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Nanopriming of Tomato (*Solanum lycopersicum*) Seeds Against Heavy Metal Stress During Germination and Seedling Formation

Anca Awal Sembada[1,2*], and *I. Wuled* Lenggoro[2,3]

[1]School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung 40132, Indonesia
[2]Chemical Engineering Program, Graduate School of Engineering, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan
[3]Department of Applied Physics and Chemical Engineering, Graduate School of Engineering, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan

Abstract. Abiotic stress can have a negative impact on plant growth. Heavy metal is one of the examples. One approach to overcome this issue is to use seed priming. The priming used in this study was nanopriming. We used colloidal silica nanoparticles (size of 10 nm) as the priming agent and copper (Cu) and barium (Ba) as the model heavy metals. This treatment was implemented for tomato (*S. lycopersicum* var. Momotaro) seed germination. The results showed that the presence of heavy metals during germination may lead to prolonging the germination time. The presence of Cu and Ba at 1 ppm could increase germination time by 28.38% and 26.9%, respectively, compared to control. When primed seeds were subjected to heavy metal stress, the use of silica nanopriming could reduce the germination time by 10.45% for Cu and 11.54% for Ba compared to the unprimed seeds. This evidence demonstrated that nanopriming could make seeds more resilient to heavy metal stress. We also found that heavy metal ions became less detectable in the seedlings when nanopriming was applied. This ion transport alteration essentially allowed seeds to cope with heavy metal stress. This method can be potentially used on various kinds of crops and heavy metals.

1 Introduction

Throughout their lives, plants are continually exposed to various abiotic stress factors, and they must adapt and respond to these challenges to survive and thrive. However, prolonged or severe abiotic stress can still lead to reduced crop yields, economic losses, and ecosystem disturbances [1]. Abiotic stressors can vary depending on the plant's environment and location. Heavy metals are indeed a significant example of abiotic stressors that can harm plants. Heavy metals are naturally occurring elements that can become pollutants when present in high concentrations in the environment [2]. The presence of heavy metals can inhibit plant growth right from the germination stage. A study from Banadka & Nagella

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[*] Corresponding author: anca@st.go.tuat.ac.jp

showed that heavy metals could inhibit the germination of *Withania somnifera* seeds and alter vegetative and biochemical parameters in the observed seedlings [3]. Phytotoxic effects (inhibiting germination and seedling growth) of heavy metals were also observed during *Pisum sativum* germination [4]. High concentrations of certain heavy metals can prevent seeds from absorbing water and breaking dormancy, leading to delayed or inhibited germination. Even if germination does occur, heavy metals can interfere with the development of the radicle (the embryonic root) and plumule (the embryonic shoot) [5]. This can result in abnormal seedling growth and poor establishment.

Seed priming is indeed a valuable technique that has been widely used to overcome various stress conditions at the germination stage. Seed priming involves pre-treating seeds under controlled conditions to enhance their germination and early seedling growth. One method of seed priming that can be applied is the use of nanoparticles as a priming material or known as nanopriming [6]. Seed priming with nano-ZnO (size of 30 ± 10 nm) improved the seedling growth of *Oryza sativa* and modulated the related physio-biochemical responses under Cd stress [6]. Another study revealed that seed priming with Si-NPs improved the germination and biomass yield of *Zea mays* under Cd stress [7]. Most of the studies conducted did not use copper as the heavy metal model, even though Cu is the most toxic metal for seed germination of tested crops [8]. In this present study, we used Cu and Ba as model heavy metals and we used Si-NPs as nanopriming agents. We also performed elemental analysis of the seedlings which was used as a basis for studying element transport during germination under stress conditions. It provides valuable insights into the movement and distribution of essential nutrients and other elements within plant tissues. Elemental analysis could also be employed to investigate how plants respond to various environmental stress factors [9].

2 Materials and methods

2.1 Preparation and characterization of nanoparticles

The Si-NPs used in this study is a product from Nissan Chemical Corporation, Japan with the commercial name Snowtex-S. The concentration of the Si-NPs suspension used was 1000 mg/L according to our previous report [10]. The pH of the suspension was also adjusted to 9.5 – 10.5. The Si-NPs suspension was characterized based on the zeta potential value and particle size distribution. Measurements were performed using a dynamic light scattering zeta-sizer instrument (DLS Zetasizer Nano-ZS, Malvern Panalytical Ltd., UK). Zeta potential measurements used a disposable folded capillary cell (DTS1070, Malvern Panalytical Ltd., UK) while particle size distribution measurements used a 12 mm square polystyrene cuvette (DTS0012, Malvern Panalytical Ltd., UK) [10].

