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Research Article

Modulation of morpho-physiological and biochemical attributes through seed priming towards improved seedling growth of *Bergenia ciliata* (Haw.) Sternb.

Kajal Mog Chaudhuri[a], Kuntal Bera[a,b], Bigyananda Mutum[a], Soham Mukherjee[a], Puspendu Dutta[a]*, Manoj Kanti Debnath[c]

[a]Department of Seed Science and Technology, Uttar Banga Krishi Viswavidyalaya, Pundibari – 736165, Cooch Behar, West Bengal, India.
[b]Plant Molecular Biology Laboratory, Department of Botany, Raiganj University, Raiganj – 733134, Uttar Dinajpur, West Bengal
[c]Department of Agriculture Statistics, Uttar Banga Krishi Viswavidyalaya, Pundibari – 736165, Cooch Behar. West Bengal, India

Abstract

Bergenia ciliata of the Saxifragaceae family is an important perennial herb that is predominantly propagated through rhizomes due to its poor seed germination potential. This study was carried out at the Department of Seed Science and Technology, UBKV, Pundibari, Cooch Behar, and a Completely Randomized Design (CRD) was followed and replicated thrice. Seed priming, a widely accepted technique, aims to enhance seed germination traits, ensure a good crop stand, and improve yield across diverse climatic conditions. Thus, the investigation aimed to assess the impact of different priming techniques on morpho-physiological and biochemical parameters of *B. ciliata* seedlings. Seeds were primed with 14 different conventional agents, depending on concentrations or duration of exposure. Upon emergence, seedlings were grown for 3 months, during which various seedling growth and physiological or biochemical parameters were analyzed. All priming treatments resulted in better seedling growth than the control. However, the GA_3 at 50 ppm treatment recorded the highest root and total length, and fresh weight of 3-month-old seedlings. The highest leaf area and leaf numbers were found in GA_3 at 100 ppm. The positive effect of different priming agents on seedling performance and morpho-physiological traits, as compared to the control treatment, would be attributed to the advancement of pre-germinative metabolisms and improved repair mechanisms involving greater activities of antioxidant systems. The PCA and cluster analysis also helped to understand that the efficacy of priming agents varied depending on their kinds and the dosages.

Article info

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Introduction

Bergenia ciliata (Haw.) Sternb. (Family—Saxifragaceae) is a promising perennial herb with notable therapeutic potential. Members of the *Bergenia* genus are characterized by their short, succulent stems and produce flowers in hues of white, pink, or purple on robust, fleshy stalks. Research has highlighted its remarkable efficacy in therapeutic applications and its role as a source of various secondary metabolites with medicinal benefits (Koul et al. 2020). It has been designated as "vulnerable" due to a rapid decline in its populations within natural habitats (Hassler 2016).

Correspondence: puspendu@ubkv.ac.in

The plant's resilient, cylindrical or barrel-shaped rhizomes are valued for their diuretic and astringent effects, making them essential for treating kidney and bladder stones (Singh and Pandey 2019).

The tiny size and poor germination rates of seeds present significant challenges for many plant species (Huh et al. 2016). Additionally, numerous medicinal plants, including *Bergenia*, face difficulties in germination and crop establishment in field conditions (Zare et al. 2011). One effective method is pre-sowing treatment, known as seed priming. This approach, utilizing various priming agents such as gibberellic acid, KNO_3, and PEG-6000, has proven to expedite germination and improve final germination rates in many species with small seeds (Mog Chaudhuri et al., 2024). Moreover, it is a cost-effective, straightforward strategy recognized for enhancing the seed's water uptake capacity, enzyme activity, and DNA repair processes, thereby promoting rapid and uniform germination in subsequent sowings (Dutta 2018).

Seed priming involves various commercial applications aimed at effective crop stress management and enhanced productivity while optimizing the use of seeds, water, and nutrients. Antioxidative properties are vital to the plant's stress response, and plants have an antioxidant system that is a critical element of their defense mechanisms against oxidative damage caused by excessive reactive oxygen species (Kong et al. 2021). Beyond boosting germination and activating pre-germinative metabolism, seed priming can also enhance antioxidant activity and reduce lipid peroxidation (Bradford 1990). Various studies demonstrate that priming positively influences germination across multiple plant species; it also promotes the synthesis of essential proteins and enzymes for cellular metabolism and offers protection against oxidative stress (Varier et al. 2010).

Considering the significance of *B. ciliata* for medicinal, therapeutic, and economic purposes, this study aims to investigate priming-induced changes in the growth and physiological or biochemical aspects of 3-month-old *Bergenia* seedlings, identifying the optimal priming treatment that enhances seedling growth and establishment.

Materials and methods

Seed source and growth conditions

Seeds of *Bergenia ciliata* were collected from the temperate Himalayas in West Bengal, India, then dried under sunlight, meticulously cleaned, and stored in airtight glass desiccators at 4 °C for future use. Given their diminutive size, a scanning electron microscopy (SEM) image of a mature seed is provided in **Supplementary Figure 1A**.

