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ORIGINAL PAPER

Seed priming and phosphorus fertilization boost nutrient biofortification of lentil plants*

Mustafa Ceritoglu[1], Murat Erman[2], Fatih Çığ[1]

[1] Department of Field Crops
Siirt University, Siirt, Türkiye
[2] Department of Field Crops
Bursa Uludağ University, Bursa, Türkiye

Abstract

This experiment investigated effects of seed priming and phosphorus fertilization on the biofortification of lentil plants grown under low-phosphorus field conditions. Four phosphorus doses and six priming treatments were used in the experiment. According to results, 15 and 30 kg P ha^{-1} significantly increased the nitrogen concentration in plants while all phosphorus doses stimulated greater magnesium accumulation over control. Higher phosphorus addition restricted potassium acquisition by 9.5% under high-potassium soils. Moreover, 15 kg P ha^{-1} application of salicylic acid, citric acid, inorganic phosphorus or plant growth-promoting bacteria (PGPB) distinctly promoted the uptake of nitrogen, phosphorus, potassium, manganese, iron and zinc. PGPB mostly promoted nitrogen and phosphorus uptake, while citric acid priming highly stimulated the acquisition of Mg, Mn and Fe. All priming treatments were lower than the control for potassium accumulation, in which the lowest value was observed in PGPB-primed plants, because it can solubilize phosphorus compounds in rhizosphere, thereby causing an antagonistic effect on potassium uptake. Seed priming with 4 mM silicon enhanced copper accumulation in tissues up to 9.4%. Priming with 100 mg kg^{-1} citric acid promoted iron, magnesium and manganese accumulation by 13.8%, 3.8% and 4.7% compared with control, respectively. In conclusion, phosphorus addition boosted macro- and micronutrient acquisition, although the 15 kg P ha^{-1} dose is recommended from an economic perspective. Also, phosphorus application and seed priming treatments exhibited synergistic effects on nutrient acquisition depending on a nutrient element. Finally, seed priming with PGPB, 4 mM salicylic acid and 100 mg kg^{-1} citric acid exhibited superior performance on nutrient uptake in lentil.

Keywords: beneficial microorganism, food security, *Lens culinaris*, phosphorus deficiency, sustainable agriculture

Mustafa Ceritoglu, Dr., Department of Field Crops, Faculty of Agriculture, Siirt University, Siirt, Türkiye, e-mail: ceritoglu@siirt.edu.tr

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INTRODUCTION

Lentil is a versatile crop considered to be a pivotal one since it can be utilized as human food and animal feed, for example to make various lentil snacks, in the medical sector and as green manure. The importance of lentil in human nutrition is already a well-established fact. In addition, research shows that it is also a valuable animal feed since lentil has higher protein and crude fiber content than other grain legumes (Açıkbaş, Özyazıcı, 2022*a*, Namba-Santiago et al. 2023). Thus, biofortification of crops, which is described as the process of improving the content and/or bioavailability of crops during the growth stage, is an increasingly substantial phenomenon. This is primarily due to the issue of micronutrient deficiency, also known as "hidden hunger". People suffer from health problems caused by micronutrient deficiencies, particularly in developing countries. Therefore, biofortification of crops is of vital importance, especially in regions such as sub-Saharan Africa, where combating hunger and poverty is a major concern (Dimkpa et al. 2023).

There are two main biofortification strategies i.e., genetic and agronomic pathways. Genetic biofortification expresses genetic engineering or classical breeding, while agronomic one provides fertilization or various practices to soil, seed, or leaves of the crop (Zulfiqar et al. 2024). Genetic biofortification including breeding, genetic engineering and gene edition, aims to boost the genotypes by promoting micronutrient accumulation (Dwivedi et al. 2023). Agronomic biofortification focuses on improving mineral solubility and mobilization, thereby boosting the micronutrient concentration of major edible crops via fertilization practices (Zulfiqar et al. 2021*a*). Thus, agronomic biofortification enables better crop growth and promotes nutrient mobilization and utilization. Fertilization applied to meet the basic nutritional needs of plants can be considered as the first example of agronomic biofortification. Therefore, it not only addresses growth retardation and yield losses due to nutrient deficiencies, but it also contributes to increasing the nutritional content. On the other hand, among agronomic biofortification methods, seed priming has gained increasing importance owing to its low additional application costs and significant effects, as well as its low labor requirement (Ashraf et al. 2023).

Seed priming is based on the principle of soaking the seeds in a solution with low osmotic potential for a certain period, followed by drying (Ceritoglu et al. 2021). Consequently, various biochemical processes are initiated in the seed, triggering the activation of germination mechanisms along with the involvement of antioxidant defense systems (Mauch-Mani et al. 2017). Therefore, seed priming promotes early seedling and root development in plants, allowing them to utilize water and soil nutrients more effectively. Additionally, it serves as an effective and simple method to enhance plant tolerance to biotic and abiotic stress factors (Rhaman et al. 2021, Açıkbaş, Özyazıcı 2022*b*, Ceritoglu et al. 2023). Haider et al. (2020) reported that zinc (Zn) priming increased seed yield and Zn content up to 60% in mung bean. Rehman et al. (2022) indicated that boron priming promoted both plant growth and grain boron concentration in *Vigna radiata*. Zulfiqar et al. (2021*b*) pointed out that Zn priming had the most profound effect on Zn biofortification of rice seed compared with other techniques, including foliar application, basal application and seed coating. Ikram et al. (2023) determined the promoting effect of iron (Fe) seed priming on Fe biofortification of tomato. Mehrabanjoubani et al. (2015) reported that silicon (Si) priming increased potassium (K), phosphorus (P), Zn and Fe concentrations in wheat seed.