2.2 Seed nanopriming

The prepared 1000 mg/L Si-NPs suspension would be used for seed nanopriming. Tomato (*Solanum lycopersicum* var. Momotaro, Takii & Co., Ltd., Japan.) seeds were chosen as model seeds in this study. The viability of seeds was tested by float test in distilled water [11]. Viable seeds were denser and tended to sink to the bottom. Seeds that floated on the surface of the water were often hollow or damaged and were unlikely to germinate. Only viable seeds were selected and then immersed in 25 mL of the Si-NPs suspension. Immersion was performed in the dark condition with 100 rpm shaker stirring (DLAB SK-L180-E, QTE Technologies Co., Ltd., Vietnam) for 24 h. Seeds were subsequently separated from the suspension and dried in an oven at 25 °C for 24 h prior to germination [12].

2.3 Seed germination and assessment of germination parameters

Seed germination took place for 7 days in dark settings which was conducted by placing the seeds on cotton puffs that had been moistened in a Petri dish [10]. Cotton puffs were moistened using 15 mL of distilled water or heavy metal solutions with a certain concentration. The heavy metal models used in this study were Cu derived from $(CH_3COOH)_2Cu{\cdot}H_2O$ (No. 036-04025, Fujifilm Wako Pure Chemical Co., Ltd., Osaka, Japan) and Ba derived from $(CH_3COOH)_2Ba$ (No. 026-00065, Fujifilm Wako Pure Chemical Co., Ltd., Osaka, Japan) with concentrations of 1, 5, and 10 ppm. The seeds used came from primed and unprimed (as controls) seeds for each treatment. The combination of treatment variables in accordance with the name of the treatment is presented in Table 1.

Table 1. Combination of different variables used in this study

Seed	Heavy metal	Concentration (ppm)	Treatment name
Unprimed	-	-	Control
	Cu	1	Cu-1
		5	Cu-5
		10	Cu-10
	Ba	1	Ba-1
		5	Ba-5
		10	Ba-10
Primed	-	-	Control-Si
	Cu	1	Cu-1-Si
		5	Cu-5-Si
		10	Cu-10-Si
	Ba	1	Ba-1-Si
		5	Ba-5-Si
		10	Ba-10-Si

Seedlings were separated from the cotton puffs at the end of the germination. Several germination parameters were used to assess seed germination performance. Seed germination percentage (SGP) is a measure of the proportion of seeds from a particular batch that successfully sprouts and grows into seedlings. Mean germination time (MGT) is a measure used in seed germination studies to describe the average amount of time it takes for a group of seeds to germinate [13]. The germination index (GI) is a measure used to provide information about the rate of germination and the uniformity of germination within a group of seeds. The coefficient of velocity of germination (CVG) is a measure used to assess the rate at which seeds germinate and it also quantifies the speed of germination [14]. The vigor index (VI) is a measure used to assess the overall vigor or health of seedlings that have germinated from a batch of seeds based on seedlings' length (VI_{LENGTH}) or seedlings' fresh weight (VI_{FW}) [10]. The following formulas were used to determine those germination parameters:

$$SGP = \frac{\text{number of seeds germinated}}{\text{total number of seeds}} \times 100\% \tag{1}$$

$$MGT = \frac{\sum n \times d}{\sum n} \tag{2}$$

$$GI = (8 \times n_1) + (7 \times n_2) + (6 \times n_3) + \ldots + (1 \times n_8) \tag{3}$$

$$CVG = \frac{(n_1 + n_2 + \ldots + n_8)}{(n_1T_1 + n_2T_2 + \ldots + n_8T_8)} \times 100 \tag{4}$$

$$VI_{LENGTH} = SGP \times \text{mean of seedlings length in cm} \quad (5)$$

$$VI_{FW} = SGP \times \text{mean of seedlings fresh weight in g} \quad (6)$$

where *d* the was the number of days since the start of the experiment and *n* was the number of seeds germinated on day *d*. The first number in each term (8, 7, 6, ..., and 1) represented the weight that was given to the number of seeds that germinated on the first, second, and every following day. The letters n_1, n_2, ..., and n_8 represented the number of seeds that germinated on the first, second, and every following day up until the eighth day. *T* was the number of days since germination first started [13, 14].

