Research Square

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Germination Response of Smoke Water Treatment in Buckwheat Fagopyrum esculentum Seeds

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Abstract

Seed germination represents a vital phase in the plant life cycle and is strongly stimulated by environmental cues, including smoke generated during fire events. The ecological phenomenon of pyrophytism, in which seeds germinate after exposure to wildfire smoke followed by rainfall, underscores the regulatory role of smoke in seed biology. Beyond stimulating germination, Smoke has been reported to promote post-germination growth and development. Traditional practices such as slash-and-burn agriculture and smoke-based seed treatments in regions including South Africa and Northeast India further highlight its agronomic relevance. Smoke water, prepared by dissolving smoke from burned plant biomass in water, contains bioactive compounds that regulate germination and seedling vigour. This research examined the influence of smoke water that is derived from paddy straw, neem leaves-wood, and mango leaves-wood on germination and early seedling performance of buckwheat (*Fagopyrum esculentum* Moench). Seeds were soaked for 12 hours in six concentrations of smoked water, with distilled water as control. Seedling vigour indices were recorded. Paddy straw smoke water significantly enhanced germination, while neem showed a dose-dependent response and mango exhibited a non-linear trend. Overall, responses were strongly source and concentration-dependent, emphasizing the need for optimization for agricultural application.

1. INTRODUCTION

Germination is a crucial event in the overall life cycle of angiospermic plants, influenced by a several external/internal factors. One such factor is the smoke produced by fire, which has an important role in enhancing germination, mainly in post-fire conditions. This phenomenon, where plants germinate after a wildfire followed by rainfall, is referred to as pyrophytism, and these plants involved are known as pyrophytic species [1; 2]. Recent studies have focused on how seeds respond to smoke water to promote germination, where plant material is burned and the resulting smoke is bubbled through water [3].

The slash-and-burn technique, which involves using fire to clear forest land for agriculture, has been a traditional practice among indigenous communities globally. De Lange and Boucher [3] were among the first to investigate the role of smoke in promoting germination, using the endangered species *Audouinia capitata* as a model. In many developing countries, crop residue is often cleared by prescribed burning, which also benefits modern farmers by speeding up field operations, lowering residue management costs, and controlling weeds [4]. Some species, such as *Leucospermum cordifolium* and *Serruria florida*, require smoke exposure for germination to occur, demonstrating the vital role of smoke in post-fire conditions [3]. While the germination influence of smoke is commonly associated with fire-prone environments, many species originating from non-fire-prone environments areas also exhibit strong responses. For example, species like lettuce [5], celery [6], red rice [7], and wild oats [8] all show enhanced germination of seeds in response to smoke.

Based on previous research suggesting that smoke can boost seedling growth and promote germination, we hypothesized that smoke water could increase germination percentages and influence seedling growth in buckwheat seeds (Figure 6). This study demonstrates the potential of using smoke water in agriculture and plant residue management by utilizing paddy straw to generate smoke in a controlled chamber to promote seed germination and improve seed vigor. Smoke produced by burning one kilogram of each material (paddy straw, neem leaves-wood and mango leaves-wood) was dissolved into one liter of distilled water under pressure using an electric motor (Figure 1). The resulting smoked stock solution was diluted to concentrations of 0.1%, 0.5%, 2%, 5%, 8%, and 10%. The seeds were soaked for 12 hours in each treatment, and various parameters like, germination frequency, seedling length (root/shoot length combined), fresh weight, dry weight, and seedling vigor indices (I and II), were recorded for each treatment. Our results will highlight the optimal concentration and soaking duration of smoke water for enhancing germination in buckwheat seeds.

2. MATERIAL AND METHODS

2.1 Preparation of smoke water:

Plant-derived smoke metabolites are known to be water-soluble, enabling their efficient extraction into aqueous solutions [9]. In the current study, smoke water was prepared using a controlled combustion and bubbling method to ensure maximum dissolution of biologically active compounds, as described previously [10]. Briefly, 1 kg each of paddy straw (PSW), neem leaves-wood (NSW), and mango leaves-wood (MSW) were combusted separately in a closed chamber (25 cm × 25 cm × 40 cm) fitted with a 9 cm × 9 cm ignition opening. The generated smoke was channeled through PEX (cross-linked polyethylene) pipe connected to an electric motor system that directed the smoke into one litre of distilled water in a conical flask, allowing continuous bubbling for effective extraction (Fig. 1). After complete combustion, the resulting stock solution, filtered twice through Whatman No. 42 filter paper to remove particulate matter. The filtered smoke water was stored at room temperature and subsequently diluted to the required concentrations for experimental use.