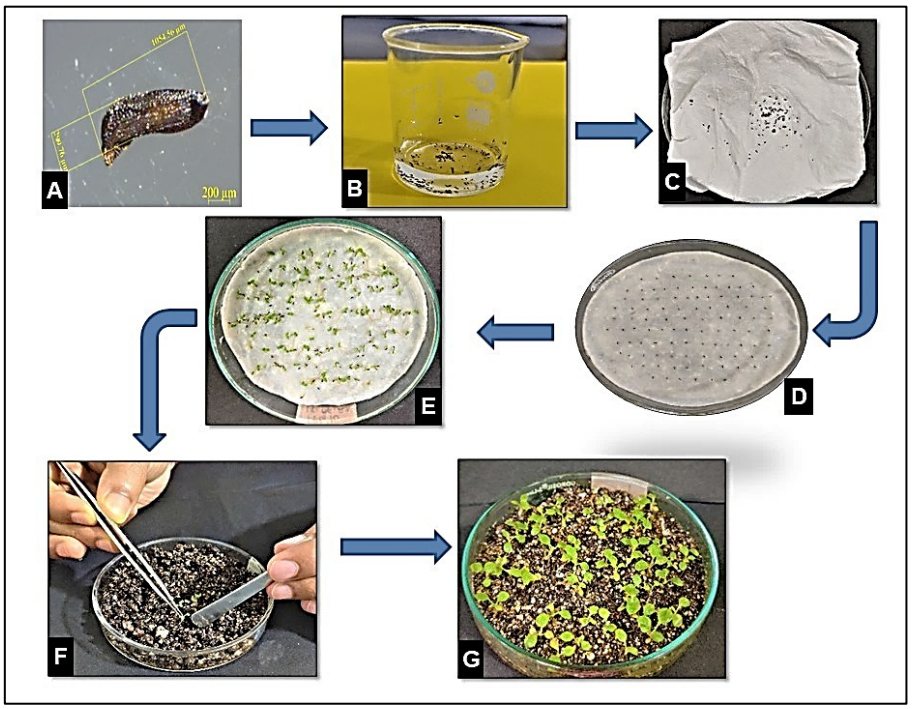

Figure 1. Photographs of each step were taken during the experiment. Stereo-microscopic photograph of an individual mature seed of *Bergenia ciliata* [A], soaking of Bergenia seeds in respective solution of various priming agents [B], drying the seeds under normal room temperature [C], placing the seeds on filter paper pre-moistened with sterile water [D], 15-day-old seedlings [E], transferring of seedlings to petri-plates pre-filled with growing media, i.e., soil rite [F], 3-month-old seedlings [G].

The experiment employed fourteen priming treatments and three replications within a completely randomized design. The treatments included: control (T1), hydro-priming for 12 hours (T2), hydro-priming for 24 hours (T3), 25 ppm gibberellic acid (GA_3) (T4), 50 ppm GA_3 (T5), 100 ppm GA_3 (T6), 25 ppm ascorbic acid (T7), 50 ppm ascorbic acid (T8), 3% KNO_3 (T9), 6% KNO_3 (T10), 0.4% sodium chloride (NaCl; T11), 0.8% NaCl (T12), 10% polyethylene glycol (PEG) (T13), and 20% PEG (T14) (Mog Chaudhuri et al. 2024). Post-priming, seeds were thoroughly rinsed with autoclaved double-distilled water and air-dried until they regained their initial weight.

The seeds were then arranged in sterilized, pre-moistened Petri plates following ISTA guidelines (2015) (**Supplementary Figure 1B**) and incubated at 20 °C in a seed germinator under standardized conditions: 12 hours of light followed by 12 hours of darkness (Mog Chaudhuri et al. 2023). The experimental workflow from priming to seedling growth over three months is detailed in Figure 1.

Growth and morphological parameters

Seedling height (measured from the growing media surface to the base of the topmost fully opened leaf), root length, and total length were assessed using a graded scale (**Supplementary Figure 1C**). Ten seedlings from each treatment were randomly selected for these measurements, with mean values expressed in cm seedling^{-1}. The same ten seedlings were weighed fresh and then subsequently dried for 24 hours or until a constant weight was achieved at 60 °C in a hot air oven, with both fresh and dry weights recorded as mg seedling^{-1}. The number of leaves and petiole lengths (PL) were also noted from the same seedlings, and PL was measured using a meter scale, reported in centimeters.

The leaf area of *B. ciliata* was determined using the Easy Leaf Area (ELA) digital image analysis application (**Supplementary Figures 1D-F**). Ten leaf areas from each treatment were calculated by assessing the ratio of red calibration pixels to green leaf pixels, multiplying the count of green by that of red pixels (Baker et al. 1996).

Physiological or biochemical parameters

Fresh seedlings from each treatment were initially rinsed with tap water and then with double-distilled water to remove dust, and a standard protocol was followed to estimate total protein content (Lowry et al. 1951). BSA served as the standard, and protein content was expressed as mg g^{-1} fresh weight.

The total phenolic content was determined using the Folin-Ciocalteu Reagent (FCR) method with modifications (Cardona et al. 2017). The O.D. was measured at 650 nm using a UV-VIS spectrophotometer (Model: SHIMADZU UV-1800). The absorbance values were compared to a standard calibration curve with gallic acid to quantify total phenolic content, expressed as mg GAE g^{-1} dry mass.

A 50 mg sample was ground with 5 mL of hydro-methanol (82:18) to estimate total antioxidant content (Cardona et al. 2017). The reactivity of a standard methanolic ascorbic acid solution with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated following Singh et al. (2002) with minor modifications. The molar concentration of DPPH was determined using an extinction coefficient of 12.509 M ¹ cm ¹. To measure the extracts' radical scavenging ability, the IC_{50} value — the concentration at which 50% of DPPH radicals are neutralized — was calculated.

$$Scavenging\ (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

To assess antioxidant enzymes, including superoxide dismutase (SOD), total peroxidase (POX), and catalase (CAT), seedlings were acclimatized at room temperature (29±2 °C) overnight before sampling. Fresh samples of 100 mg were ground in liquid nitrogen, and 2 mL of a grinding medium was added, containing phosphate buffer (50 mM, pH 7.0), EDTA (2 mM), mercaptoethanol (5 mL), and PVP-40 (4%). The homogenate was then centrifuged at 30,000 g at 4 °C for 30 minutes to obtain supernatants rich in the enzymes. Modifications were made to the techniques outlined by Teranishi et al. (1974) for measuring catalase and peroxidase activity. As per Dhindsa et al. (1981), SOD activity was determined by monitoring the decrease in optical density of formazan produced by superoxide radicals reacting with nitroblue tetrazolium dye.

Statistical analysis

Data on seedling growth and physiological or biochemical attributes were analyzed using SPSS Statistics (Ver. 23). Tukey's HSD test was applied to evaluate significant differences among means and interactions ($p<0.05$). Visualization included generating graphs, box plots, and heatmaps with GraphPad Prism (Version 8.0.2.263). Moreover, R software (R Core Team 2012) was used for Principal Component Analysis (PCA) to obtain treatment scores and for cluster analysis to group

treatments based on their effectiveness in promoting germination and seedling growth.

Results

Seedling growth and morphological parameters

Table 1 illustrates the effects of various priming treatments on seedling parameters such as root length (RL), seedling height (SH), total length (TL), fresh weight (FW), and dry weight (DW) of 3-month-old *B. ciliata*. The results revealed a significant influence of the priming treatments on seedling growth, with RL and TL showing greater enhancement than SH. The highest RL (7.01 cm) and TL (7.62 cm), along with FW (9.82 mg), were recorded with GA_3 at 50 ppm, while the maximum SH (0.663 cm) was observed with GA_3 at 100 ppm. Conversely, seedlings from the control (dry seed) treatment exhibited the lowest RL (4.68 cm), SH (0.480 cm), and TL (5.16 cm). Additionally, both GA_3 at 50 ppm and NaCl at 0.4% yielded the greatest dry weight (1.58 mg), whereas the control treatment had the lowest dry weight (1.11 mg).