2.4 Seedling chlorophyll and carotenoid analysis

A total of 0.1 g of seedling leaves was ground with mortar and pestle and 5 mL of 96% ethyl alcohol (C_2H_6O) (Wako Pure Chemical Corporation, Japan) was added [15]. The extract was left for 10 min and then filtered with quantitative filter paper (Advantec® 4A, Advantec Toyo Kaisha, Ltd., Tokyo, Japan). The resulting filtrate was then poured into a 5 mL volumetric flask and added with 96% ethanol until the volume reached 5 mL. About 200 µL of the filtrate was taken and placed into a 96-well microplate (Nikkei Products Co. Ltd., Japan) and then the absorbance (470, 647, 663, and 750 nm) was measured using a microplate reader (INFINITE M1000 Pro, Tecan Group Ltd., Switzerland). The calculation formulas for determining the pigment content of leaves were shown as follows:

$$A_X = \frac{(OD_X - OD_{750})}{\ell} \quad (7)$$

$$\text{Chl a} = (11.24 \times A_{662}) - (2.04 \times A_{645}) \times \frac{v}{\omega} \quad (8)$$

$$\text{Chl b} = (20.13 \times A_{645}) - (4.19 \times A_{662}) \times \frac{v}{\omega} \quad (9)$$

$$\text{Crt} = \frac{(1000 \times A_{470}) - (1.9 \times Chl\ \text{a}) - (63.14 \times \text{Chl b})}{214} \times \frac{v}{\omega} \quad (10)$$

where A_X was absorbance at x nm, OD_X was measured optical density at x nm, ℓ was path length of microplate (= 0.876887 cm), Chl a was chlorophyll a (mg/g DW), Chl b was chlorophyll b (mg/g DW), Crt was total carotenoids (mg/g DW), v was volume of solvent (mL), and ω was weight of sample (mg) [16].

2.5 Seedling elemental and statistical analysis

Seven-day-old seedlings were taken from the cotton puff and dried in the oven for 48 h at 50 ºC [17]. Elemental analysis measurements were made using an X-ray fluorescence (XRF) spectrometer (JSX-3100RII, JEOL Ltd., Japan) under the following conditions: tube voltage = 30 kV, collimator = 1, and live-time value = 100 sec. Pellets (ø = 0.8 cm) for analysis were prepared by pressing 0.001 g of dried seedlings under 100 kgf/cm^2 in a mechanical press machine [10]. Intensity data obtained from the XRF was visualized using a heat map. All of the collected data were analyzed by using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) in IBM SPSS Statistics with *p*-values ≤ 0.05 considered as significant [18].

3 Results and discussions

3.1 Characteristics of nanoparticles

The silica nanoparticles used in this study were characterized based on particle size distribution and zeta potential as shown in Fig. 1. The measured average particle size was 10.35 ± 0.11 nm. This value was in accordance with information from the manufacturer which stated that the particle size is in the range of 8 – 11 nm [19]. The consistency of particle size in nanoparticles could be an indicator of their stability. When nanoparticles maintained a uniform size, it often implied that they were less prone to aggregation or agglomeration, which could lead to changes in their properties and performance. The measured zeta potential value was 30.87 ± 1.04 mV. Colloidal systems with zeta potentials around ± 30 mV were usually considered to be stable. Particles in such systems tended to repel each other strongly due to electrostatic forces, minimizing the risk of aggregation [10].