2.2 Plant material and treatment:

The seeds of buckwheat (*Fagopyrum esculentum*) genotype PRB1 were obtained from National Bureau of Plant Genetic Resources (NBPGR), India. The filtered stock solution of prepared smoke water (diluted with distilled water) so as to obtain six concentrations of, 0.1%, 0.5%, 2%, 5%, 8%, and 10%. These dilutions constituted six treatments along with a control (distilled water), resulting in a total of seven treatments. Seeds were soaked in the respective treatment solutions for 12 hours to ensure adequate exposure to smoke-derived bioactive compounds (Fig. 2). Germination percentage and seedling parameters were recorded.

2.3 The determination of germination frequency:

Germination tests were conducted using the between-paper (BP) method in accordance with the guidelines of the International Seed Testing Association. Seeds, placed evenly between the two sheets of moistened germination paper, covered with an additional moist sheet, and rolled carefully to maintain uniform contact. The were incubated in a germination chamber for incubation at controlled temperature and humidity conditions [11] and final germination counts were recorded after 7 days. The trial done was arranged in Completely Randomized Design having four replications of 100 seeds each per treatment. Germination frequency was assessed for each replication, and the mean germination percentage was determined following ISTA procedures using the formula:

Germination (%) = (Number of seeds germinated ÷ Total number of seeds sown) × 100.

2.4 Determination of Seedling Parameters:

On the seventh day, ten seedlings from each treatment were randomly selected for growth assessment. Root and shoot lengths were measured using a graduated scale, and total seedling length was calculated. Fresh weight was recorded using a precision electronic balance by weighing ten randomly selected seedlings from each treatment. For dry weight determination, seedlings oven-dried at 100°C for 1 h to obtain constant weight [12]. Seedling vigour indices calculated where, Vigour Index-I = Germination (%) × Total seedling length (cm) and Vigour Index-II = Germination (%) × Seedling dry weight (g).

2.5 Statistical Analysis:

A completely randomized design with factorial treatment arrangement was adopted for the experiment. Two-way analysis of variance (ANOVA) was performed to determine the main and interaction effects [13]. To evaluate the relationship, correlation analysis was performed to determine relationships among measured traits [14], and Principal Component Analysis (PCA) was done to detect the major influencing variables and treatment patterns [15].

3. RESULTS

This study evaluated the effects of different smoke-water sources and concentrations on buckwheat germination and seedling growth. Parameters including germination, biomass, seedling length, and vigour indices showed source-specific and dose-dependent responses. Lower concentrations enhanced seedling performance, whereas higher concentrations produced variable or inhibitory effects, indicating a hormetic regulatory response.

3.1 Two-way Analysis of Variance (ANOVA):

A two-way analysis of variance (ANOVA) was performed to assess the individual and interactive effects of smoke-water type (PSW, MSW, and NSW) and concentration (0.1–10%) on germination percentage, seedling growth traits, and vigour indices of buckwheat (*Fagopyrum esculentum* Moench). It was observed that smoke-water type significantly influenced germination percentage, dry weight, and both vigour indices, while its effect on fresh weight and seedling length was not significant.

3.1.1 Effect of Factor-1 (Smoke-water Types):

The results of the two-way ANOVA indicated a significant impact of smoke-water type (Factor-1) on germination rate, dry biomass, Vigour Index I, and Vigour Index II ($P \leq 0.05$), whereas fresh weight and seedling length were not significantly affected ($P > 0.05$) (Fig. 3). Among treatments, F1:1 recorded the highest germination (80.57%) and vigour indices, while F1:2 showed comparatively lower values (TableT1).

3.1.2 Effect of Factor-2 (Concentration of Smoke-water Levels):

ANOVA further indicated that smoke-water concentration (Factor-2) had significant main effect on all of the measured parameters ($P \leq 0.05$) (Fig. 4). Germination, biomass accumulation (fresh and dry weight), vigour indices, and seedling length varied markedly across concentration levels. The highest germination (89.89%) and VI I (957.17) was recorded at F2:7, while F2:1 showed the lowest germination (12.00%) (Table T2).