The findings depicted in Table 1 provide evidence regarding the impact of different priming agents on leaf parameters, i.e., leaf area, leaf number, and petiole length of 3-month-old seedlings, which was statistically significant. The highest leaf area (0.439 cm^2 $seedling^{-1}$) was measured in GA_3 at 100 ppm, followed by NaCl at 0.8% (0.424 cm^2 $seedling^{-1}$), ascorbic acid at 25 ppm (0.403 cm^2 $seedling^{-1}$), GA_3 at 25 ppm (0.398 cm^2 $seedling^{-1}$), and HP at 24 hours (0.385 cm^2 $seedling^{-1}$). The highest petiole length and leaf number were observed in ascorbic acid at 25 ppm (0.390 cm) and GA_3 at 100 ppm (5.50 $seedlings^{-1}$), respectively. However, the lowest leaf area (0.246 cm2 $seedling^{-1}$), leaf number (4.00 $seedlings^{-1}$), and petiole length (0.290 cm) were found in the control.

Table 1. Effect of various priming agents on seedling growth and morphological parameters of *Bergenia ciliata*

Treatments	Root length (cm)	Seedling height (cm)	Fresh weight (mg)	Dry weight (mg)	Total length (cm)	Leaf area (cm^2 $seedling^{-1}$)	Leaf Number ($seedling^{-1}$)	Petiole length (cm)
Control	4.68^{g}	0.480^{g}	7.21^{k}	1.11^{f}	5.16^{h}	0.246^{b}	4.00^{b}	0.290^{e}
HP (12h)	5.66^{e}	0.570^{bcde}	8.28^{f}	1.31^{bcd}	6.23^{ef}	0.385^{a}	5.00^{ab}	0.310^{de}
HP (24h)	5.29^{f}	0.510^{fg}	7.50^{j}	1.13^{ef}	5.80^{g}	0.373^{ab}	4.50^{ab}	0.380^{ab}
GA_3 (25ppm)	5.89^{d}	0.533^{def}	$8.22f^{g}$	1.34^{bc}	6.42^{de}	0.398^{a}	4.50^{ab}	0.330^{bcde}
GA_3 (50ppm)	7.01^{a}	0.617^{ab}	9.82^{a}	1.58^{a}	7.62^{a}	0.349^{ab}	4.50^{ab}	0.360^{abcd}
GA_3 (100ppm)	6.54^{c}	0.663^{a}	8.90^{d}	1.41^{b}	7.20^{bc}	0.439^{a}	5.50^{a}	0.320^{cde}
Ascorbic Acid (25ppm)	6.51^{c}	0.593^{bc}	9.24^{c}	1.37^{b}	7.10^{bc}	0.403^{a}	5.00^{ab}	0.390^{a}
Ascorbic Acid (50ppm)	5.90^{d}	0.553^{cdef}	7.75^{i}	1.29^{bcd}	6.45^{de}	0.326^{ab}	4.00^{b}	0.340^{abcde}
KNO_3 (3%)	6.39^{c}	0.597^{bc}	7.97^{h}	1.28^{bcde}	6.98^{c}	0.338^{ab}	4.00^{b}	0.350^{abcd}
KNO_3 (6%)	5.39^{f}	0.620^{ab}	7.76^{i}	1.17^{def}	6.01^{fg}	0.318^{ab}	4.50^{ab}	0.350^{abcd}
NaCl (0.4%)	6.80^{ab}	0.590^{bc}	9.62^{b}	1.58^{a}	7.39^{ab}	0.347^{ab}	4.50^{ab}	0.370^{abc}
NaCl (0.8%)	6.62^{bc}	0.573^{bcd}	8.12^{g}	1.31^{bcd}	7.19^{bc}	0.424^{a}	5.00^{ab}	0.360^{abcd}
PEG (10%)	6.02^{d}	0.520^{efg}	8.50^{e}	1.34bc	6.54^{d}	0.388^{a}	5.00^{ab}	0.360^{abcd}
PEG (20%)	6.06^{d}	0.560^{cdef}	7.58^{j}	1.21^{cdef}	6.62^{d}	0.379^{ab}	5.00^{ab}	0.380^{ab}
SEm(±)	0.056	0.011	0.033	0.033	0.065	0.028	0.063	0.011
CD (p≤0.05)	0.162	0.032	0.097	0.096	0.191	0.081	0.185	0.032

N.B. Arcsine transformation of counted data was done for ANOVA and post hoc test. The different letters (superscripts) on the same columns indicate significant differences between treatments as per *Tukey's* Honest Significant Difference test at $p < 0.05$.

Physiological or biochemical parameters

Total protein content

Total protein content in 3-month-old seedlings of *B. ciliata* ranged from 14.75 to 30.75 mg g^{-1} FW (Figure 2A). All priming treatments significantly enhanced protein levels, with notable variations among them (**Supplementary Table 1**). The highest total protein (30.75 mg g^{-1} FW) was observed with GA_3 at 25 ppm, while the control (without priming) had the lowest amount (14.75 mg g^{-1} FW).

Total phenolics content

Total phenolic content in 3-month-old *B. ciliata* seedlings varied from 10.58 to 18.43 mg GAE g^{-1} DM (Figure 2B). Results in **d** revealed significant variations in phenolic content due to different priming agents. Seeds treated with GA_3 at 25 ppm exhibited the highest phenolic accumulation (18.43 mg GAE g^{-1} DM), while untreated control seeds had the lowest (10.58 mg GAE g^{-1} DM).