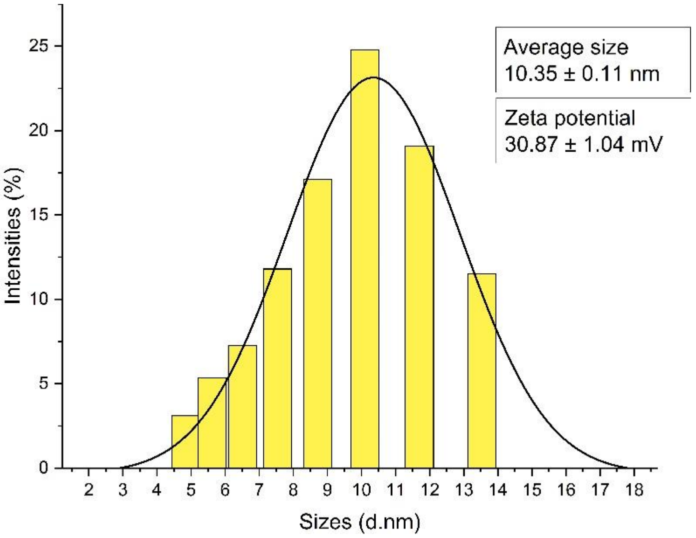
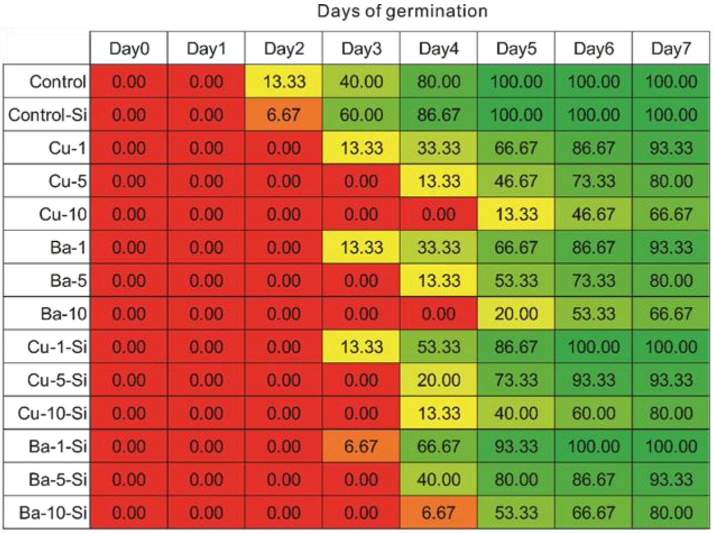


Fig. 1. Characteristics of nanoparticles as indicated by particle size distribution and dispersity.

3.2 Performance of seed germination

Analyzing the performance of seed germination is essential as it provides valuable information about the quality and viability of seeds. The first parameter analyzed was the seed germination percentage (SGP) as shown in Fig. 2. Control seeds, both primed and unprimed, were fully germinated on the last day. The presence of heavy metals (Cu or Ba) in the germination environment might hinder the germination process, consequently leading to not all seeds germinating on the last day. Heavy metals are known to generate reactive oxygen species (ROS) in plant cells [20]. ROS can cause oxidative stress, damaging cellular components such as proteins, lipids, and DNA. This oxidative damage can disrupt critical metabolic processes required for germination [20]. Nanopriming with silica could increase the percentage of seeds that germinate on the last day. Nanopriming can enhance the antioxidant defense systems within seeds and seedlings. Nanopriming can help scavenge ROS and reduce oxidative stress, mitigating the toxic effects of heavy metals [7]. A study conducted by Gupta et al. revealed the regulation mechanism of ROS in *Basella alba* seed germination under lead metal stress [21].



Days of germination

	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Control	0.00	0.00	13.33	40.00	80.00	100.00	100.00	100.00
Control-Si	0.00	0.00	6.67	60.00	86.67	100.00	100.00	100.00
Cu-1	0.00	0.00	0.00	13.33	33.33	66.67	86.67	93.33
Cu-5	0.00	0.00	0.00	0.00	13.33	46.67	73.33	80.00
Cu-10	0.00	0.00	0.00	0.00	0.00	13.33	46.67	66.67
Ba-1	0.00	0.00	0.00	13.33	33.33	66.67	86.67	93.33
Ba-5	0.00	0.00	0.00	0.00	13.33	53.33	73.33	80.00
Ba-10	0.00	0.00	0.00	0.00	0.00	20.00	53.33	66.67
Cu-1-Si	0.00	0.00	0.00	13.33	53.33	86.67	100.00	100.00
Cu-5-Si	0.00	0.00	0.00	0.00	20.00	73.33	93.33	93.33
Cu-10-Si	0.00	0.00	0.00	0.00	13.33	40.00	60.00	80.00
Ba-1-Si	0.00	0.00	0.00	6.67	66.67	93.33	100.00	100.00
Ba-5-Si	0.00	0.00	0.00	0.00	40.00	80.00	86.67	93.33
Ba-10-Si	0.00	0.00	0.00	0.00	6.67	53.33	66.67	80.00

Fig. 2. Seed germination percentage observed during germination. Different colors in the matrix represent a percentage range from 0 to 100%