3.1.3 Interaction Effect (Factor-1 × Factor-2):

The interaction between smoke-water type and concentration (F1 × F2) was also highly significant for all parameters as revealed by two-way ANOVA ($P \leq 0.05$). This indicates that the effect of concentration on germination and seedling growth depended on the type of smoke-water applied (Fig. 5). The highest germination (96.67%) and VI I (1208.03) was observed under specific treatment combinations, whereas the lowest germination (12.00%) occurred at F2:1 across types. Notably, enhanced germination, biomass accumulation, and vigour were observed under specific smoke-water type × concentration combinations rather than under individual factor effects alone (Table T3).

3.2 Germination percent:

Germination percentage, recorded on the 7th day after treatment using a seed purity working board. Germination increased markedly from 12% in the control to 94% at T1 (0.1%) in PSW-treated seeds and remained relatively stable across higher concentrations, ranging from 90.33% at T3 (2%) to 91.33% at T6 (10%). In MSW-treated seeds, germination increased from 12% in the control to 55% at T1 (0.1%) and gradually rose with increasing concentration, reaching 81.67% at T6 (10%), although a reduction to 28.67% was observed at T2 (0.5%). NSW treatments showed variable responses, with germination increasing to 93% at T1 (0.1%), 90% at T4 (5%), and a maximum of 96.67% at T6 (10%), while a significant decline to 25.33% occurred at T5 (8%). Factorial analysis indicated that smoke-water type (Fig. 3a), treatment concentration (Fig. 4a), and their interaction (Fig. 5a), significantly influenced germination percentage ($p < 0.05$).

3.3 Fresh weight:

Fresh weight was measured by weighing 10 seedlings per replicate and expressed in grams. In the control, seedling fresh weight was 0.29 g. Exposure to PSW resulted in a marked increase in fresh weight across all treatments compared with the control. The highest fresh weight (1.47 g) was recorded at T2 (0.5%) and T4 (5%), followed by a decline to 0.85 g at T6 (10%). In MSW-treated seedlings, fresh weight showed concentration-dependent variation, with maximum values at T1 (0.1%, 1.20 g) and T3 (2%, 1.17 g), while the lowest value was observed at T2 (0.5%, 1.06 g), indicating sensitivity to lower concentrations. NSW treatments exhibited greater variability, with the highest fresh weight at T1 (0.1%, 1.30 g) and the lowest at T5 (8%, 0.94 g). Factorial analysis showed that treatment concentration significantly affected fresh weight (Fig. 4b), whereas smoke-water type alone was not significant ($p = 0.097$) (Fig. 3b). However, the interaction between smoke-water type and concentration (Fig. 5b) significantly influenced fresh weight ($p < 0.05$).

3.4 Dry weight:

Seedling dry weight in the control was consistently low (0.04 g). Exposure to PSW resulted in a notable increase in dry weight across treatments, with a maximum of 0.11 g recorded at T4 (5%), followed by a decline at T3 (2%, 0.04 g) and T2 (0.5%, 0.05 g). MSW treatments also enhanced dry weight, with relatively higher values at T1 (0.1%, 0.09 g) and T3 (2%, 0.09 g), and a reduction to 0.05 g at T5 (8%). In contrast, NSW treatments showed greater variability, with the highest dry weight observed at T1 (0.1%, 0.13 g), while T3 (2%) and T4 (5%) recorded the lowest values (0.07 g). Factorial analysis indicated that both smoke-water type and treatment concentration significantly affected dry weight ($p < 0.05$) (Fig. 3c and 4c). Similar to fresh weight, the interaction between smoke-water type and concentration (Fig. 5c) was also highly significant ($p = 0.000$) (Fig. 5c).