Most of the available phosphorus in the soil forms chelates with aluminum (Al) and iron (Fe) in acidic soils, while in alkaline soils it forms compounds with calcium (Ca) and becomes unavailable to plants (Owino-Gerroh, Gascho, 2005). MacDonald et al. (2011) pointed out that more than 29% of agricultural soils available globally are P deficient. Moreover, plants can use only a small amount of phosphorus-based fertilizers (about 10-25%) due to being precipitated by metal-cation complexes and rapidly fixed in soils (Sharma et al. 2013). Thus, this study will examine the effects of increasing P fertilizer doses on the uptake of phosphorus and other nutrients. In summary, the aim of this study was to determine the effects of seed priming with different materials on nutrient uptake in lentil under varying P concentrations. Seed priming of lentil seeds with the tested materials on macro- and micronutrient accumulation is an original aspect of the experiment. Moreover, this research will contribute to understanding the interactive effects of seed priming with different materials and phosphorus fertilization on nutrient biofortification in lentil.

MATERIALS AND METHODS

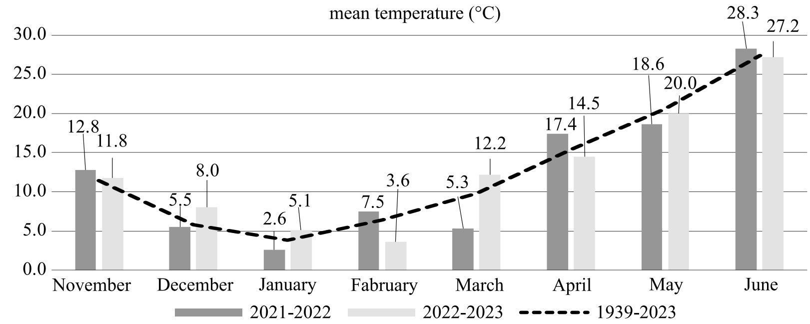
Experimental materials

Tigris, a red lentil cultivar (*Lens culinaris* Medik.) used in this experiment, was developed via selective breeding by the GAP International Agricultural Research and Training Center (GAPUTAEM) in 2013. In the experiment, inorganic phosphorus (Pi), silicon (Si), citric acid (CA), salicylic acid (SA) and PGPB were used for seed priming since each of them plays a direct or indirect critical role in P acquisition by plants. *Brevibacillus choshinensis* (TV53D) and *Paenibacillus xylanilyticus* (KF63C) consortia were used for bio-priming. The biological materials were isolated from the Van Lake Basin in 2008 (TV53D) and from Siirt region in 2020 (KF63C).

Ecological conditions of experimental area

The research was conducted at the Faculty of Agriculture of Siirt University, in the 2021-2022 and 2022-2023 seasons. Siirt is located in Southeastern Anatolia, at 41°57′ east longitude and 37°55′ north latitude. The elevation of the research area is 590 meters above sea level.

Siirt has a predominantly continental climate that is characterized by hot and dry summers. There is a significant temperature difference between day and night throughout the province. Climate data for the 2021-2022 and 2022-2023 seasons, including monthly average temperatures and monthly total precipitation, along with the long-term averages (LTA), in the Siirt region are shown in Fig 1. Total temperatures throughout the plant growing



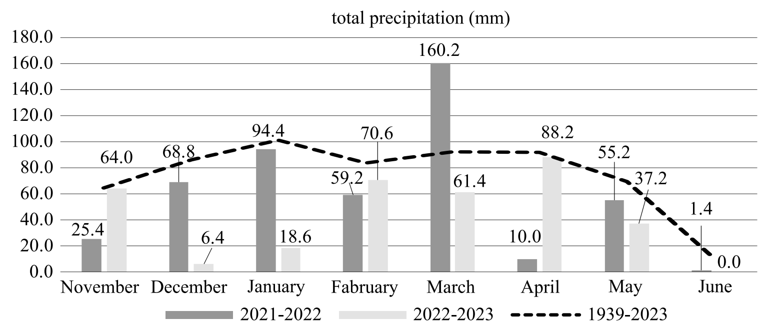


Fig. 1. The long-term (1939-2023) and research years (2021-2023) average temperature and total precipitation data for the province of Siirt

period were 2940°C and 3072°C in the 2021-2022 and 2022-2023 seasons, respectively. In addition, the lowest air temperatures in November, December, January, and February during the 2021-2022 season were 6.4°C, -3.5°C, -9.0°C, and -0.8°C, respectively, while being 5.5°C, 0.7°C, -2.2°C, and -5.4°C, respectively, in the 2022-2023 season. Total precipitations during the entire plant growing period from the crop stand establishment to harvest were recorded as 474.6 mm and 346.4 mm for the 2021-2022 and 2022-2023 seasons, respectively.