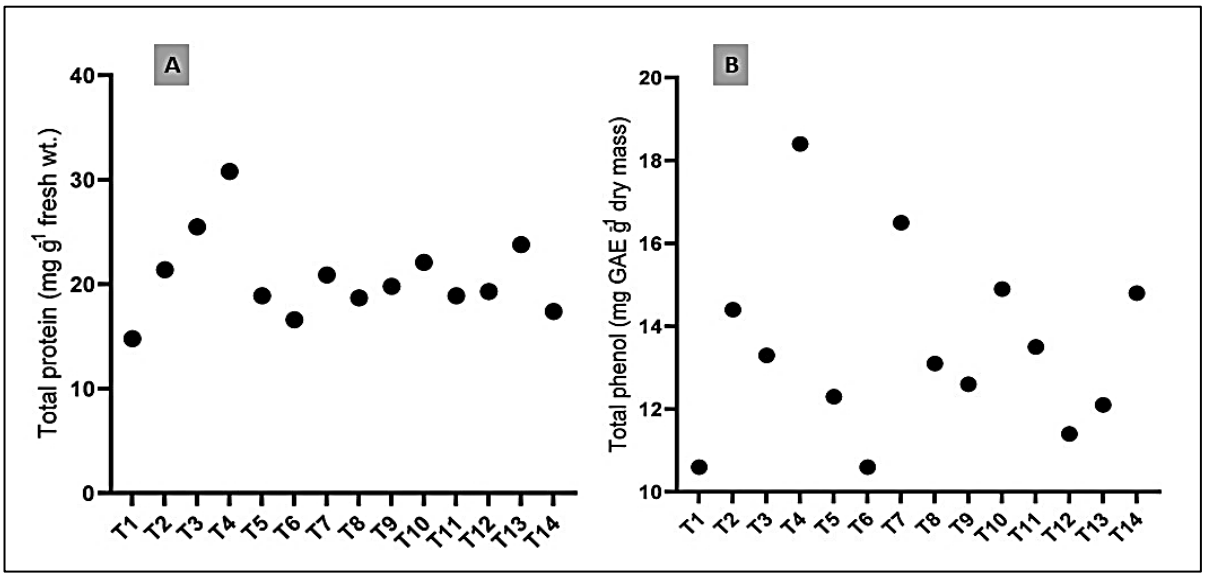

Figure 2. Changes in total protein (mg g^{-1} fresh weight) [A] and total phenol content (mg g^{-1} fresh weight) [B] of 3-month-old seedlings of *Bergenia ciliata* under the influence of various priming agents.

Total antioxidant content

Total antioxidant activity ranged from 89.31% to 91.96%, while IC50 values varied between 41.57 µg mL^{-1} and 114.68 µg mL^{-1} (Figure 3A & 3B). Results indicated significant effects of different priming agents on total antioxidant activity and IC_{50} values in these seedlings (**Supplementary Table 1**). The highest antioxidant activity (91.96%) was observed with KNO_3 at 6%, followed closely by NaCl at 0.4% (91.79%). In contrast, the control treatment showed the lowest scavenging potential (89.31%). Seed priming with PEG at 20% resulted in the lowest IC50 (41.57 µg mL^{-1}), while the control had the highest IC_{50} (114.68 µg mL^{-1}). A lower IC_{50} value indicates greater antioxidant activity.

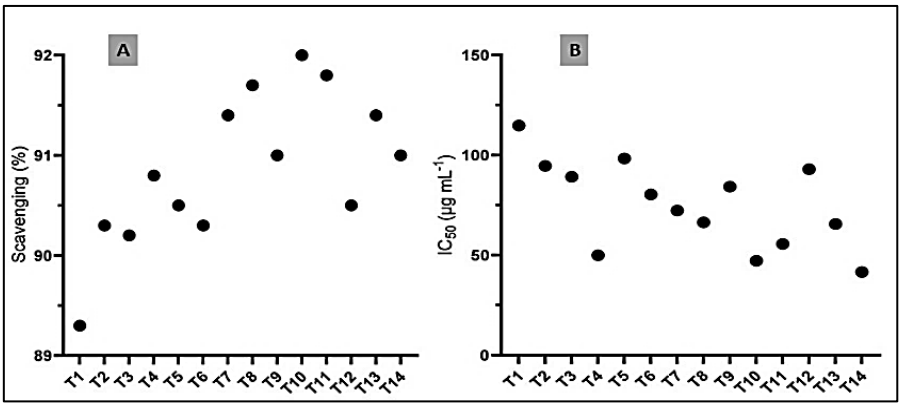

Figure 3. Changes in total antioxidant activity (% scavenging) [A] and IC_{50} (µg mL^{-1}) [B] of 3-month-old seedlings of *Bergenia ciliata* under the influence of various priming agents.

Antioxidant enzymes activity

The highest CAT activity (6.21 µmol H_2O_2 decomposed mg^{-1} protein min^{-1}) was found in GA_3 at 100 ppm and it was followed by NaCl at 0.8% (6.09) (Figure 4A). The lowest activity (5.05) was recorded in KNO_3 at 6% (**Supplementary Table 2**).

The highest POD activity (27.21 µmol tetra-guaiacol formed mg^{-1} protein min^{-1}) was found in HP at 12 h (Figure 4B). Higher POD activity was also observed in KNO_3 at 6% (23.95), followed by PEG at 10% (23.75), ascorbic acid at 25 ppm (23.48), and NaCl@0.4% (22.62), respectively. The lowest activity (16.04) was recorded in non-priming or control, but it was statistically at par with ascorbic acid @50 ppm (16.22) and PEG@10% (16.36).

The highest SOD activity (158.5 units mg^{-1} protein) was found in GA_3 at 100 ppm, which was closely followed by NaCl at 0.8% (156.6 units mg^{-1} protein) (Figure 4C). However, control or non-primed seedlings recorded the lowest SOD activity (146.4 units mg^{-1} protein).

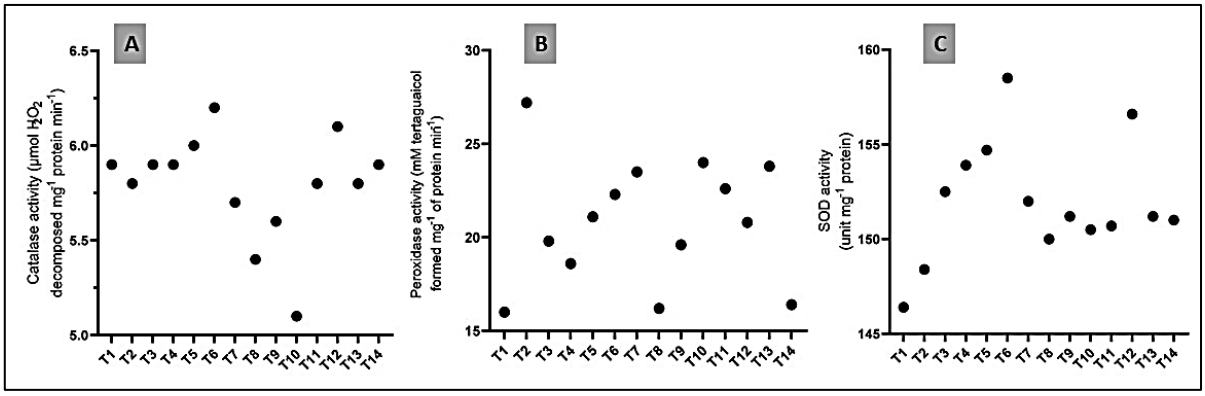

Figure 4. Changes in activities of antioxidant enzymes in 3-month-old seedlings of *Bergenia ciliata* under the influence of various priming agents. Catalase activity (µmol H_2O_2 decomposed mg^{-1} protein min^{-1}) [A], peroxidase (POD) activity (µm tetra-guaiacol formed mg^{-1} protein min^{-1}) [B], superoxide dismutase (SOD) activity (unit mg^{-1} protein) [C].

