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Article

Comprehensive Metal-Based Nanopriming for Improving Seed Germination and Initial Growth of Field Pea (*Pisum sativum* L.)

Gordana Tamindžić [1,*], Sergei Azizbekian [2], Dragana Miljaković [1], Jan Turan [3], Zorica Nikolić [1], Maja Ignjatov [1], Dragana Milošević [1] and Sanja Vasiljević [1]

[1] Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, 21000 Novi Sad, Serbia; dragana.bjelic@ifvcns.ns.ac.rs (D.M.); zorica.nikolic@ifvcns.ns.ac.rs (Z.N.); maja.ignjatov@ifvcns.ns.ac.rs (M.I.); dragana.milosevic@ifvcns.ns.ac.rs (D.M.); sanja.vasiljevic@ifvcns.ns.ac.rs (S.V.)
[2] Institute of Physical Organic Chemistry, National Academy of Sciences of Belarus, 220072 Minsk, Belarus; s.az@mail.ru
[3] Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Serbia; jturan@polj.edu.rs
* Correspondence: gordana.tamindzic@ifvcns.ns.ac.rs; Tel.: +381-64-870-6115

Abstract: Nanopriming is a newly developed seed technology that improves seed germination, initial plant growth, and crop yield by enabling plants to withstand a variety of abiotic stresses. The objective of this study was to evaluate the effectiveness of comprehensive metal-based (Co, Mn, Cu, Fe, Zn, Mo, and Se) nanopriming as compared to hydro- and non-primed seeds of three different pea cultivars in a germination test. Seed priming with nanoparticles (NPs) improved field pea quality via significant increase in germination energy (cv. E-244), final germination (cv. E-244, cv. Dukat), shoot length (cv. E-244, cv. Partner), root length (cv. E-244, cv. Dukat, cv. Partner), fresh shoot weight (cv. Partner), dry shoot weight (cv. Partner), seedling vigor index (cv. E-244, cv. Partner), and chlorophyll content (cv. Dukat, cv. Partner), as compared to both hydropriming and the control. Moreover, nanopriming led to significant improvements in shoot length, fresh shoot length, dry shoot length, seedling vigor index (cv. Dukat), and dry root weight (cv. E-244) as compared to the control only. In general, the highest effect on the examined parameters was achieved by nanopriming, indicating that this treatment may be utilized to raise field pea quality performance. To optimize the method, it is necessary to conduct extensive laboratory and field trials.

Keywords: nanopriming; metal-based nanoparticles; hydropriming; seed quality performance; initial plant growth and development; *Pisum sativum* L.

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1. Introduction

Field pea (*Pisum sativum* L.) is a grain legume of great importance for food and feed and one of the most important legumes next to soybean, groundnut, and beans. This pulse crop is a rich source of protein (21–25%), with a wealth of amino acids such as tryptophan, lysine, arginine, aspartic acid, and glutamic acid, as well as carbohydrate, fibers, minerals, vitamins C, B, B3, and E, and significant quantities of beta-carotene, lutein, and zeaxanthin, confirming the outstanding nutritional value of this crop [1–3]. Pea has the lowest trypsin inhibitory activity of any legume, making it an essential food source for humans and livestock [3]. Moreover, field pea has proven to be an excellent crop species for the intercropping system due to its particularly favorable effect on soil structure and quality as well as nitrogen fixation [4]. In 2021, the global production of peas has reached over 7 million hectares with an average yield of 1.76 t ha^{-1} [5]. However, various biotic and abiotic factors have been identified to have an impact on pea productivity. Peas have proven to be sensitive to unfavorable conditions such as drought, salinity and heat stress in the initial stages of plant development, which leads to a significant yield loss.

Seed germination and initial plant growth are the most important stages in plant development. Because sowing on a large scale demands quick and uniform seed germination, fine-tuning seed germination at an adequate time is essential for crop yield. Exposure to various stresses decrease seed germination and early seedling growth traits, damage morpho-physiological parameters—which are commonly associated with biochemical, physiological, and molecular changes—and subsequently reduce crop yield [6,7]. Modern agriculture management is one weapon of choice for combating the harmful effects caused by environmental stresses. Precision agriculture technologies require that every single seed must be healthy and germinate quickly to ensure a high yield. These technologies in seed production have developed new study approaches to increase seed vigor, and recently, seed enhancement techniques have evolved. Among these techniques, seed priming stands out as the most important method of physiological seed improvement, which enables controlled hydration of seeds and induces pre-germination (phases I and II) but prevents radicle protrusion [8,9].

Recently, much attention has been paid to the development of nanomaterials for use in agriculture due to their multiple functions as a promising alternative to overcome stress and improve sustainable agriculture. Previous research has shown that seed priming improves seed germination and the initial growth of many crops under optimal conditions but also under stress [10–14]. Many priming techniques have been used in recent decades, such as hydropriming, halopriming, osmopriming, hormopriming, and biopriming. Moreover, seed priming with nanoparticles has attracted a lot of attention due to the attributes and physical characteristics of nanoparticles such as high surface area-to-volume ratios and high reactivity, which make them suitable for agriculture applications [12]. Nanoparticles (NPs) are being employed as priming agents to improve seed quality, such as greater seed germination, seedling growth, stress resistance or tolerance, and ultimately higher yields and food nutritional value [9,15–17]. In addition, NPs alter significant, beneficial changes in seed metabolism as well as physiological and biochemical changes, gene expression, antioxidant enzyme activity, and signaling pathways [9,15,18,19]. The beneficial effect of NPs is attributed to their small size ranging between 1–100 nm and unique physio-chemical properties, which make them suitable for seed priming [20]. Nanomaterials have a wide range of physical-chemical properties depending on their shape, size, surface, surface area/volume ratio, chemical behavior, particle charge, production method, and coating [8]. Moreover, their unique properties, such as a high surface area to mass ratio, allow them to improve catalysis and deliver materials of interest, as well as adsorb substances of interest. Several studies have been concerned with the application of metal-based, carbon-based, and polymeric nanoparticles as seed priming agents for the improvement of seed germination and plant growth under various conditions [18,21–25].