Soil characteristics of experimental area

In both years, soil samples were taken from A horizon at the experimental area before sowing. The experimental soil was determined to be an Entisol according to the US Soil Taxonomy (USDA, 2014). Soil samples were analyzed at Siirt University for texture, soil reaction (pH), electrical conductivity (EC), lime, organic matter, available phosphorus, and potassium contents. The pH and EC were determined using the 1:2.5 soil-water mixture method. Lime was detected using the calcimeter method. The Bouyoucus hydrometer method was used for texture analysis (Bouyoucos 1951). Soil organic matter was determined using the Walkley Black wet combustion method (De Vos et al. 2007). Available phosphorus was calculated with the sodium bicarbonate method using a spectrophotometer (Schoenau, O'Halloran 2007), and available potassium was determined using the ammonium acetate method with a flame photometer (Mason 1963).

Soil texture was clayey-loamy (sand %20-40 and clay %27-40) and its pH was slightly alkaline in both years. The soil was non-saline and the lime content was moderate in both seasons. Organic matter content and available phosphorus were low in 2021-2022 and very low in 2022-2023. Available potassium content was high in both seasons (Table 1).

Table 1

Characteristics of experimental soil in the 2021-2022 and 2022-2023 growing seasons

Year	Texture	EC (dS m^{-1})	pH	Lime (%)	OM (%)	P (kg da^{-1})	K (kg da^{-1})
2021-22	clay-loam	0.16	8.02	8.44	1.55	3.07	55.1
2022-23	clay-loam	0.13	7.82	7.89	1.12	2.24	82.2

Physiochemical characteristics of experimental soil were determined in Siirt University Science and Technology Application and Research Center. OM – Organic matter

Preparation of solutions and seed priming process

The density of bacterial solutions (10^9 cfu) was determined using the turbidimetric method. Chemical solutions, i.e., Pi, Si, SA and CA, were set in concentrations at 1%, 4 mM, 0.2 mM and 100 mg kg^{-1}, respectively. Concentrations of seed priming solutions were determined according to pre-experiments. Seeds were treated by surface sterilization with 70% ethyl alcohol for 1 minute and 10% sodium hypochlorite (NaOCl) for 5 min before the priming process. Seeds were carefully washed with distilled water and then rinsed while surface water was remove using sterile filter paper. Appropriate concentrations of Pi, Si, SA, and CA solutions were added at a seed: solution ratio of 1:1.5 (kg L^{-1}). Priming duration was set at 20 hours for chemical materials (Farooq et al. 2020), while it was set at 4 hours for bio-priming (Erman et al. 2022).

Experimental design and layout of two-year field trails

This experiment covers part of a Ph.D. thesis. Another part of the dissertation including agronomy, yield and quality attributes has already been published (Ceritoglu et al. 2024). This manuscript deals with the question of nutrient uptake. The field trials were set up on 19 and 21 November in the 2021-2022 and 2022-2023 seasons, respectively. Sowing was done on slightly moist soil, and rainfall occurred within 48 hours after planting in both seasons. The experiment was laid out in a split-plot design with four replications. Main plots were assigned phosphorus levels, while subplots received priming treatments. Phosphorus levels were set as P0 (control), P1 (15 kg P ha^{-1}), P2 (30 kg P ha^{-1}), and P3 (60 kg P ha^{-1}). Phosphorus doses were applied under the seedbed with the planting using triple superphosphate (TSP) fertilizer. Nitrogen fertilization of 20 kg ha^{-1} pure nitrogen in urea form was treated in all plots.

The distance between plots and blocks was set at 1.5 meters. Row spacing was set at 20 cm, and each plot consisted of 5 rows. With a plot width of 1 m and a plot length of 5 m, each plot was established as 5 m^2. Seeds were manually sown to avoid infection. The planting rate was adjusted to 250 seeds per square meter (Togay, Anlarsal 2008).

Weed populations were control manually to ensure they did not reach the critical threshold that could cause damage to lentil. Herbicides or insecticides were not used in either season. Plant samples were collected at the flowering stage (first week of March in both years) at the highest point of nodulation and biological nitrogen fixation (BNF) that affect nutrient uptake.

Macro- and microelement composition in lentil leaves

Plant samples were collected from each plot during at the flowering stage. Samples were air-dried after collecting. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) concentrations were determined in plant materials. P, Ca, Mg, Fe, Mn, Zn and Cu elements were determined by the ICP-OES spectrophotometry method (Hansen et al. 2012). The total N content in the plant was determined using the Dumas method (Wang, Daun 2006). The K content in the plant was determined using the ammonium acetate method with a flame photometer (Mason 1963).

Statistical analysis

The data from the two-year experiment were subjected to the Levene's test for homogeneity. Once homogeneity was confirmed, analysis of variance (ANOVA) was conducted based on the split-plot design. The Tukey's Honestly Significant Difference (HSD) test was used for the grouping of means for dependent variables using "*agricolae*" package R software (Cornillon et al. 2012).