Principal component analysis and clustering

Principal component analysis (PCA) of morpho-physiological attributes in 3-month-old *B. ciliata* seedlings under various priming agents is illustrated in Figure 4. Key metrics, including eigenvalue, variability, cumulative variability, and the PCA loading matrix, were computed (**Supplementary Table 3**). Five principal components with eigenvalues greater than 1 accounted for a cumulative variability of 88.09%. Notably, the control treatment (T1) exhibited higher PC1 scores, positioning it distinctly on the right side of the PC1 axis (Figure 5).

Treatments were organized on the right side based on increasing PC1 scores: T3 > T8 > T10 > T2 > T9 > T14 > T4. PC1 indicated significant negative loadings for root length (RL), total length (TL), dry weight (DW), leaf area (LA), fresh weight (FW), superoxide dismutase (SOD), and shoot height (SH), while the IC_{50} parameter showed a positive loading. In contrast, PC2 displayed strong positive loadings for scavenging percentage, total phenols, and total proteins, but high negative loadings for IC_{50} and catalase activity.

Additionally, a hierarchical cluster analysis (HCA) dendrogram was done. It helped to evaluate the similarity of the priming treatments by using Ward's method. The HCA resulted in the formation of 3 groups from the fourteen priming treatments on all morpho-physiological and biochemical traits of 3-months old *Bergenia* seedlings (Figure 6). The precise distribution of various priming treatments in different clusters has been shown in Table 2a. The first group comprised five treatments, viz. T6 (100 ppm GA_3), T12 (0.8% NaCl), T5 (50 ppm GA_3), T7 (25 ppm ascorbic acid), and T11 (0.4% NaCl). The control treatment, i.e., T1, formed a second group individually, and it explained the minimal values of almost all seedlings' parameters except for IC_{50} and CAT activity (Table 2b). Whereas the treatments like T10 (6% KNO3), T8 (50 ppm ascorbic acid), T9 (3% KNO_3), T2 (HP@12h), T13 (10% PEG), T4 (25 ppm GA_3), T3 (HP@24h), and T14 (20% PEG) formed cluster III. Cluster II also indicated that the enhanced effect for all the seedling traits came under the influence of priming, which was a bit less when compared with cluster I.

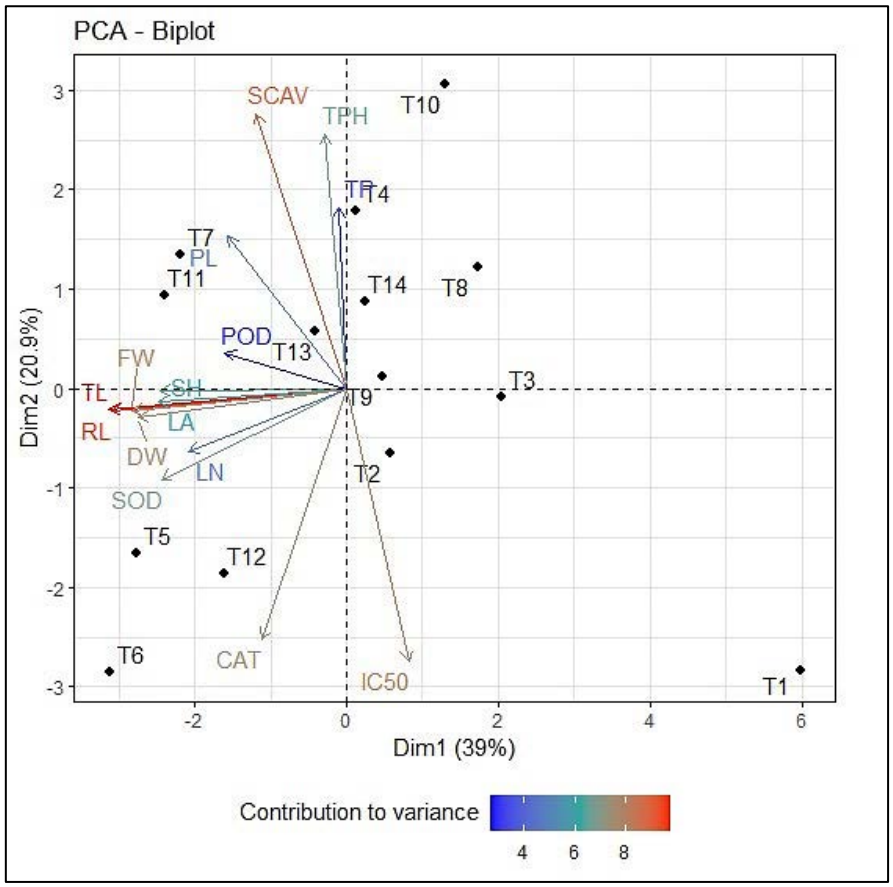

Figure 5. PCA-Biplot of different germination traits and seedling growth parameters of 3-months old seedling as influenced by various priming agents in *Bergenia ciliata*. SH: shoot height; RL: root length; TL: total length; FW: fresh weight; DW: dry weight; LA: leaf area; LN: leaf number; PL: petiole length; TP: total protein; TPH: total phenol; SCAV: scavenging %; IC_{50}: concentration for 50% scavenging; CAT: catalase; POD: peroxidase; SOD: superoxide dismutase.