Table 2. Mean germination time, germination index, and coefficient of velocity of germination observed during germination. Different letters indicate significance according to Duncan's test

Treatment	MGT (days)	GI	CVG
Control	3.53 ± 0.41^{a}	27.33 ± 2.05^{a}	28.7 ± 3.46^{a}
Control-Si	3.4 ± 0.16^{a}	28 ± 0.82^{a}	29.48 ± 1.42^{a}
Cu-1	4.93 ± 0.75^{bc}	19.33 ± 5.19^{bc}	20.71 ± 2.86^{bcd}
Cu-5	5.44 ± 0.63^{cd}	14.67 ± 4.99^{cd}	18.6 ± 1.98^{de}
Cu-10	6.08 ± 0.12^{d}	9.67 ± 0.94^{d}	16.44 ± 0.31^{e}
Ba-1	4.83 ± 0.29^{bc}	19.33 ± 1.25^{bc}	20.76 ± 1.22^{bcd}
Ba-5	5.31 ± 0.27^{cd}	15 ± 4.08^{cd}	18.9 ± 0.97^{cde}
Ba-10	5.92 ± 0.3^{d}	10.33 ± 2.05^{d}	16.94 ± 0.82^{e}
Cu-1-Si	4.47 ± 0.25^{b}	22.67 ± 1.25^{ab}	22.46 ± 1.23^{bc}
Cu-5-Si	4.98 ± 0.18^{bc}	18.67 ± 1.25^{bc}	20.09 ± 0.75^{bcde}
Cu-10-Si	5.59 ± 0.07^{cd}	13.67 ± 2.87^{cd}	17.9 ± 0.22^{de}
Ba-1-Si	4.33 ± 0.25^{b}	23.33 ± 1.25^{ab}	23.16 ± 1.37^{b}
Ba-5-Si	4.82 ± 0.31^{bc}	19.67 ± 3.3^{bc}	20.84 ± 1.27^{bcd}
Ba-10-Si	5.42 ± 0.31^{cd}	14.33 ± 1.25^{cd}	18.52 ± 1.09^{de}

The second parameter analyzed was the mean germination time (MGT) as shown in Table 2. The results indicated that the presence of Cu or Ba during germination might result in a longer germination period. The presence of Cu and Ba at 1 ppm could increase germination time by 28.38% and 26.9%, respectively, compared to control. Heavy metals can directly inhibit the activity of enzymes that are essential for various metabolic processes during germination [22]. Slower enzymatic activity can delay the processes necessary for germination to occur. The use of nanopriming could reduce the germination time by 10.45% for Cu and 11.54% for Ba compared to the unprimed conditions under heavy metals environments. Nanoparticles can improve water uptake by seeds [23], facilitating the imbibition process during germination. A study conducted by Rai-Kalal & Jajoo showed that nanopriming with zinc oxide could increase the water uptake during seed germination of wheat cultivars [23]. Improved water uptake can help dilute the concentration of heavy metals within the seed and reduce their toxic effects. The third and fourth parameters were germination index (GI) and coefficient of velocity of germination (CVG) as shown in Table 2. The results indicated that the presence of Cu or Ba during germination might result in a decrease of the GI and CVG values. The presence of Cu and Ba at 1 ppm could decrease the GI value by 41.38% compared to the control. The presence of Cu and Ba at 1 ppm could also decrease the CVG value by 38.62% and 38.25%, respectively, compared to the control.

Table 3. Vigor index based on seedling length and fresh weight observed during germination. Different letters indicate significant according to Duncan's test

Treatment	**VI_{LENGTH}**	**VI_{FW}**
Control	1526 ± 62.76^{a}	2.1 ± 0.03^{a}
Control-Si	1638.67 ± 24.78^{a}	2.2 ± 0.06^{a}
Cu-1	982.17 ± 92.54^{c}	1.51 ± 0.02^{cd}
Cu-5	724.83 ± 46.76^{d}	1.33 ± 0.07^{def}
Cu-10	614.44 ± 62.68^{d}	1.11 ± 0.17^{g}
Ba-1	981 ± 26.24^{c}	1.49 ± 0.09^{cde}
Ba-5	731.11 ± 38.9^{d}	1.3 ± 0.14^{efg}
Ba-10	614.17 ± 17.36^{d}	1.12 ± 0.04^{fg}
Cu-1-Si	1250 ± 109.99^{b}	1.77 ± 0.12^{b}
Cu-5-Si	1025.33 ± 141.58^{c}	1.6 ± 0.05^{bc}
Cu-10-Si	892.11 ± 75.76^{c}	1.27 ± 0.08^{fg}
Ba-1-Si	1235.33 ± 54.95^{b}	1.78 ± 0.04^{b}
Ba-5-Si	1017 ± 8.04^{c}	1.61 ± 0.08^{bc}
Ba-10-Si	896.67 ± 56.21^{c}	1.24 ± 0.14^{fg}