3.5 Seedling length:

Seedling length was enhanced significantly by treatments of smoke-water compared to the control, demonstrating a clear stimulatory effect on early growth. Paddy straw smoke water (PSW) produced the greatest increase at moderate concentrations (0.5–2%), indicating optimal stimulation of cell division and elongation under balanced exposure. Mango smoke water (MSW) also improved seedling length across concentrations, with peak elongation at 5%, suggesting effective growth promotion at intermediate doses. In contrast, neem smoke water (NSW) showed variable responses, with stimulation restricted to low concentration (0.1%) and reduced growth at higher levels, likely due to inhibitory constituents. Factorial analysis revealed that treatment concentration significantly affected seedling length (Fig. 4d), whereas smoke water source alone was not significant (Fig. 3d), emphasizing concentration as the principal determinant of elongation. The near-significant interaction between smoke type and concentration indicates emerging source-specific trends (Fig. 5d), supporting the concept that smoke-induced growth responses are dose-dependent.

3.6 Vigor indices:

4.6.1 Vigor index I (VI I)

The mean values of VI I at different concentrations varies for PSW, MSW, and NSW. In PSW, Vigor index I value varied gradually from T2 (0.5%) having the highest value of 1129.23 and T5 (8%) having lowest value i.e., 752.27. In MSW, there was a fluctuating trend, with the values peaking at T6 (10%) to be 888.34 and lowest for T2 (0.5%) which is 325.65. For NSW, the mean values showed a mixed pattern, with value reaching its peak at T2 (0.5%) i.e., 1208.02 and for the last treatment T6 (10%) i.e., 1119.49 and the values decreasing at T5 (8%) i.e. 184.633.

3.6.2 Vigor index II (VI II)

In PSW, the treatment T4 demonstrated its highest mean expression at (5%) to be 10.12, and decreases for T1 (0.1%) and T5 (8%) which are 7.54 and 7.40 respectively as compared to the control i.e., 0.48. For MSW, the mean values fluctuated across concentrations between 2.08 for T2 (0.5%) and 5.67 for T6 (10%). Interestingly, the highest mean expression occurred at T6 (10%) for the last treatment i.e., 5.67. Moving to NSW, the VI II ranged from 1.96 of T5 (8%) to 10.48 for T1 (0.1%). Particularly, the first treatment displayed its peak expression at T1 (0.1%) to be 10.47.

Factorial analysis reveals that both factors, type of Smoke-water and Treatment concentration, significantly affect both VI I and VI II (Fig. 3e, 4f and 4e, 4f), and the interaction between Smoke and Treatment is also significant for both Vigor indices (Fig. 5e, 5f) as evidenced by their low p-values (< 0.05).

3.7 Correlation:

Pearson's correlation: this analysis revealed strong and predominantly positive associations among seed germination and vigour-related traits (Fig. 6). Correlation coefficients ranged from 0.75 to 0.97, with most relationships being statistically significant ($p < 0.05$ to $p < 0.001$). Seed dry weight (DW) and imbibition-related traits (W_1 and W_2) showed very strong and highly significant correlations with germination percentage (GP) and vigour indices (VI_1 and VI_2). Fresh weight (FW) and vigour index I exhibited a stronger positive association ($r = 0.89$, $p < 0.01$) (Table T4). In contrast, the correlation between germination percentage and seedling length (SL) was not statistically significant.

3.8 Principal Component Analysis (PCA):

Principal component analysis (PCA) resulted in a marked reduction in data dimensionality (Fig. 7). The screen plot showed that 1st principal component (PC1) contributed for approximately 89–90% of the overall variance, whereas the second principal component (PC2) explained for around 6–7%, resulting in a cumulative variance explanation of > 95% by the first two components (Fig. 7a). All remaining components individually explained < 3% of the variance and had eigenvalues below unity.

The loading plot showed that Seedling Length (SL) and Fresh Weight (FW) had strong positive loadings on PC1, while Germination Percentage (GP), Dry Weight (DW), Vigour Index I (VI-I), and Vigour Index II (VI-II) also exhibited positive associations, indicating strong correlations among growth and vigour traits (Fig. 7b). The PCA score plot demonstrated that lower smoke-water concentrations (0.1–1%),

particularly PSW and MSW, were distributed on the positive side of PC1, whereas higher concentrations (5–10%), especially NSW and concentrated PSW treatments, clustered on the negative side (Fig. 7c).

The PCA biplot (Fig. 7d) revealed clear associations between treatments and growth traits. Low smoke-water concentrations (0.1–1%), particularly PSW and MSW, aligned with seedling length, fresh weight, germination, and vigour indices, indicating enhanced growth. In contrast, higher concentrations (5–10%) were negatively associated with these traits, showing inhibitory effects.