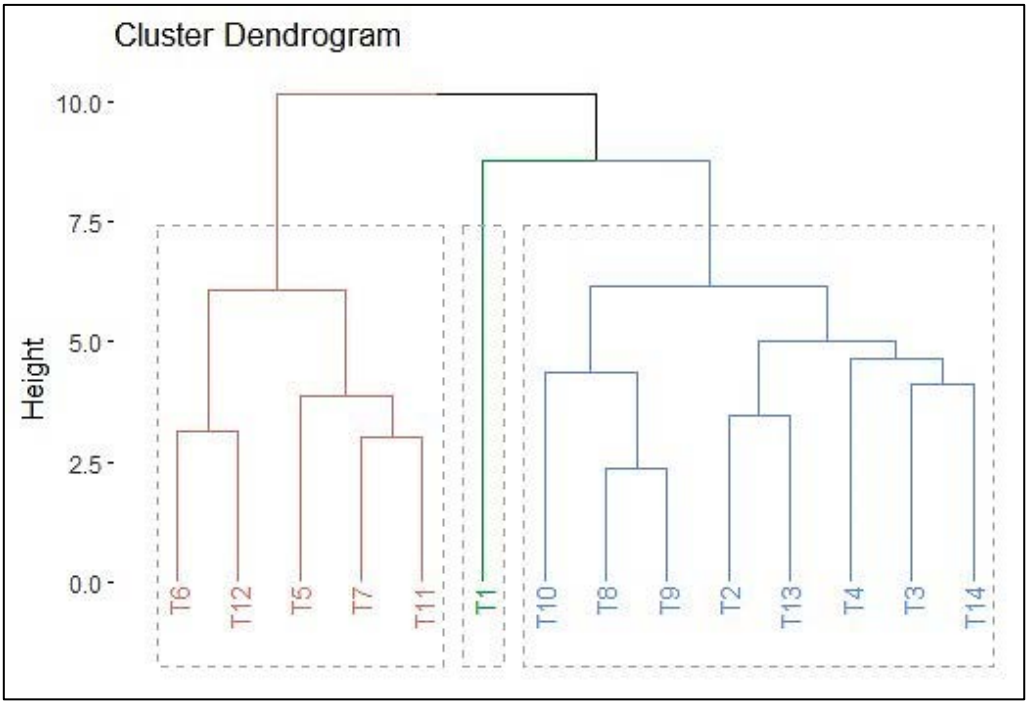

Figure 6. Cluster dendrogram of seed priming treatments using Ward's method of systematic classification.

Table 2a. Distribution of different priming agents in different clusters based on morpho-physiological parameters of 3-month-old *Bergenia* seedlings

Cluster	No of treatments	Name of treatments
I	5	GA_3 (100 ppm), NaCl (0.8%), GA_3 (50 ppm), Ascorbic Acid (25 ppm), NaCl (0.4%)
II	1	**Control**
III	8	KNO_3 (6%), Ascorbic Acid (50 ppm), KNO_3 (3%), HP (12 hrs), PEG (10%), GA_3 (25 ppm), HP (24 hrs), PEG (20%)

Table 2b. Cluster means for different morpho-physiological parameters of 3-month-old seedlings under the influence of various priming agents

Parameters	Cluster I	Cluster II	Cluster III
RL	6.70	4.68	5.83
SH	0.607	0.480	0.558
TL	7.30	5.16	6.38
FW	9.14	7.21	7.95
DW	1.45	1.11	1.26
LA	0.392	0.246	0.363
LN	4.90	4.00	4.56
PL	0.360	0.290	0.350
TP	18.92	14.75	22.42
TPH	12.86	10.58	14.19
SCAV	90.90	89.31	91.04
IC_{50}	79.89	114.68	67.32
CAT	5.96	5.90	5.65
SOD	154.5	146.4	151.1
POD	22.07	16.04	20.68

N.B. RL: root length; SH: shoot height; TL: total length; FW: fresh weight; DW: dry weight; LA: leaf area; LN: leaf number; PL: petiole length; TP: total protein; TPH: total phenol; SCAV: scavenging %; IC_{50}: concentration for 50% scavenging; CAT: catalase; POD: peroxidase; SOD: superoxide dismutase.

Discussion

Among the priming treatments, GA_3 at 50 ppm resulted in the longest root and total lengths in 3-month-old seedlings, while the control group exhibited the shortest. This enhancement can be attributed to the role of GA_3 in synthesizing hydrolytic enzymes during germination and its ability to promote embryonic growth through cell elongation (Aziz and Pekşen 2020). Researchers also noted that cell elongation occurred through signaling mechanisms recognized by GA_3 via receptor molecules (Pallardy 2010). This interaction facilitates the formation of cell wall-loosening factors or inhibits wall-stiffening factors, thereby promoting cell expansion.

Earlier studies have reported that priming enhances photosynthetic area and biomass by increasing leaf number and area (Singhal et al. 2021). Our findings align with Shahzad et al. (2014), who demonstrated that GA_3 at 10^{-4} M significantly boosts leaf count and area in sponge gourd. Additionally, Aziz and Pekşen (2020) reported that GA_3 seed priming improved chickpea seedling growth under chilling stress.

The current investigation indicated that seed priming may enhance antioxidant enzyme activity. Enhanced germination, root traits, and seedling growth in *Bergenia* under priming treatments can be attributed to increased antioxidant potential, primarily reflected by elevated total phenol content, SOD, and POD activity, along with reduced IC_{50} values. Phenolic compounds are vital for scavenging radicals by absorbing and neutralizing oxygen and peroxide radicals (Osawa 1994).

Improvements in CAT, SOD, and POD activities were noted in rice seedlings subjected to priming (Zheng et al. 2016). Primed seeds exhibit significantly increased activities of CAT, ascorbate

peroxidase (APX), glutathione peroxidase (GPOX), and SOD compared to unprimed seeds (Sen and Puthur 2020).

Enhanced germination rates due to priming are linked to increased activities of SOD, CAT, and POD, which are critical for processes like germination and repair. Researchers have suggested that the antioxidant role of peroxidases is closely related to the physiological state during late germination and early seedling growth (Gomes and Garcia 2013). Free radical scavenging enzymes, including SOD, POD, and CAT, operate more efficiently when seeds are primed, boosting germination rates while reducing lipid peroxidation (Sharma et al. 2014). Huang et al. (2019) noted that as time progresses post-germination, the sites generating reactive oxygen species (ROS) transition within organelles, underlining the importance of a robust antioxidant system for normal photosynthesis and stress response. Overall, our findings suggest that seed priming positively modifies metabolic pathways to enhance cellular equilibrium, supporting better seedling establishment. Seed priming with GA_3 was reported to lower the oxidative damage triggered by salt in *Isatis indigotica* fort seedlings through enhancement of enzyme activities for CAT, POD, and SOD (Jiang et al. 2020). Furthermore, Khan et al. (2022) reported that pre-treatment of the seeds using KNO_3 enhanced antioxidant activities, including peroxidase, in maize plants grown under alkalinity stress.