Seeds that are exposed to heavy metals may remain in a dormant state longer, as the environmental conditions needed to break dormancy may not be met due to the stress imposed by heavy metals. Heavy metals (Hg^{2+}, Pb^{2+}, Cu^{2+}, and Zn^{2+}) could induce seed dormancy in *Arabidopsis thaliana* seeds [24]. This was related to fewer seeds germinating which resulted in lower GI and CVG values under stress conditions caused by the presence of heavy metals. The use of nanopriming could increase the GI value by 14.71% for Cu and 17.14% for Ba compared to the unprimed seeds. The use of nanopriming could also increase CVG value by 7.79% for Cu and 10.34% for Ba compared to the unprimed conditions under heavy metals environments. Nanopriming has the potential to influence seed dormancy and it might help shorten seed dormancy periods. The presence of nanoparticles could help soften or weaken these seed coats [10], making it easier for water and gases to penetrate the seed. This could effectively break physical dormancy and promote germination. A study revealed that nanopriming with zinc oxide could regulate the H_2O_2 signal which acted as a signaling molecule in breaking seed dormancy in *Triticum aestivum* [25]. From these four germination parameters, it could be concluded that nanopriming was able to provide tolerance to heavy metal stress.

The fifth and sixth parameters were vigor index based on seedling length (VI_{LENGTH}) and vigor index based on seedling fresh weight (VI_{FW}) as shown in Table 3. Plant vigor can be significantly affected by heavy metal contamination in the environment. The presence of Cu and Ba at 1 ppm could reduce vigor seedling length by 55.37% and 55.56%, respectively, compared to the control. The use of nanopriming could increase the vigor seedling length by 21.43% for Cu and 20.59% for Ba compared to the unprimed conditions. Nanopriming can stimulate cell elongation in plant tissues, including root and shoot tissues [26]. This elongation contributes to increased seedling length. The presence of Cu and Ba at 1 ppm could also reduce vigor fresh weight by 39.36% and 40.71%, respectively, compared to the control. The use of nano priming could increase the vigor of fresh weight by 14.68% for Cu and 15.8% for Ba compared to the unprimed conditions. Nanopriming treatments could improve nutrient uptake by seeds and seedlings [27]. A study conducted by El-Badri et al. showed that *Brassica napus* seeds nanoprimed with zinc oxide showed increased nutrient absorption during germination [27]. Enhanced nutrient availability supports overall seedling growth and biomass accumulation, leading to higher fresh weight.

3.3 Profile of leaves pigments

Leaf pigments, particularly chlorophylls and carotenoids, play important roles during seed germination, although their functions primarily become prominent after germination [28]. As the seedling emerges and its leaves expand, chlorophyll allows the seedling to start photosynthesis, providing the energy and carbon compounds needed for growth. We analyzed the chlorophylls and carotenoids content of seedling leaves as shown in Fig. 3.

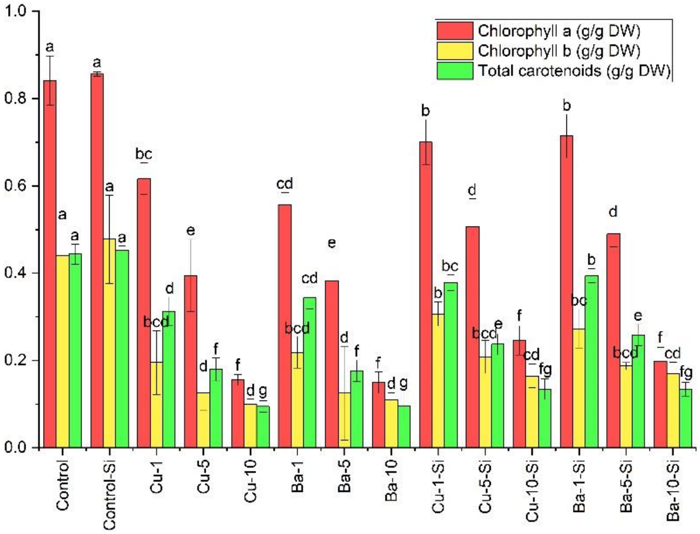


Fig. 3. Chlorophylls and carotenoids of seedling leaves observed during germination. Different letters indicate significance according to Duncan's test