However, to our knowledge, there is a lack of information on the effect of comprehensive metal-based NPs seed priming on germination and initial growth of field pea. Therefore, the present study was carried out to examine the impact of comprehensive seed priming with metal-based NPs (Co, Mn, Cu, Fe, Zn, Mo, and Se) on seed germination and initial plant growth and development of three field pea cultivars under optimal conditions.

2. Materials and Methods

2.1. Priming Nanomaterial and Characterization of Nanoparticles

A commercial nanomaterial, Nanoplant Ultra (JSC "ECO—Vlit", Trakai, Lithuania), was used. The nanomaterial was originally developed at the National Academy of Sciences of Belarus and represents an innovative micronutrient product intended for crop nutrition and plant stimulation. The sample under study is a complex nanomaterial–aqueous colloidal solution of the following high-quality nanosize mineral elements: cobalt (Co)—0.036% (7 mM), manganese (Mn)—0.036% (7 mM), copper (Cu)—0.043% (8 mM), iron (Fe)—0.06% (10 mM), zinc (Zn)—0.025% (4 mM), molybdenum (Mo)—0.045% (5 mM), and selenium (Se)—0.045% (6 mM). The priming solution was prepared in accordance with the manufacturer's instructions by adding 0.7 mL of Nanoplant Ultra to 1 L of water. The pH of the nanomaterial's colloidal solution was 7 ± 1. The pH values of the solutions for hydropriming and nanopriming were the same and corresponded to the pH of the distilled water used. To stabilize the structure of the colloidal solution, nanoparticles of microelements are encapsulated by organic polymers (polyvinpyrrolidone K17 and dextran D20).

The concentration of elements in the nanomaterial solution was measured by inductively coupled plasma spectrometry (ICP, VISTA PRO (Varian, Palo Alto, CA, USA)) at the Institute of Physical Organic Chemistry, National Academy of Sciences of Belarus, Minsk. The traditional transmission electron microscopy method for measuring the size of nanoparticles implies a dry sample, while the water removal from volumetric nanoparticles leads to incorrect results that differ from the actual sizes of nanoparticles in the initial structure of an aqueous colloidal solution [26]. Therefore, dynamic scattering spectroscopy was used to measure the size of nanoparticles in the colloidal solution [27]. The measurements were carried out in two accredited laboratories: Center for Research and Testing of Materials of the Institute of Powder Metallurgy (Minsk, Belarus) and Fraunhofer Institute for Ceramic Technologies and Systems IKTS (Dresden, Germany). A laser analyzer based on the dynamic scattering method called "Zetasizer Nano ZSP" (Malvern, UK) was used to measure the granulometric composition of the colloidal solution in both laboratories. The standard technique for measuring colloids includes a procedure for checking the presence of associates and aggregates in the test sample. To do this, measurements are carried out at various degrees of sample dilution in deionized water (from 1/10 to 1/1000) to identify associates. The procedure also provides for each dilution's additional processing of the colloidal solution in an ultrasonic field to identify aggregates. The measurement results in two laboratories were identical and showed the presence of nanoparticles in the nanomaterial under study that are not destroyed by dilution and exposure to ultrasound. The results are prepared in the form of a table, which shows the presence of nanoparticles in a wide range of measured sizes (Table 1), and a histogram, which shows the size distribution of nanoparticles for visual aids (Figure 1).

The size of the nanoparticles is plotted along the logarithmic x-axis, while the percentage of presence is plotted along the y-axis.

Measurements revealed the presence of nanoparticles in the studied sample, with sizes ranging from 6 to 105 nm. The range of approximately 18–24 nm was where the maximum peak in the nanoparticle size distribution was found.

Studies on the toxicology and safety of the synthesized material were carried out at the Scientific and Practical Center for Hygiene, Minsk, Belarus. The nanoparticles of microelements are synthesized in a cover of biogenic polymers, which are gradually absorbed by enzymes in plant cells with a dosed release of atoms of elements, leading to prolonged action and the absence of inhibition of plant development. It has been established that the presented nanomaterial is low-toxic, non-eco- and phytotoxic, and does not cause long-term consequences such as mutagenicity, genotoxicity, and effect on reproductive function [28,29].

Table 1. The presence of nanoparticles of various sizes in the sample under study.