RESULTS AND DISCUSSION

Results

According to the ANOVA, year (Y), phosphorus fertilizer (P), priming treatments (Pr) and their various interactions have led to statistically significant differences ($p<0.05$ or $p<0.01$) in traits. The experimental seasons were distinctly different due to climatic indices (Figure 1), which caused major differences in all traits. Phosphorus fertilizer was statistically effective on N, K, Ca and Mg concentrations in plants. Priming treatments led to statistically significant differences in all nutrients. YxP interactions significantly affected Ca, Cu, Fe and Mg concentrations, while YxPr interaction also affected the plant's K and Mn content. PxPr and YxPxPr interactions led to significant differences in all macro and micronutrients in lentil plants (Table S1).

The experimental years indicated a noteworthy variation in all characteristics. Potassium and iron concentrations were higher in the 2022-2023 season, whereas the content of the other elements was higher in the 2021-2022 growing season. The content of macronutrients, i.e., N, P, K, Ca and Mg, varied between 2.89-3.56%, 2616-3046 mg kg^{-1}, 3127-4370 mg kg^{-1}, 8846-11 173 mg kg^{-1} and 2274-2840 mg kg^{-1}, respectively. On the other hand, micronutrients, i.e., Cu, Fe, Mn and Zn, changed between 8.79-13.07 mg kg^{-1}, 716-883 mg kg^{-1}, 40.3-53.4 mg kg^{-1} and 24.2-31.4 mg kg^{-1}, respectively (Figure 2).

Increasing the dose of phosphorus added to experimental soil significantly promoted N, K, Ca and Mg accumulation in plant tissues, and the content of theseelements varied between 3.02-3.44%, 3565-3946 mg kg^{-1}, 9501-10362 mg kg^{-1} and 2428-2654 mg kg^{-1}, respectively. Potassium accumulation decreased by 9.5% with increasing phosphorus addition. Increasing phosphorus doses promoted Ca accumulation in plants whereas they caused a reduction in K concentration in plants. In terms of N concentration, 15 and 30 kg P ha^{-1} significantly increased N concentration, while 60 kg P ha^{-1} decreased it to the control level. Although phosphorus addition significantly affected Mg accumulation in plants over control, all doses were in the same statistical group. Micronutrients such as Cu, Fe, Mn and Zn varied between 10.75-11.54 mg kg^{-1}, 724-836 mg kg^{-1}, 43.9-48.5 mg kg^{-1} and 27.5-27.6 mg kg^{-1}, respectively (Figure 3).

Seed priming positively affected the uptake of all nutrients except K, which was the highest in the control plants. PGPB priming caused the highest N and P content, whereas it led to the lowest K, Ca and Cu content in plants. All priming treatments resulted in the lower K accumulation than in the control, and the lowest value of this element was observed in PGPB-primed plants. The highest Ca was observed in Si-primed plants, while the highest levels of Fe, Mg and Mn were determined in the CA-priming treatment plants. Seed priming with 4 mM silicon enhanced copper accumulation in tissues up to 9.4%. The lowest Zn concentration was determi-