The presence of Cu and Ba at 1 ppm could reduce the chlorophyll a content by 36.46% (0.62 ± 0.04 g/g DW) and 51.03% (0.56 ± 0.03 g/g DW), respectively, compared to control (0.84 ± 0.06 g/g DW). The use of nanopriming could increase the chlorophyll a content by 11.94% (0.7 ± 0.05 g/g DW) for Cu and 22.02% (0.71 ± 0.05) for Ba compared to the unprimed seeds. Heavy metals can directly inhibit enzymes involved in chlorophyll biosynthesis [28]. These enzymes include those responsible for the conversion of precursor molecules into chlorophyll. When these enzymes are inhibited, the synthesis of chlorophyll is disrupted, leading to reduced chlorophyll content in plant tissues. Nanoparticles may enhance the uptake of essential nutrients by plants [29], which can counteract the negative effects of heavy metals on nutrient absorption. Adequate nutrient levels are essential for chlorophyll a synthesis [30]. The presence of Cu and Ba at 1 ppm could reduce the chlorophyll b content by 125.99% (0.2 ± 0.07 g/g DW) and 101.97% (0.22 ± 0.04 g/g DW), respectively, compared to control (0.44 ± 0.07 g/g DW). The use of nanopriming could increase the chlorophyll b content by 36.3% (0.31 ± 0.03 g/g DW) for Cu and 19.92% (0.27 ± 0.04 g/g DW) for Ba compared to the unprimed seeds. Heavy metals can generate reactive oxygen species (ROS) within plant cells, causing oxidative stress. ROS can damage chlorophyll molecules and disrupt the photosynthetic machinery [31]. This damage leads to the degradation of chlorophylls. Nanoparticles can influence water uptake and retention in plants [32]. Maintaining optimal water balance is crucial for photosynthesis, including chlorophyll b synthesis [33]. The presence of Cu and Ba at 1 ppm could reduce the total carotenoid content by 41.78% (0.31 ± 0.03 g/g DW) and 29.12% (0.34 ± 0.03 g/g DW), respectively, compared to control (0.44 ± 0.02 g/g DW). The use of nanopriming could increase the total carotenoid content by 17.36% (0.38 ± 0.02 g/g DW) for Cu and 12.87% (0.39 ± 0.02 g/g DW) for Ba compared to the unprimed conditions under heavy metal stress. Nanoparticles might confer stress tolerance to plants, enabling them to better cope with heavy metal stress [34]. Improved stress tolerance can help maintain the overall health of the plant, including the synthesis and retention of carotenoids [35]. Nanoparticles could also potentially influence metabolic processes in plants, including those related to the synthesis of secondary metabolites such as carotenoids [36].

3.4 Elemental information of the seedlings

Elemental analysis is a valuable tool in the study of the physiological function of plants as it can determine the levels of essential nutrients in plant tissues [37]. This information helps the study of nutrient uptake, transport, and distribution within plants, shedding light on the role of these elements in plant growth and development. Analysis using XRF on the seedlings detected 11 elements (Mg, Si, P, S, K, Ca, Mn, Fe, Cu, Zn, Ba) as shown in Fig. 4. Si was not detected in unprimed seeds, therefore the Si detected in primed seeds must come from the silica nanoparticles that we used in this study.