Size d (nm)	6.5	7.5	8.7	10.1	11.7	13.5	15.7	18.2	21.0	24.4
Mean Volume (%)	0.3	1.6	3.5	4.3	4.3	4.8	6.2	7.4	7.4	6.6
Size d (nm)	28.2	32.7	37.8	43.8	50.8	58.8	68.1	78.8	91.3	105.7
Mean Volume (%)	5.5	3.7	3.7	3.1	2.6	2.1	1.7	1.3	1.0	0.8

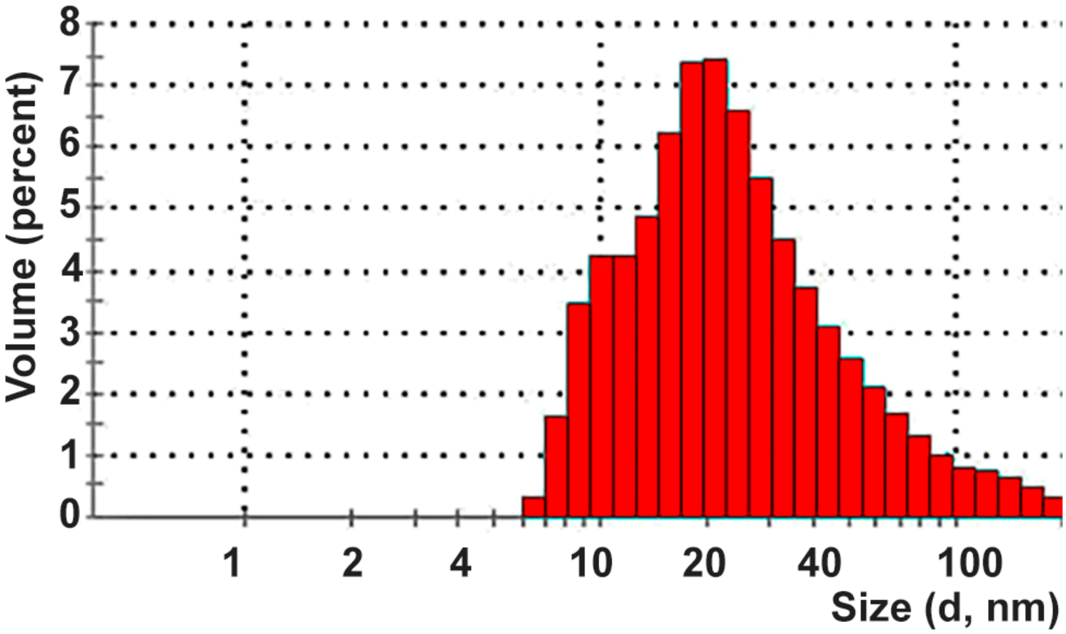

Figure 1. Histogram of nanoparticle size distribution in the sample under study.

2.2. Seed Materials

Three different field pea cultivars, namely E-244, Dukat, and Partner, were obtained from the Institute of Field and Vegetable Crops, National institute of the Republic of Serbia, Novi Sad, Serbia. E-244 is an experimental line of spring field pea used for grain production. It is mid-early line, characterized by an aphyllous type of leaf, a white flower, uniform ripening, lodging tolerance, and high genetic potential for grain yield. The genetic potential of this line reaches 6 t ha^{-1} of grain with approximately 27% of crude protein. The 1000-grain weight is approximately 200 g. Dukat is a mid-early cultivar of spring field pea, with a determinate growth (50–75 cm), an ordinary leaf type, a white flower, uniform ripening, and lodging tolerance. Grain yield ranges from 4 to 5 t ha^{-1} with 25–27% of crude proteins. The 1000-grain weight varies between 210 and 225 g. Partner is a cultivar of spring field pea used for grain production, with uniform ripening and lodging tolerance. It is characterized by a tall stem height of 50–70 cm, aphyllous leaf type and a white flower color. Grain yield ranges from 4.5–5.5 t ha^{-1} with 25–27% of crude protein. The 1000-grain weight varies between 200–220 g.

2.3. Priming Treatments

Seeds of field pea cultivars were primed with distilled water (hydropriming) and a solution of the selected, comprehensive nanomaterial (nanopriming), while non-primed seeds were taken as the control. The pea seeds were fully immersed in priming media for 10 h in the dark at room temperature [30]. Priming was carried out in the following ratio: 5 mL of solution per 1 g of seeds. Thereafter, pea seeds were rinsed thrice with distilled water and dried back near to the original weight.

2.4. Assessment of Seed Germination and Germination-Related Parameters of Field Pea Seeds

To assess the seed germination and germination-related parameters, the laboratory experiment was conducted at the Laboratory for Seed testing, Institute of Field and Vegetable Crops, Novi Sad, Serbia. The germination study was conducted in a germination chamber at 20 °C with three treatments: control (non-primed), hydroprimed, and nanoprimed seeds. Working samples consisted of 3 × 100 seeds. The pea seeds were placed in 240 × 150 mm plastic boxes. The moistened sterilized sand was used as substrate. Energy of germination was determined five days after seed sowing, while seed germination and percentage of abnormal seedlings were determined 8 days after seed sowing [31]. To obtain shoot and root length, 10 seeds were places in rolled filter paper. The length of shoot and root were determined on the fifth and eight days after seed placement. Fresh shoot and root weight were determined eight days after seed placement in filter paper using laboratory balance. To obtain dry shoot and root weight, seedlings were oven dried at 80 °C till constant mass.

Seedling vigor index (SVI) was determined using the formula of Abdul-Baki and Anderson [32]:

$$\text{SVI} = \text{Seedling length (cm)} \times \text{Seed germination (\%)}.$$

Shoot elongation rate (SER) and root elongation rate (RER) were determined using the formula of Channaoui et al. [33]:

$$\text{SER} = (\text{SLE} - \text{SLS})/(\text{TE} - \text{TS}),$$

where
SLS—shoot length measured five days after sowing,
SLE—shoot length measured eight days after sowing,
TE-TS—time duration between two measurements (days).

$$\text{RER} = (\text{RLE} - \text{RLS})/(\text{TE} - \text{TS}),$$

where
RLS—root length measured five days after sowing,
RLE—root length measured eight days after sowing,
TE-TS—time duration between two measurements (days).