Principal Component Analysis (PCA) is a crucial statistical technique for multivariate analysis, valuable in identifying patterns and revealing interrelationships among variables. It aids in understanding the associations between parameters and treatments while simplifying data dimensions to highlight relationships influenced by priming treatments. Notably, the biochemical parameter IC_{50} significantly contributed to PC1, as indicated by the vector length; a longer vector signifies a greater influence on the principal component. IC_{50} was negatively correlated with scavenging percentage and total phenol content, suggesting that a lower IC_{50} value—or higher antioxidant scavenging capacity and phenol content—is a vital seedling parameter positively affected by seed priming, promoting cellular homeostasis.

Moreover, cluster analysis illustrated distinct groupings of data, showing that primed seeds formed a separate cluster from control seeds (T1), indicating significant differences. These findings align with previous studies by Gour et al. (2023). Consequently, results from these multivariate analyses imply that seed priming enhances 'pre-germinative metabolisms' and bolsters antioxidant defense systems through various agents.

Conclusion

This study is the first to examine the effectiveness of seed priming techniques to enhance seedling establishment in this valuable medicinal herb, which faces challenges in germination due to its small size and low viability. The positive effects of Various seed priming agents were demonstrated by improvements in attributes such as total protein, total phenol content, scavenging percentage, as well as SOD and POD activities, accompanied by a decrease in IC_{50} values. PCA revealed favorable changes in seedling characteristics resulting from different priming treatments. Cluster analysis highlighted the varied effects of priming agents, particularly GA_3, ascorbic acid, and NaCl, based on their concentrations, with non-priming treatment (control or T1) forming a distinct cluster. The findings of this study have significant practical implications for the mass propagation of high-quality planting materials of this important medicinal plant and offer valuable insights for researchers in this field.

Authors' contributions

Both the authors listed above have contributed substantially and intellectually, and approved it for publication. KMC – executed the original experiment, data analysis, and drafted the original manuscript; KB, BM & SM – performed investigation, data analysis, manuscript drafting and editing; PD – originally conceived the idea, interpreted the data, critically revised the work, reviewed and edited the manuscript; MKD – statistical analysis, Drawing Graphs & Plots.

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Conflict of Interest

The authors declare that this publication does not have any conflicts of interest.

Data Availability Statements

However, all the data supporting the findings of this study can be made available on request from the Corresponding author.

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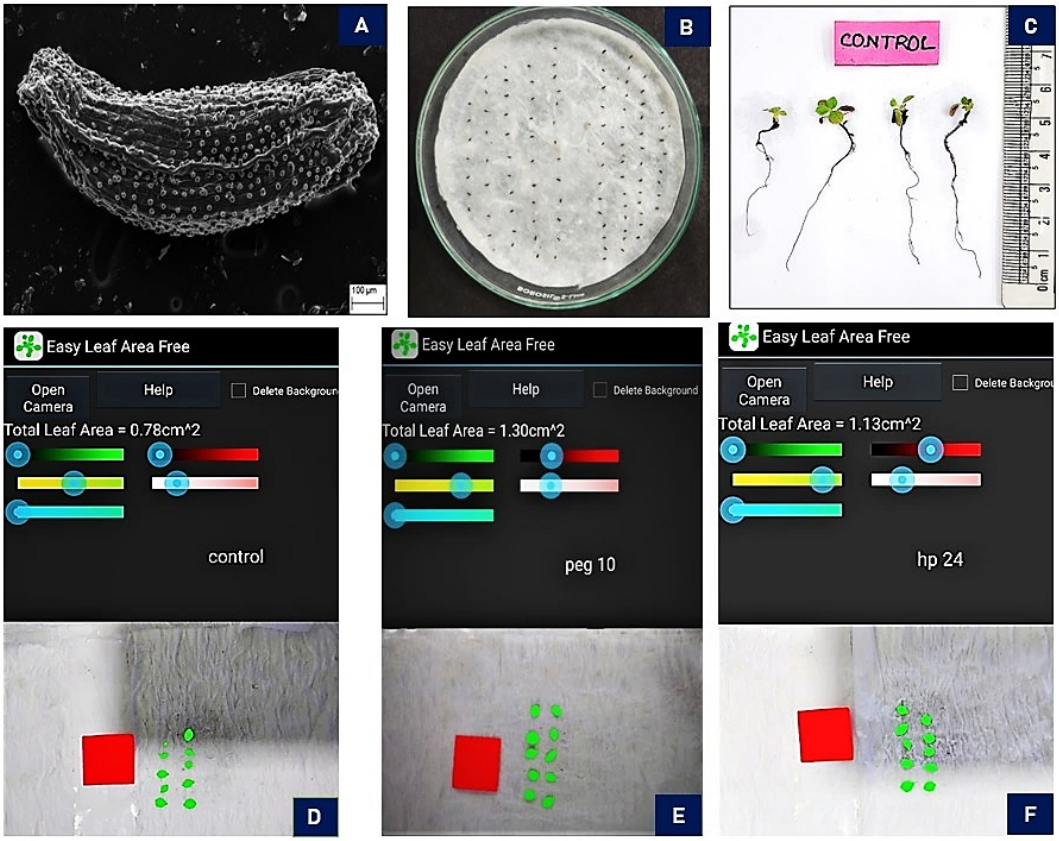

Supplementary Figure 1. Scanning Electron Microscopic photograph of a single *Bergenia* seed [A]; Placing of seeds on Petri dish for germination [B]; 3-month-old *Bergenia* seedling grown from non-primed (control) treatment [C]; Leaf area measurements using the Easy Leaf Area Free app [D-F].