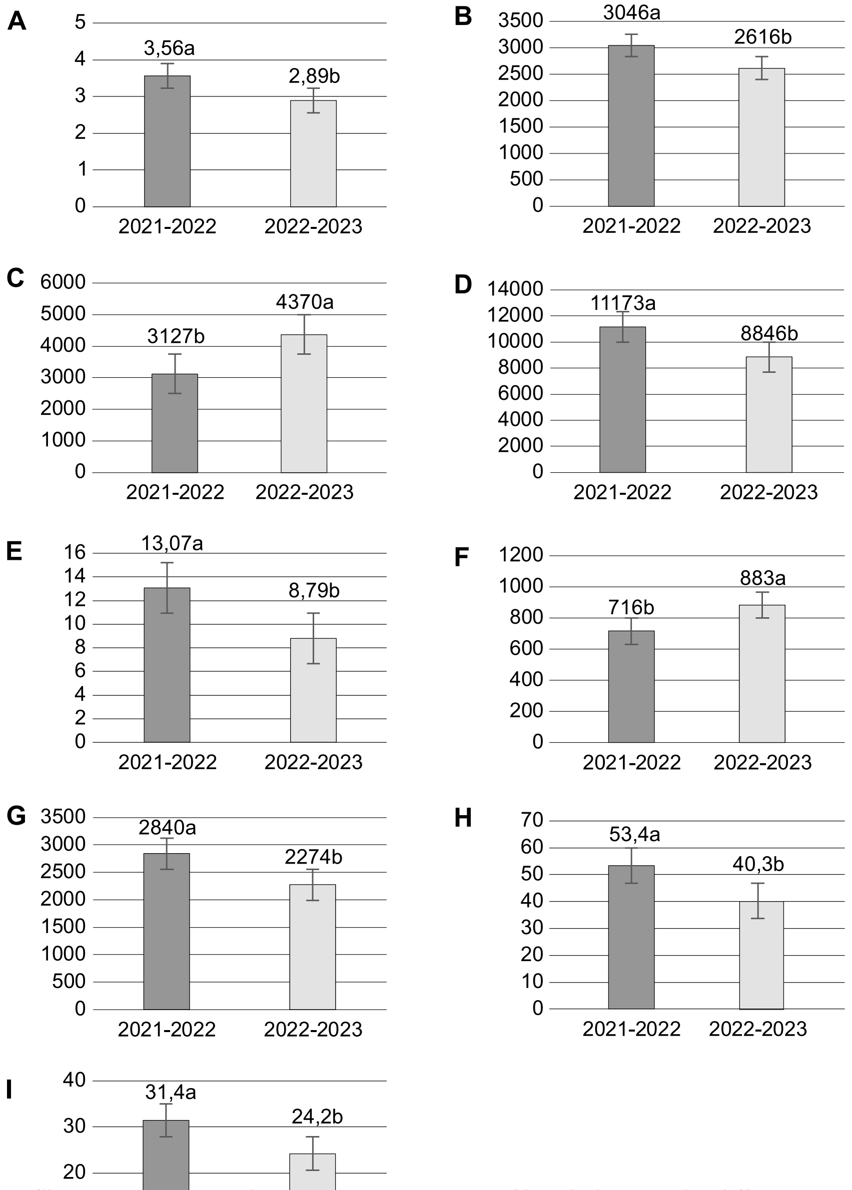


Fig. 2. Changes in micro- and macro nutrient content of lentil plants under different growing seasons: A – nitrogen (%), B – phosphorus (mg kg^{-1}), C – potassium (mg kg^{-1}), D – calcium (mg kg^{-1}), E – copper (mg kg^{-1}), F – iron (mg kg^{-1}), G – magnesium (mg kg^{-1}), H – manganese (mg kg^{-1}), I – zinc (mg kg^{-1})

ned in the CA-priming variant, whereas the highest one was observed in Pi-primed plants. Macronutrients such as N, P, K, Ca and Mg changed between 2.98-3.43%, 2681-2916 mg kg^{-1}, 3437-4028 mg kg^{-1}, 9637-10070 mg kg^{-1} and 2473-2627 mg kg^{-1}, while micronutrients, i.e., Cu, Fe, Mn and Zn,