2.5. Assessment of Physiological Response of Pea Seeds to Priming

To evaluate the physiological response of pea seeds to priming treatments, the chlorophyll content was determined according to the method of [34]. For determination of chlorophyll content, 0.1 g of the leaf sample was mixed with 10 mL of 80% ethanol in test tubes, stirred at vortex for 5–10 s, and then heated in a water bath at 100 °C for 3–5 min. Reading of the obtained extract solution was performed at 666 nm using a spectrophotometer (Thermo Scientific, Genesys 10S UV-VIS Spectrophotometer, Waltham, MA, USA). The chlorophyll content (Chl) was determined using the following formula:

$$\text{Chl (mg/g of FW)} = (\text{Abs} - 0.01) \times 1/92.6474 \times 10/\text{FW (g)}$$

where,
FW—fresh weight (g)
Abs—absorbance at 666 nm.

2.6. Statistical Analysis

Data were analyzed statistically using the software STATISTICA 10.0 software package (StatSoft Inc., Tulsa, OK, USA). The significance between treatments was tested using Duncan's multiple range test at 0.05 probability level. The results were expressed as means ± standard error of the mean (n = 3). Principal component analysis (PCA) was employed to ascertain the impact of seed priming treatments on the examined parameters for field pea cultivars. For data visualization, Microsoft Excel (https://www.microsoft.com/en-us/microsoft-365/excel) was used.

3. Results and Discussion

The germination test was used in the current study primarily to characterize seed quality in relation to seed priming treatment under optimal conditions and to predict the field emergence of primed pea seeds. The purpose of this test was to assess the percentage of viable seeds that germinate and produce normal seedlings with well-developed essential structures, as well as to identify abnormal seedlings. The results presented in Table 2 clearly show that the examined parameters of different field pea cultivars were significantly altered by priming treatments, excluding abnormal seedlings and dry root weight. The significant effect of the cultivar on all examined parameters was observed, while cultivar × treatment interaction significantly influenced all parameters, excluding abnormal seedlings and fresh and dry root weight.

Table 2. Analysis of variance for the examined parameters of three pea cultivars after hydropriming and nanopriming under optimal laboratory conditions.

Traits	Factors		
	Cultivar (C)	**Treatment (T)**	**C × T**
Germination Energy	***	***	***
Seed Germination	***	***	***
Abnormal Seedlings	***	ns	ns
Shoot Length	***	***	***
Root Length	***	***	***
Fresh Shoot Weight	***	***	***
Fresh Root Weight	**	*	ns
Dry Shoot Weight	**	***	**
Dry Root Weight	***	ns	ns
Root/Shoot Ratio	***	***	***
Shoot Elongation Rate	***	***	***
Root Elongation Rate	***	***	***
Seedling Vigour Index	***	***	***
Chlorophyll Content	***	***	***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns—not significant.

Generally, priming treatments led to an increase in the examined parameters of all pea cultivars (Tables 3–5). The results indicated that the tested nanopriming treatment did not significantly affect the germination energy of Dukat and Partner pea cultivars in comparison to the control, while hydropriming led to a decrease in the germination energy of cv. Dukat (Table 3). However, the energy of germination in cv. E-244 was significantly improved by nanopriming, followed by hydropriming. Furthermore, significantly increased seed germination was observed by nanopriming in cv. E-244 and nanopriming and hydropriming in cv. Dukat compared to the control seeds, while no significant differences were observed for pea cv. Partner. In this regard, nanopriming led to an increase of seed germination between 3.19% (cv. Dukat) and 4.51% (cv. E-244) in comparison to the control. Recently published studies also revealed the beneficial effect of seed priming metal-based NPs such as zinc oxide (ZnO) NPs, copper oxide (CuO) NPs, silver (Ag) NPs, and cerium oxide (CeO_2) NPs on seed germination of different crops [8,35–38]. Positive effects of priming with metal-based nanoparticles such as TiO_2, ZnO, and FeO NPs on seed germination under various conditions were observed in wheat [39,40], rapeseed [41], and rice [42]. Nanoparticles of metals such as Zn, Ag, Cu, and Fe have been proven to be more effective in improving seed germination in many plant species than metal salts, without adverse effects on the appearance of abnormal seedlings [22,36]. Moreover, Chau et al. [43] also reported the beneficial effects of Co and Mo NPs on the seed germination of soybean. These benefits of seed nanopriming have been attributed to nanoparticle exposure-induced changes in seed metabolism as well as several physiological, biochemical, and signaling pathways in the seed [15].

Table 3. Germination and initial growth of non-primed, hydroprimed, and nanoprimed seeds of field pea cultivars.