During heavy metal stress, plants undergo several elemental alterations or changes in their elemental composition. These alterations are responses to the presence of toxic levels of heavy metals in the environment and are part of the plant's strategy to cope with and adapt to stress [38]. One of the most significant elemental alterations is the increased accumulation of the specific heavy metal(s) causing the stress. Plants may absorb these metals from the soil and transport them to various plant tissues, such as roots, stems, and leaves. We could not detect Cu and Ba in control seeds, but we did detect them in treated seeds. P and K were macronutrients that changed significantly when heavy metals were present. The presence of Cu and Ba at 1 ppm could reduce the P intensity by 67.77% (5475.67 ± 188.56) and 66.84% (5506.33 ± 97.35), respectively, compared to control (9186.67 ± 192.67). The use of nanopriming could increase the P intensity content by 24.91% (7292.33 ± 72.82) for Cu and 24.91% (7333 ± 154.38) for Ba compared to the unprimed conditions under heavy metal stress. Heavy metals could compete with macronutrients like P for uptake by plant roots. When heavy metals are present at elevated concentrations, they can outcompete P for binding sites on the root surfaces, leading to reduced P uptake by the plants [39]. Heavy metals can inhibit or disrupt the activity of enzymes involved in P metabolism within plant cells. This disruption can hinder P uptake and utilization [32]. The presence of Cu and Ba at 1 ppm could reduce the K intensity by 62.88% (3970.33 ± 188.56) and 64.18% (3938.67 ± 97.35), respectively, compared to control (6466.67 ± 192.67). The use of nanopriming could increase the K intensity by 22.77% (5140.67 ± 72.82) for Cu and 26.73% (5375.67 ± 154.38) for Ba compared to the unprimed seeds. Nanopriming might help reduce the toxic effects of heavy metals on plant roots and their impact on nutrient uptake [40]. A study conducted by Benerjee et al. demonstrated that the use of zinc oxide as a nano priming agent could confer *Vigna mungo* seeds tolerance under arsenic stress by triggering the production of antioxidant defense systems [40]. By improving plant stress tolerance, nanopriming could enable plants to maintain more normal nutrient uptake even when heavy metals are present. It was important to note that the effectiveness of nano priming in increasing P and K content under heavy metal stress might vary depending on the specific nanoparticles used, their concentration, the plant species, and the environmental conditions.

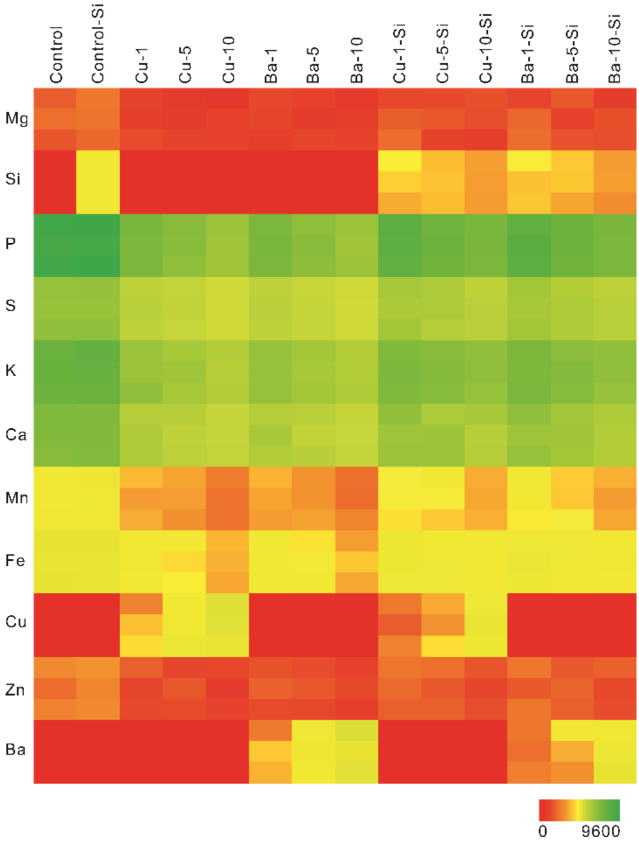


Fig. 4. Heat map of elements detected on seedlings observed during germination

4 Conclusion

The presence of heavy metals, both Cu and Ba, could inhibit seed germination and negatively impact seedling establishment as it reduced the mean germination time and declined several germination parameters such as seed germination percentage, germination index, coefficient of the velocity of germination, and vigor index. However, we have demonstrated that nanopriming was a potential solution to mitigate the inhibitory effects of heavy metals on seed germination and early seedling growth. Nanopriming could enhance the germination percentage of seeds and accelerate the germination process, allowing seeds to sprout more quickly. Nanopriming treatments might increase the tolerance of germinating seeds and young seedlings to heavy metal stress as indicated by the leaves' pigments and the elemental status of the seedlings. Nanopriming methods have the potential to be applied to various seed crops, including a wide range of plant species used in agriculture and horticulture.

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