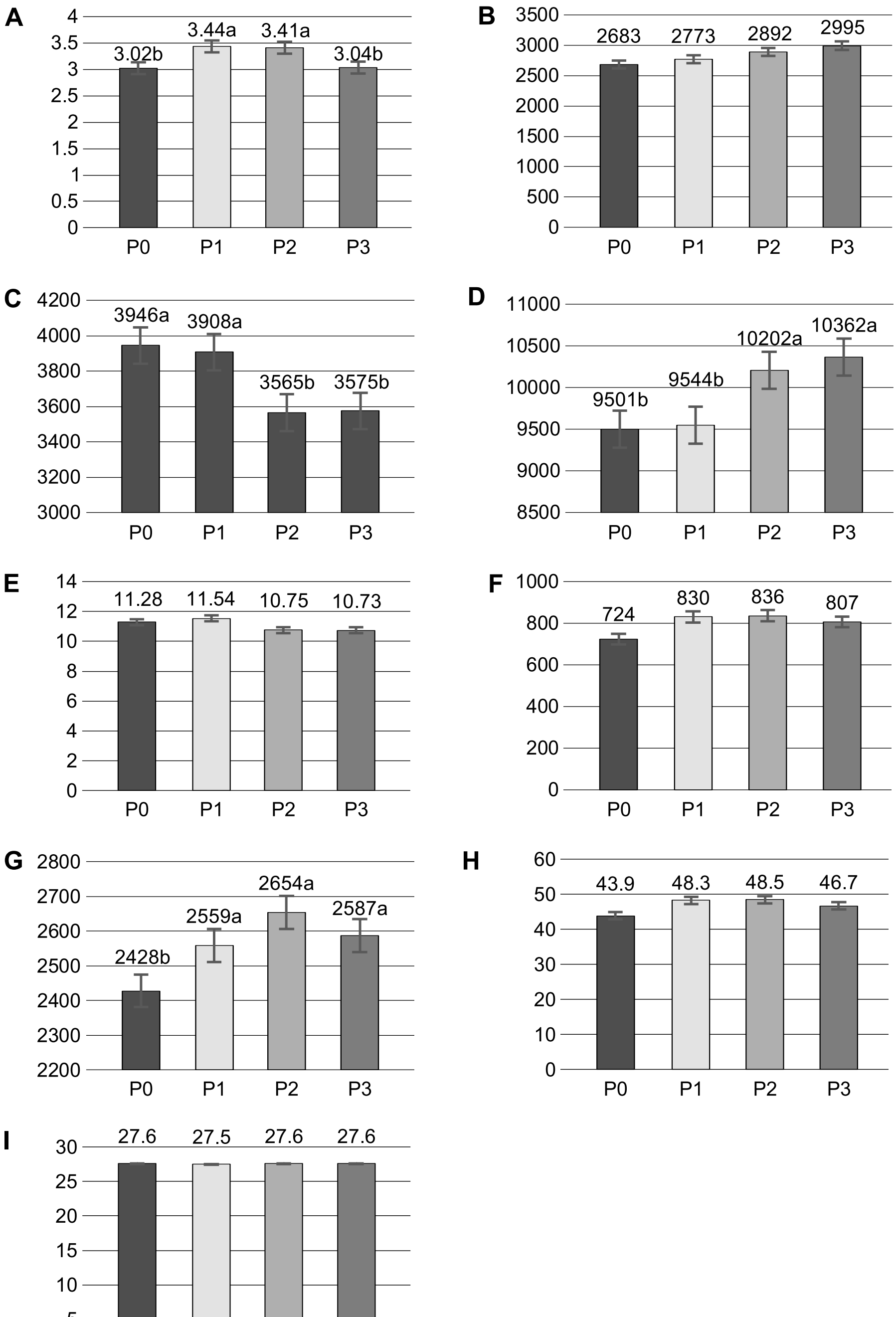


Fig. 3. Changes in micro- and macro nutrient content of lentil plants under different phosphorus fertilizers: A – nitrogen (%), B – phosphorus (mg kg^{-1}), C – potassium (mg kg^{-1}), D – calcium (mg kg^{-1}), E – copper (mg kg^{-1}), F – iron (mg kg^{-1}), G – magnesium (mg kg^{-1}), H – manganese (mg kg^{-1}), I – zinc (mg kg^{-1})

varied between 10.47-11.86 mg kg^{-1}, 747-900 mg kg^{-1}, 44.5-48.8 mg kg^{-1} and 25.7-30.0 mg kg^{-1}, respectively (Figure 4).

The lowest N concentration (2.09%) was determined in non-primed plants treated with phosphorus in 2021-2022, whereas the highest N (4.26%) was observed in non-primed plants treated with 30 kg P ha^{-1} phosphorus in 2021-2022. The highest phosphorus content (3629 mg kg^{-1}) was determined in SA-primed plants treated with 60 kg P ha^{-1} phosphorus in 2021-2022, whereas the lowest one (2368 mg kg^{-1}) was observed in CA-primed plants treated with 60 kg P ha^{-1} phosphorus in 2022-2023. According to results, the lowest K concentration (1701 mg kg^{-1}) was determined in PGPB-primed plants treated with 30 kg P ha^{-1} phosphorus in the first experimental season, while the highest one (5548 mg kg^{-1}) was observed in SA-primed plants with no phosphorus treatment in the second experimental season (Table S2).

While Pi-priming together with 60 kg P ha^{-1} phosphorus treatment caused the highest Ca (12 700 mg kg^{-1}) in 2021-2022, SA-priming without phosphorus fertilization led to the lowest Ca (7948 mg kg^{-1}) accumulation in 2022-2023. SA-priming in 2022-2023 and CA-priming in 2021-2022 together with 60 kg P ha^{-1} phosphorus treatment caused the highest (3216 mg kg^{-1}) and the lowest (2033 mg kg^{-1}) Mg content, respectively. Mn concentration was the highest (61.7 mg kg^{-1}) in SA-primed plants treated with 60 kg P ha^{-1} phosphorus in the first experimental season, whereas it fell the lowest (33.8 mg kg^{-1}) under the same treatment in the second season (Table S2).

The lowest Cu content (7.86 mg kg^{-1}) was determined with no treatment (priming or phosphorus) in 2022-2023, whereas the highest one (18.64 mg kg^{-1}) was observed in Si-primed and 60 kg P ha^{-1} phosphorus-treated plants in 2021-2022. Fe and Zn exhibited an antagonistic relationship in that Pi-primed and 15 kg P ha^{-1} phosphorus-treated plants in 2021-2022 revealed the lowest Fe (521 mg kg^{-1}) and the highest Zn (46.7 mg kg^{-1}) levels. On the other hand, CA-primed and 15 kg P ha^{-1} phosphorus-treated plants had the highest Fe (1546 mg kg^{-1}) while CA-primed and 60 kg P ha^{-1} phosphorus-treated plants in the 2022-2023 contained the lowest Zn (19.1 mg kg^{-1}) in the 2022-2023 season (Table S3).

DISCUSSION

First of all, unlike other nutrient elements, the N content in plants varies depending on BNF. It is so because legumes can fix atmospheric nitrogen (N_2) and convert it into ammonia in nodules located in the root zone through symbiotic nitrogen fixation in the presence of appropriate rhizobium bacteria in the soil (Baber et al. 2023). Therefore, environmental factors affecting BNF can directly influence the amount of N acquisition by plants. The total precipitation in the first and second experimental years until April