Pea Cultivar	Treatment	Germination Energy (%)	Seed Germination (%)	Abnormal Seedlings (%)	Shoot Length (mm)	Root Length (mm)
E-244	Control	73.00 ± 0.58 c	91.67 ± 0.33 b	2.00 ± 0.58 a	43.63 ± 0.48 b	85.93 ± 0.55 c
	HP	82.33 ± 0.33 b	91.00 ± 0.58 b	5.00 ± 1.53 a	50.50 ± 0.25 a	113.77 ± 0.50 b
	NP	85.67 ± 0.33 a	96.00 ± 0.58 a	3.00 ± 1.15 a	50.67 ± 0.44 a	123.77 ± 0.69 a
	p value	0.00000	0.00087	0.25193	0.00003	0.00000
Dukat	Control	53.67 ± 0.33 a	73.33 ± 0.33 c	7.33 ± 0.33 a	51.83 ± 0.17 c	98.33 ± 0.44 b
	HP	47.33 ± 0.33 b	78.33 ± 0.88 a	7.67 ± 0.33 a	78.00 ± 1.53 a	102.67 ± 0.44 b
	NP	53.67 ± 0.33 a	75.67 ± 0.33 b	7.00 ± 1.15 a	71.00 ± 2.36 b	104.00 ± 1.61 a
	p value	0.00001	0.002614	0.81304	0.00007	0.01619
Partner	Control	82.33 ± 0.67 a	93.33 ± 0.33 a	1.33 ± 0.67 b	54.50 ± 0.29 c	106.83 ± 1.17 c
	HP	82.00 ± 0.58 a	94.33 ± 0.88 a	2.33 ± 0.33 ab	67.17 ± 0.93 b	118.33 ± 0.67 b
	NP	82.67 ± 0.67 a	94.00 ± 0.58 a	3.67 ± 0.33 a	82.50 ± 0.58 a	143.67 ± 1.09 a
	p value	0.77026	0.56152	0.03505	0.00000	0.000001

Data are presented as mean ± SE (*n* = 3). Means ± SE denoted by the different lowercase letters are significantly different at $p \leq 0.05$. HP: hydropriming; NP: nanopriming.

Table 4. Accumulation of fresh and dry shoot and root biomass of non-primed, hydroprimed, and nanoprimed seeds of field pea cultivars.

Pea Cultivar	Treatment	Fresh Shoot Weight (g)	Fresh Root Weight (g)	Dry Shoot Weight (g)	Dry Root Weight (g)
E-244	Control	1.93 ± 0.02 a	2.10 ± 0.14 a	0.178 ± 0.002 a	0.178 ± 0.008 b
	HP	2.04 ± 0.06 a	2.23 ± 0.04 a	0.185 ± 0.005 a	0.187 ± 0.003 ab
	NP	1.91 ± 0.08 a	2.37 ± 0.05 a	0.177 ± 0.005 a	0.204 ± 0.007 a
	p value	0.38019	0.16797	0.57373	0.07827
Dukat	Control	2.05 ± 0.02 b	1.63 ± 0.08 a	0.154 ± 0.007 b	0.131 ± 0.008 a
	HP	2.70 ± 0.04 a	1.71 ± 0.11 a	0.199 ± 0.016 a	0.151 ± 0.009 a
	NP	2.74 ± 0.07 a	1.83 ± 0.06 a	0.214 ± 0.006 a	0.148 ± 0.007 a
	p value	0.0001	0.31085	0.018315	0.22694
Partner	Control	2.22 ± 0.12 b	2.40 ± 0.20 a	0.191 ± 0.013 b	0.198 ± 0.018 a
	HP	2.43 ± 0.16 b	2.20 ± 0.03 a	0.191 ± 0.011 b	0.180 ± 0.002 a
	NP	3.19 ± 0.05 a	2.60 ± 0.06 a	0.248 ± 0.008 a	0.184 ± 0.007 a
	p value	0.00283	0.15800	0.01484	0.53089

Data are presented as mean ± SE (*n* = 3). Means ± SE denoted by the different lowercase letters are significantly different at $p \leq 0.05$. HP: hydropriming; NP: nanopriming.

Moreover, in cultivars E-244 and Dukat, no significant differences were observed regarding the appearance of abnormal seedlings, while in cv. Partner, an increased percentage of abnormal seedlings was observed when priming with the nanoparticle solution (3.67%) compared to the control. It can be assumed that it is the result of phytotoxicity, considering the primary roots were stunted, retarded, and/or deeply broken. As stated by previous research [22,36], nanoparticles are absorbed on the surface of the seed and are gradually released over a germination period, which can lead to stress in the germination process. Moreover, the accumulation of metals such as Zn from the treatment of nanoparticles is much higher compared to the value from an aqueous solution of the same concentration, which can cause phytotoxicity to occur, and it depends on the plant species, as well as the cultivar, together with nanoparticle size [35].

Table 5. Shoot and root elongation rate, seedling vigor index, and chlorophyll content of non-primed, hydroprimed, and nanoprimed seeds of field pea cultivars.