4. DISCUSSION

Seed germination is a tightly regulated developmental phase of the plant life cycle, governed by endogenous hormonal signals and environmental cues [16]. Among natural bio-stimulants, smoke from plant derived materials has received considerable attention for its ability to enhance seed germination and vigour by early seedling growth across diverse species [17;18]. Smoke-water, produced through biomass combustion, contains biologically active compounds such as karrikins and related butanolides that stimulate germination by enhancing seed sensitivity to germination-promoting hormones and activating early metabolic processes [19;20]

Karrikins also promote embryo growth, enhance cell cycle activation, stimulate β-tubulin accumulation, and modulate reactive oxygen species (ROS) homeostasis, collectively leading to radicle protrusion and dormancy release. Recent reviews further substantiate the vital role of smoke-water and karrikins in enhancing germination and early seedling vigor across species [10]. Karrikins are widely reported to exhibit maximal stimulatory effects on seed germination at low concentrations [21]. Similarly, smoke water derived from paddy straw (PSW), neem leaves-wood (NSW), and mango leaves-wood (MSW) significantly influenced germination and early seedling vigour of buckwheat (*Fagopyrum esculentum* Moench) in a concentration-dependent manner. Lower concentrations (0.1–1%) enhanced germination percentage, seedling length, biomass accumulation, and vigour indices, whereas higher concentrations (5–10%) reduced performance. Variation among PSW, NSW, and MSW treatments likely reflects differences in biomass composition and resultant pyrolysis products, as supported by statistical separation through correlation and principal component analyses.

4.1 Germination percent:

Germination percentage is an integrative indicator of seed viability and early physiological competence, making it a key parameter for assessing bio-stimulant efficacy [23]. Germination was significantly influenced in the present study by smoke-water source, concentration, and their interaction, with PSW showing the strongest and most consistent stimulatory effect (Fig. 8). Similar promotive effects of smoke treatments have been documented across various species [24;25]. Mango smoke water (MSW) exhibited a non-linear trend, suggesting a balance between stimulatory and inhibitory constituents depending on concentration. Neem smoke water (NSW) enhanced germination only at lower concentrations, whereas higher doses reduced germination, likely due to the inhibitory action of neem-

derived allelochemicals at elevated levels [26]. The significant interaction between smoke water type and concentration underscores the need for source- and dose-specific optimization to maximize germination responses in buckwheat (Fig. 5a). This promotory effects at optimal doses may be associated with smoke-derived bioactive compounds, particularly karrikin-like butenolides, which are known to stimulate germination by modulating the gibberellic acid (GA)–abscisic acid (ABA) balance and activating germination-related metabolic pathways [18;27;10].

4.2 Fresh and Dry weight:

Seedling fresh and dry weight are reliable indicators of early biomass accumulation, reflecting water uptake, cell expansion, and reserve mobilization during initial growth [16;28]. In current study, fresh weight was significantly influenced by smoke-water treatments, with concentration exerting a greater effect than source, while PSW at moderate concentrations produced the highest increase, indicating enhanced seedling vigour and water uptake. Comparable increases in seedling biomass following smoke application have been reported in other species [24;25]. Seedling dry weight also significantly affected by smoke water source and concentration, indicating differences in biomass accumulation and reserve utilization. The marked increase under PSW (notably at 5%) suggests improved metabolic conversion of seed reserves into structural biomass. Similar enhancements in early growth have been recorded previously [29;25]. The significant interaction effect indicates that the influence of treatment concentration on fresh and dry weight was dependent on smoke-water type, suggesting differential sensitivity of seedlings to specific smoke-derived compounds.

4.3 Seedling length:

Seedling length, an important indicator of early growth and vigour, exhibited a clear concentration-dependent response to smoke water treatments [12]. PSW significantly enhanced seedling elongation at low to moderate concentrations (0.5–2%), suggesting effective stimulation of cell elongation and early developmental processes. However, the reduced response at higher concentration (8%) indicates potential inhibitory effects at elevated doses. MSW similarly promoted elongation, with maximum stimulation at moderate levels, supporting the concept that optimal smoke concentrations enhance growth whereas excessive amounts suppress it. In contrast, NSW showed variable responses, with stimulation confined to very low concentrations, likely reflecting source-specific chemical composition and possible allelopathic effects at higher doses. Factorial analysis demonstrated that treatment concentration exerted a significant effect on seedling length, while the main effect of smoke source was not significant, indicating the dominant influence of concentration over smoke type. These findings are consistent with earlier reports of enhanced shoot and root elongation following smoke treatments [30].