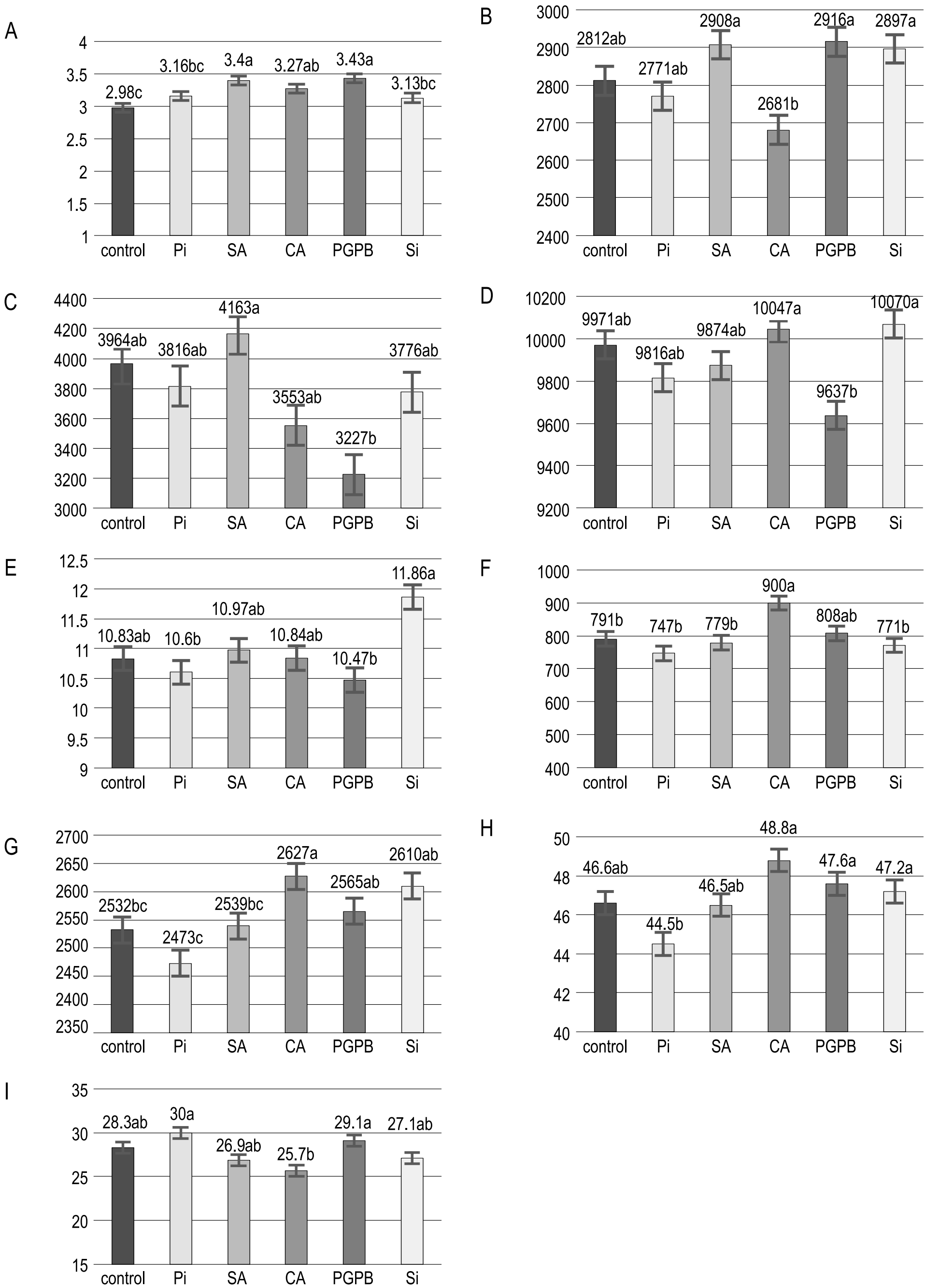


Fig. 4 Changes in micro- and macro nutrient content of lentil plants under different priming treatments: A – nitrogen (%), B – phosphorus (mg kg^{-1}), C – potassium (mg kg^{-1}), D – calcium (mg kg^{-1}), E – copper (mg kg^{-1}), F – iron (mg kg^{-1}), G – magnesium (mg kg^{-1}), H – manganese (mg kg^{-1}), I – zinc (mg kg^{-1})

(plant samples were taken during this period) was 408 and 220.8 mm, respectively (Figure 1). Higher soil moisture during the 2021-2022 season led to elevated photosynthetic activity, and more efficient transport of nutrients to the roots via the phloem, thus allowing for higher rates of BNF (Jumrani et al. 2023). Moreover, higher nitrogen fixation and photosynthetic activity facilitate increased root and shoot development and uptake of nutrients. Indeed, all nutrient elements except K and Fe were taken up more by plants in the 2021-2022 season. Higher uptake of K and Fe in the second year resulted from the competition during the uptake of other nutrient elements and antagonistic effects due to the presence of certain nutrient elements such as P, Ca, and Mg (Rodenas et al. 2019). Printz et al. (2016) reported that Cu and Fe uptake in plants occurs through the same mechanism, thus competition arises for their uptake. The higher Cu concentration detected in the first year led to the conclusion that it had an antagonistic effect on the Fe uptake. On the other hand, higher organic matter and phosphorus content were detected in the first year, while higher potassium content was determined in the second year, supporting the findings regarding nutrient uptake (Table 1).