Pea Cultivar	Treatment	Root/Shoot Ratio	Shoot Elongation Rate	Root Elongation Rate	Seedling Vigour Index	Chlorophyll Content (mg/g of FW)
E-244	Control	1.97 ± 0.03 c	6.31 ± 0.37 a	8.13 ± 0.37 c	1187.7 ± 4.8 c	1.61 ± 0.011 a
	HP	2.25 ± 0.00 b	6.49 ± 0.23 a	10.63 ± 0.34 b	1494.9 ± 13.0 b	1.65 ± 0.021 a
	NP	2.44 ± 0.03 a	7.32 ± 0.41 a	14.31 ± 0.25 a	1674.6 ± 12.6 a	1.65 ± 0.033 a
	p value	0.0001	0.1246	0.0000	0.0000	0.4212
Dukat	Control	1.90 ± 0.00 a	10.18 ± 0.33 c	13.89 ± 0.11 b	1101.2 ± 1.3 c	1.93 ± 0.018 c
	HP	1.32 ± 0.03 c	17.68 ± 0.42 a	16.37 ± 0.37 a	1415.3 ± 21.0 a	2.05 ± 0.029 b
	NP	1.47 ± 0.06 b	15.47 ± 0.71 b	11.70 ± 0.84 c	1324.3 ± 21.9 b	2.14 ± 0.033 a
	p value	0.0001	0.0001	0.0025	0.0000	0.0027
Partner	Control	1.96 ± 0.02 a	10.79 ± 0.09 c	8.78 ± 0.32 b	1505.7 ± 7.7 c	1.31 ± 0.027 c
	HP	1.76 ± 0.03 b	12.91 ± 0.18 b	7.50 ± 0.29 c	1749.9 ± 14.7 b	1.44 ± 0.022 b
	NP	1.74 ± 0.01 b	16.40 ± 0.03 a	11.94 ± 0.24 a	2126.2 ± 28.2 a	1.63 ± 0.022 a
	p value	0.0009	0.0000	0.0001	0.0000	0.0002

Data are presented as mean ± SE (n = 3). Means ± SE denoted by the different lowercase letters are significantly different at $p \leq 0.05$. HP: hydropriming; NP: nanopriming; FW: fresh weight.

Pea cultivars differed in their shoot length in control (Table 3). The results indicated that both priming treatments significantly improved the shoot length of the tested pea cultivars compared to the control. Nanopriming markedly improved shoot length up to 51.38% (cv. Partner), while hydropriming increased shoot length up to 50.49% (cv. Dukat) compared to the control. Regarding early seedling growth, the root length of seedlings varied among pea cultivars in the control and ranged between 85.93 mm (cv. E-244) and 106.83 mm (cv. Partner). The obtained results indicated that root length generally increased due to both priming treatments compared to the control. However, the highest root length was discovered in the nanopriming treatment, which was significantly improved prior to the control and hydropriming treatments. Priming with nanoparticles led to an increase of root length up to 44.04% (cv. E-244) as compared to the control seeds, while hydropriming increased root length up to 32.40% (cv. E-244). As stated by [15], nanopriming has also been proven to have an effect on plant growth, stability, and physiology, in addition to modulating seed germination. Similarly, previous studies reported augmentation in plant growth of maize primed with TiO_2 NPs [44], rapeseed primed with ZnO NPs [41], fenugreek plants primed with Ag NPs [45], wheat, pea, and mustard primed with SiO_2 NPs [46], and chickpea primed with Fe_2O_3 NPs [47]. Nanoparticles, as the main actors in plant morphology, growth, and physiology, affect physiological characteristics through changes in the formation of reactive oxygen species (ROS), peroxidase, superoxide dismutase (SOD), catalase (CAT), and enzymatic activities and modify leaf protein, chlorophyll, and total phenolic content (TPC) [48–50].

Regarding biomass accumulation, fresh and dry shoot and root weights of the tested pea cultivars differed from the control (Table 4). According to the obtained results, fresh and dry shoot weight of cv. Dukat were considerably higher in both tested treatments compared to the non-primed treatment, while the same parameters of cv. Partner were significantly improved only in the priming treatment with comprehensive nanoparticles. However, no statistical difference regarding the fresh root weight in all treatments was observed. Dry root weight of cv. E-244 seedlings was significantly higher when primed with NPs solution as compared to non-primed seedlings, while for other pea cultivars, no difference was observed. Previous studies have reported similar results to our findings on the seedling biomass accumulation of SeO NPs-treated wheat seeds [51]. Likewise, beneficial effects of ZnO NPs, CuO NPs, and FeO NPs on plant biomass accumulation was also reported by [52–54], respectively. Moreover, Chau et al. [43] also reported a significant increase in dry biomass of soybean due to seed priming with Co and MoO_3 NPs. Our findings are consistent with the findings of [55], who regarded metal NPs as an agent that promotes microelements to infiltrate plant cells and participate in enzymatic activities, hence increasing the rate of plant growth and development.