Supplementary Table 1. Effect of various priming agents on total protein content (mg g^{-1} fresh weight), total phenolic content (mg GAE g^{-1} DM), total antioxidant activity (% Scavenging) and IC_{50} (µg mL^{-1}) of 3-month-old seedlings *of Bergenia ciliata*

Treatment	Total protein	Total phenol	Scavenging (%)	IC_{50} (µg mL^{-1})
Control	14.75^{g}	10.58^{j}	$89.31(9.48)^{g}$	114.68^{a}
HP (12h)	21.44^{cd}	14.40^{d}	$90.27(9.53)^{ef}$	94.60^{c}
HP (24h)	25.50^{b}	13.32^{e}	$90.16(9.52)^{fg}$	89.09^{e}
GA_3 (25ppm)	30.75^{a}	18.43^{a}	$90.83(9.56)^{cdef}$	49.88^{k}
GA_3 (50ppm)	18.94^{def}	12.28^{h}	$90.49(9.54)^{ef}$	98.31^{b}
GA_3 (100ppm)	16.56^{fg}	10.62^{j}	$90.27(9.53)^{ef}$	80.35^{g}
Ascorbic Acid (25ppm)	20.88^{cde}	16.50^{b}	$91.39(9.59)^{abcd}$	72.31^{h}
Ascorbic Acid (50ppm)	18.69^{def}	13.05^{f}	$91.67(9.60)^{abcd}$	66.36^{i}
KNO_3 (3%)	19.75^{def}	12.57^{g}	$91.00(9.57)^{bcde}$	84.23^{f}
KNO_3 (6%)	22.13^{bcd}	14.90^{c}	$91.96(9.62)^{a}$	47.19^{l}
NaCl (0.4%)	18.88^{def}	13.53^{e}	$91.79(9.61)^{ab}$	55.58^{j}
NaCl (0.8%)	19.31^{def}	11.35^{i}	$90.55(9.54)^{def}$	92.92^{d}
PEG (10%)	23.75^{bc}	12.07^{h}	$91.45(9.59)^{abc}$	65.64^{i}
PEG (20%)	17.38^{efg}	14.78^{c}	$91.00(9.57)^{bcde}$	41.57^{m}
SEm(±)	0.828	0.060	0.008	0.279
CD (p≤0.05)	2.411	0.175	0.024	0.812

N.B. *Arcsine* transformation of percentage data was done for ANOVA and post hoc test. The different letters (superscript) on the same columns indicate significant differences between treatments as per Tukey's Honest Significant Difference test at $p < 0.05$.

Supplementary Table 2. Effect of various priming agents on catalase (CAT) activity (µmol H_2O_2 decomposed mg^{-1} protein min^{-1}), superoxide dismutase (SOD) activity (unit mg^{-1} protein), and peroxidase (POD) activity (µm tetra-guaiacol formed mg^{-1} protein min^{-1}) in 3-month-old seedlings of *Bergenia ciliata*

Treatment	CAT	SOD	POD
Control	5.90^{cde}	146.4^{e}	16.04^{h}
HP (12h)	5.78^{def}	148.4^{de}	27.21^{a}
HP (24h)	5.85^{def}	$152.5b^{cde}$	19.80^{efg}
GA_3 (25ppm)	5.93^{cd}	153.9^{abcd}	18.57^{g}
GA_3 (50ppm)	6.04^{bc}	154.7^{abc}	21.06^{de}
GA_3 (100ppm)	6.21^{a}	158.5^{a}	22.34^{cd}
Ascorbic Acid (25ppm)	5.73^{f}	152.0^{bcde}	23.48^{bc}
Ascorbic Acid (50ppm)	5.36^{h}	150.0^{cde}	16.22^{h}
KNO_3 (3%)	5.57^{g}	151.2^{bcde}	19.57^{fg}
KNO_3 (6%)	5.05^{i}	150.5^{bcde}	23.95^{b}
NaCl (0.4%)	5.75^{f}	150.7^{bcde}	22.62^{bc}
NaCl (0.8%)	6.09^{ab}	156.6^{ab}	20.84^{ef}
PEG (10%)	5.76^{ef}	151.2^{bcde}	23.75^{bc}
PEG (20%)	5.92^{cd}	151.0^{bcde}	16.36^{h}
SEm(±)	0.033	1.211	0.322
CD (p≤0.05)	0.096	3.527	0.938

N.B. The different letters (superscript) on the same columns indicate significant differences between treatments as per Tukey's Honest Significant Difference test at $p < 0.05$.

Supplementary Table 3. Eigen value, variability (%), cumulative variability (%), and PCA loading of different morpho-physiological parameters of 3-month-old seedlings influenced by various priming agents in *Bergenia ciliata*

Characters	PC1	PC2	PC3	PC4	PC5
RL	-0.382	-0.037	-0.176	0.192	0.065
TL	-0.386	-0.035	-0.182	0.165	0.037
DW	-0.339	-0.049	-0.237	-0.008	0.397
LA	-0.306	-0.023	0.441	0.008	-0.145
TP	-0.014	0.307	0.443	-0.004	0.439
TPH	-0.036	0.431	0.249	0.011	0.294
SCAV	-0.147	0.466	-0.230	-0.016	-0.172
IC50	0.100	-0.461	-0.067	-0.153	0.247
CAT	-0.136	-0.423	0.291	0.240	0.171
SOD	-0.301	-0.153	0.230	0.225	-0.177
POD	-0.197	0.060	0.085	-0.733	0.077
SH	-0.303	-0.003	-0.210	-0.235	-0.339
FW	-0.348	-0.037	-0.193	-0.123	0.373
LN	-0.256	-0.105	0.379	-0.249	-0.336
PL	-0.194	0.257	0.016	0.367	-0.115
Eigen value	5.843	3.130	1.958	1.243	1.040
Variability (%)	38.95	20.87	13.05	8.29	6.93
Cumulative variability (%)	38.95	59.82	72.88	81.16	88.10

Supplementary Table 4. Coefficient matrix of component scores of various priming agents in 3-month-old seedlings of *Bergenia ciliata*

Treatments	PC1	PC2	PC3	PC4	PC5
T1	5.988	-2.839	-0.886	-0.068	0.433
T2	0.574	-0.644	0.896	-2.668	0.651
T3	2.051	-0.083	1.960	0.741	0.182
T4	0.116	1.794	2.369	0.846	1.731
T5	-2.760	-1.651	-1.643	0.427	1.345
T6	-3.113	-2.853	0.699	-0.626	-1.479
T7	-2.199	1.343	0.186	-0.307	0.234
T8	1.731	1.224	-1.695	0.900	-0.405
T9	0.467	0.119	-1.477	0.453	-0.085
T10	1.295	3.060	-0.714	-1.624	-1.366
T11	-2.388	0.943	-1.967	0.087	0.007
T12	-1.604	-1.862	0.900	0.752	-0.729
T13	-0.412	0.581	0.753	-0.525	0.163
T14	0.252	0.869	0.619	1.613	-1.562