricultural, Food and Agri-environmental Sciences, University of Pisa, 56124 Pisa, Italy*

**Emad A. Alsherif** − *Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Beni Suef 62511, Egypt*

**Shereen Magdy Korany** − *Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia*

**Abdelrahim H. A. Hassan** − *School of Biotechnology, Nile University, Giza 12588, Egypt;* orcid.org/0000-0001-7905-6821

**Hamada AbdElgawad** − *Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Beni Suef 62511, Egypt; Integrated Molecular Plant Physiology Research, Department of Biology, University of Antwerp, 2020 Antwerp, Belgium*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c03002


### Author Contributions

A.Z.: writing—original draft preparation, formal analysis, writing—review and editing, conceptualization, resources. B.N.: writing—review. A.H.A.H.: Preparing the original manuscript draft, analyzed the results statistically, validation and visualization. S.M.K., A.H.A.H., E.A.A., S.S.: Validation and visualization. H.M.: supervision.


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