In addition, the root/shoot ratio was also assessed, and the results revealed that no clear pattern was observed with respect to this parameter (Table 5). The root/shoot ratio varied among pea cultivars as well as among treatments within the same pea cultivar. The highest value of root/shoot ratio in cv. E-244 was observed in nanopriming (2.44), while for cv. Dukat, the highest root/shoot ratio was observed in the control (1.90). In cv. Partner, both priming treatments led to a significant decrease of root/shoot ratio in comparison to the control. Also, shoot elongation rate (SER) varied among pea cultivars and ranged between 6.31 (cv. E-244) and 10.79 (cv. Partner) in the control (Table 5). The results indicated that all pea cultivars responded differently to priming treatments. For cv. E-244, no statistical difference was observed, while for two other cultivars, priming treatment significantly increased SER compared to the control. However, in cv. Dukat, the highest value of SER was observed in hydropriming, while nanopriming had the best effect in cv. Partner. Moreover, root elongation rate (RER) in the control treatment also differed between pea cultivars (Table 5). Nanopriming led to a significant increase of RER in cv. E-244 and cv. Partner compared to the control treatment and hydropriming. Contrary to this, it was observed that nanopriming significantly reduced RER in cv. Dukat compared to other treatments. In regard to these results, numerous studies report a beneficial effect of seed priming on the seedling growth, especially under stressful conditions. For instance, SiO_2 NPs were noticed to improve photosynthetic parameters, maintain biochemical balance, and amplify biomass production in wheat seedlings under drought [37]; ZnO NPs caused an increase in the shoot height and root-shoot biomass in wheat seedlings facing salinity stress [56]; and application of TiO_2 NPs induced an increment in the root-shoot length of seedlings and their fresh and dry biomass of maize in both optimal and salinity stress conditions [44]. Priming with ZnO NPs has been reported to have numerous advantages over other nanoparticles such as Fe_2O_3 NPs, CuO NPs, Ag NPs, CeO NPs, etc. in terms of plant growth, especially root growth, considering the fact that it is an important transitional metal and is an essential micronutrient that plays a vital role in the growth and yield of plants by maintaining cell membrane integrity, cell elongation, and protein synthesis [35,57]. However, the results of the RER reduction in cv. Dukat can be justified by the fact that this genotype is more sensitive to the metal-based nanoparticles that were tested, and the concentration of nanoparticles might affect the agronomic effectiveness of nanoparticles. In this regard, Li et al. [58] reported that Fe_2O_3 NPs at concentration of 20 mg L^{-1} significantly promoted root elongation by 11.5%, while concentrations of 50 and 100 mg L^{-1} remarkably decreased root length. Moreover, it has been reported that zero-valent Zn and Fe NPs at higher concentration could lead to reduced water flow and limit root hydraulic conductivity, thereby inhibiting the root elongation of maize and mung bean, respectively [59,60].

In addition, the seedling vigor index (SVI) was markedly improved by nanopriming, followed by hydropriming in cv. E-244 and cv. Partner. In pea cv. Dukat, both priming treatments significantly improved SVI compared to the control, but in nanopriming to a lesser extent than hydropriming. Janmohammadi and Sabaghnia [61] also reported beneficial effects of nanopriming with Si on SVI in sunflower. Moreover, Raja et al. [62] revealed a significant increase in the SVI of black gram seeds primed with ZnO NPs and Cu NPs, which is in agreement with our findings. Prasad et al. [63] also found that ZnO NPs seed priming had a beneficial effect on the SVI of peanut seeds. Additionally, Dehkourdi and Mosavi [64] and Zheng et al. [65] have demonstrated that the SVI of parsley and spinach seeds were positively influenced by priming with TiO_2 NPs.

The effect of priming treatments on chlorophyll content of the studied pea cultivars is shown in Table 4. In cv. Dukat and cv. Partner, the highest values of chlorophyll content were observed in nanopriming followed by hydropriming. The relative increase in chlorophyll content due to nanopriming was 10.88% in cv. Dukat and 24.43% in cv. Partner.

Contrary to this, no significant difference in priming treatments in terms of this parameter was observed in cv. E-244. In this regard, it has been shown that priming treatment with ZnO NPs [56,66], MnO NPs [67], and Fe_2O_3 NPs [68] positively affect the increase of chlorophyll content and photosynthetic pigments in plants. Besides, it is suggested that Mn, as an essential metal for plant growth, has an important role in the organization of the thylakoid membrane and photosynthetic electron transport [69]. However, Kasote et al. [67] showed that MnO NPs had no considerable effect on the chlorophyll content of watermelon seedlings. In addition, Faraz et al. [53] indicated that *Brassica juncea* had a significantly higher chlorophyll content when the seeds were primed with CuO NPs. No available research data was found on the effect of seed priming with CoO_3 NPs and Mo NPs on chlorophyll content. However, available data shows that the activity of photosystem I, and thus the Hill reaction, is inhibited by Co ions in peas, but the role of cobalt in photosystem I has not yet been elucidated [70–72]. Cobalt has also been found to harm the chloroplast membrane [73]. Regarding Mo NPs, the application of Mo NPs by root irrigation has been shown to have a beneficial effect on the chlorophyll content of tobacco [74].

Correlation analysis verified the favorable effects of seed priming treatments in optimal conditions (Table 6). Overall, a positive interrelationship was established between seed germination and other parameters, with an exception of abnormal seedlings, shoot and root elongation rate, and chlorophyll content, while the interrelationship between seed germination and shoot growth and biomass accumulation was not significant. Moreover, research revealed a strong correlation between fresh root weight, dry shoot and root weight, and root and shoot length. A positive interrelationship was also established between chlorophyll content and abnormal seedlings, and fresh and dry root weight, while for germination parameters, a negative relationship was established. In addition, the seed vigor index was significantly positively correlated with all examined parameters, except abnormal seedlings and shoot and root elongation rate. Therefore, seed nanopriming treatments could result in enhanced seed vigor and quality, early plant growth, and subsequent grain production at later developmental stages.

Table 6. Pearson correlation of germination, initial plant growth and chlorophyll content of a pea.