Phosphorus fertilization significantly affected N, K, Ca and Mg uptake; however, it left the other nutrients unaffected. BNF requires high energy and involves a complex mechanism. Since phosphorus is one of the key elements in cellular energy mechanisms, the application of phosphorus has positive effects on BNF (Singh, Singh 2016). Singh et al. (2017) examined the effects of P and Mo applications on nutrient contents in lentil, and reported an increase in N content in the plant compartment with increasing doses of Jindal et al. (2008) found that increasing P doses up to a certain level increased nodule formation and nodule dry weight, but when they were higher than 80 kg P ha^{-1}, the number of nodules decreased. This explains the decrease in N content in plants at the P3 level. There was a negative correlation between K content in plants and increasing P doses. As mentioned earlier, an increase in P density in the environment negatively affects K uptake. However, the systems and mechanisms regarding how plants detect nutrients in the rhizosphere and accordingly determine how much of other nutrient elements needs to be taken up have not been fully elucidated yet (Wang et al. 2020). Additionally, parallel to increasing P doses, the amount of Ca taken up by plants has increased. Ca^{+2}, Mg^{+2}, and K^{+} ions are quite similar in size and charge, and, therefore, the cation exchange sites on roots cannot distinguish between ions. Thus, higher Ca uptake due to P fertilization in soils limits K uptake. The reason for the increase in Ca uptake by plants and its transport to aerial parts due to P fertilizer is attributed to the higher uptake of Ca from roots under high P conditions and possibly facilitating rapid transpiration due to increased shoot growth rate, thus being transported to the aerial parts through xylem flow (Kumar et al. 2015). Ding et al. (2018) reported an increase in Ca content taken up by plants due to P applications in bitter melon varieties. Phosphorus fertilization increased Mg uptake compared to the control group. Phosphorus facilitates Mg absorption, allowing for more Mg acquisition (Lu et al. 2022).

Seed priming was extremely effective in stimulating the macro- and micronutrient uptake and accumulation in plant tissues. Plants exhibiting more efficient root development have been observed to utilize water and nutrient contents in the soil more effectively. Additionally, the highest N content in plants obtained through PGPB-priming suggests that N acquisition through non-symbiotic pathways occurs besides symbiotic nitrogen fixation. PGPB strains used for bio-priming are also capable of facilitating the mineralization of phosphate-containing compounds bound in the soil. Therefore, the highest P uptake may be achieved through PGPB-priming. Furthermore, besides inducing indole acetic acid (IAA) and siderophore production, PGPB strains' ability to synthesize organic acids facilitates more efficient utilization of water and nutrient sources, thereby boosting the uptake of micronutrients (Cheng et al. 2022). The significant increase in uptake of Fe, Mn, and Zn due to PGPB-priming applications supports this phenomenon. Also, SA and Si-priming have also significantly increased nutrient uptake. SA and Si applications have been particularly effective in terms of N, P, Ca, and Cu uptake. Si application stimulates nutrient uptake through mechanisms such as organic acid synthesis in soils with low P concentrations, translocation of P to aerial parts, and binding to the surface of Fe/Al oxides (Etesami et al. 2021). Silica strengthens a cell's structure and enhances root development, thereby enabling plants to benefit more from a supply of water and nutrients (Misuthova et al. 2021). While some researchers have indicated that Si applications protect plants against Cu toxicity (Keller et al. 2015), others have reported an increase in Cu uptake with Si application (Patil et al. 2018). CA-priming application has also been found to significantly increase the uptake of Ca, Cu, Fe, Mg, and Mn in plants. Citric acid, one of the most important organic acid compounds secreted by plant roots, plays a key role in nutrient uptake (Zeng et al. 2021). It is believed that CA facilitates the breakdown of calcium phosphate ($Ca_3(PO_4)_2$) compounds in the rhizosphere, thereby allowing plants to uptake more P and Ca ions left available. Organic acids reduce the pH in the root zone, promoting increased uptake of nutrient elements such as Mg in low pH conditions (Vilkinson et al. 1990). Li et al. (2023) have stated that CA application increases Mn uptake in sunflowers.

CONCLUSIONS

It has been determined that N and P contents in lentil plants are higher following SA and PGPB priming compared to the control and use of other priming materials. Compared to SA and PGPB priming, Si-priming was more effective in increasing the P concentration in lentil plants. SA-priming significantly promoted the K concentration in plants, while potassium uptake is limited when more than 15 kg P ha^{-1} is added through P fertilization. With increasing doses of P fertilizer, the Ca content in lentil plants increased, and both CA and Si-priming had similar effects. Among cationic nutrient elements, CA-priming stimulated particularly the Fe, Mg, and Mn uptake, with positive results also observed after PGPB and Si-priming. In conclusion, phosphorus addition boosted macro- and micronutrient acquisition, although economically 15 kg P ha^{-1} is recommended. Besides, seed priming with PGPB (*Brevibacillus choshinensis* + *Paenibacillus xylanilyticus*), 4 mM SA and 100 mg kg^{-1} CA were more effective in terms of nutrient uptake and accumulation in lentil tissues. The main problems of seed priming are the infrequent use of the technique in practice, especially over large areas. In addition to further research, in order to promote the use of seed priming by farmers, it is necessary to provide trainings and mechanization of this technology.

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Author contributions

M.C. and M.E. – methodology, M.C., M.E. and F.Ç. – conceptualization, data curation, formal analysis, investigation, resources, M.E. and F.Ç. – supervision; M.C. – funding acquisition; project administration, M.C. and M.E. – visualization, writing – original draft preparation, writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability statement

Data are available on request from the authors. The raw data supporting this study's findings and sample information are available from the corresponding author upon reasonable request.

Supplementary material

The manuscript contains supplementary material available only online: https://jsite.uwm.edu.pl/issue/view/1-2025/

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