Variable	GE	FG	AS	SL	RL	FSW	FRW	DSW	DRW	SER	RER	SVI	CHL
GE	1.00	0.95	−0.76	−0.28	0.55	−0.20	0.85	0.11	0.82	−0.47	−0.54	0.62	−0.87
FG		1.00	−0.81	−0.20	0.49	−0.15	0.82	0.17	0.83	−0.39	−0.51	0.65	−0.85
AS			1.00	0.30	−0.17	0.21	−0.64	−0.03	−0.64	0.38	0.67	−0.36	0.82
SL				1.00	0.51	0.92	0.01	0.72	−0.22	0.95	0.29	0.55	0.33
RL					1.00	0.50	0.64	0.55	0.40	0.29	0.12	0.94	−0.27
FSW						1.00	0.14	0.81	−0.16	0.86	0.17	0.54	0.26
FRW							1.00	0.36	0.84	−0.18	−0.38	0.71	−0.71
DSW								1.00	0.20	0.60	−0.03	0.63	0.04
DRW									1.00	−0.37	−0.37	0.50	−0.72
SER										1.00	0.32	0.33	0.42
RER											1.00	−0.07	0.68
SVI												1.00	−0.42
CHL													1.00

Moreover, the biplot of the principal component analysis (PCA) (Figure 2) illustrated the relationship between priming treatments and field pea cultivars. Priming treatments were clearly separated from the control, except nanopriming in cv. E-244, due to its lower effects on the examined pea parameters. Besides, PCA showed that pea cultivars were separated from each other within the same group. Thus, the results indicated that the comprehensive nanopriming treatment had the greatest effect on cv. Partner, followed by hydropriming treatment, while hydropriming and nanopriming treatment effects were more alike/uniform in cv. E-244 and cv. Dukat. The obtained results clearly emphasized the role of seed nanopriming treatment in improving seed germination and enhancing the initial growth of field pea plants. However, the different response of pea cultivars to nanopriming treatment implies the possibility of improving seed germination and pea seedling growth by adjusting the appropriate nanopriming seed treatment, which includes the concentration of the priming solution and the duration of priming treatment, to the requirements of each cultivar in the laboratory and in the field.

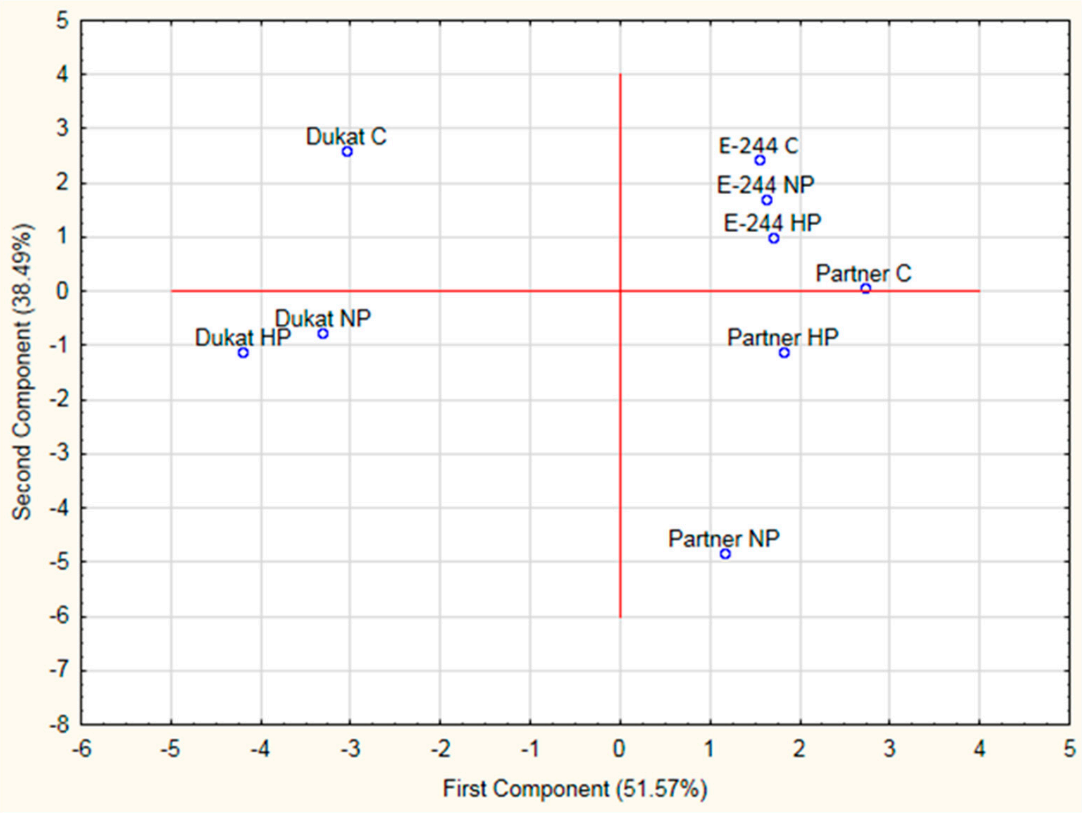

Figure 2. Principal component analysis (PCA) for the effect of hydropriming and nanopriming treatments on the examined parameters of field pea cultivars.

4. Conclusions

In the present study, comprehensive metal-based nanopriming showed great potential for improving seed germination, initial plant growth, and development. Two of the three examined pea cultivars enhanced seed germination without a significant appearance of abnormal seedlings and improved initial growth due to seed priming with comprehensive metal-based nanopriming. The beneficial effect of nanopriming on biomass accumulation as well as chlorophyll content of pea cultivars was also observed. Overall, results confirmed the valuable application of NPs through seed priming for the improvement of seed germination and the initial growth of pea cultivars. This priming technique could be recommended as an eco-friendly strategy to enhance seed quality and performance. By conducting laboratory experiments as well as extensive field trials, future studies should evaluate the effect of comprehensive metal-based nanopriming on seed quality performance under different environmental conditions.

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