4.4 Vigor indices:

Seedling vigour parameters (VI I and VI II), which combine germination performance with subsequent seedling growth, were significantly influenced by smoke water source, concentration, and their interaction, indicating their collective impact on early establishment [12]. PSW produced the highest vigour indices at moderate concentrations, reflecting enhanced germination accompanied by improved

seedling elongation and physiological activation. The decline at higher concentrations suggests reduced growth efficiency due to more exposure to smoke-derived compounds. Similar concentration-dependent effects of smoke-water in enhancing seedling vigour have been reported and are attributed to the balance between growth-promoting and inhibitory constituents in smoke solutions [24;29]. MSW exhibited variable responses, with relatively higher vigour at elevated concentrations, indicating dose-specific activation of bioactive components. In contrast, NSW enhanced vigour at lower concentrations but reduced it at higher doses, likely due to allelopathic effects. These results emphasize the need for source- and concentration-specific optimization to maximize seedling vigour in buckwheat.

4.5 Correlation:

Correlation analysis is a useful statistical approach for understanding the interrelationships among germination and seedling vigour traits and for identifying key attributes contributing to overall seed performance [13;31]. In the present study, Pearson's correlation revealed strong and predominantly positive associations among most germination and vigour related traits, with correlation coefficients varying from 0.75 to 0.97. Seed dry weight and imbibition-related traits (W_1 and W_2) showed highly significant positive correlations with germination percentage and both vigour indices, indicating that improved reserve utilization and water uptake strongly contribute to enhanced seed vigour (Fig. 3). Fresh weight was also strongly associated with vigour index I, suggesting that increased biomass accumulation directly influences seedling vigour. In contrast, the lack of a significant correlation between germination percentage and seedling length indicates that successful germination does not necessarily translate into greater seedling elongation, highlighting the independent regulation of these traits during early growth.

4.6 Principal component analysis (PCA):

Principal component analysis (PCA) serves as a multivariate approach for summarising complex datasets by reducing dimensionality and identifying key traits contributing to overall variability among treatments [15;32]. In the current study, PCA resulted in a substantial reduction in data dimensionality, with the first two principal components explaining more than 95% of the total variation (Fig. 4). The dominance of PC1, accounting for nearly 89–90% of the variance, indicates that a single underlying factor largely governed treatment response. Strong positive loadings of germination percentage, seed dry weight, fresh weight, and both vigor indices along PC1 suggest that these traits were the primary determinants of seed performance and early seedling vigor. This confirms that improvements in biomass accumulation and reserve utilization were closely associated with enhanced germination and vigor under smoke water treatments. In contrast, seedling length loaded more strongly on PC2, which explained a relatively small proportion of variance, indicating that seedling elongation contributed less to overall treatment differentiation.

5. CONCLUSION

The findings of this study clearly indicate that smoke water exerts a significant and concentration-dependent influence during seed germination and early seedling vigour of buckwheat (*Fagopyrum esculentum* Moench) genotype PRB1. Among the tested sources, paddy straw smoke water proved most effective, particularly at lower to moderate concentrations, by consistently enhancing germination, seedling growth, biomass accumulation, and vigour indices. Neem and mango smoke water exhibited variable responses. Collectively, these findings highlight the potential of optimized smoke water treatments as an eco-friendly and cost-effective seed priming approach for improving buckwheat establishment and early growth, while emphasizing the need for source- and dose-specific standardization for practical application.

Declarations

Permissions to collect plant material:

Buckwheat (*Fagopyrum esculentum* Moench) seeds were obtained from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, under an institutional Material Transfer Agreement. No field collection of plant material was undertaken by the authors.

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Conflict of interest

The authors declare no conflict of interest.

Compliance with ethical standards

This study was conducted in accordance with institutional and ethical guidelines. All experimental procedures were performed following standard laboratory and biosafety protocols.

Clinical Trial Registration

Not applicable. This study does not involve a clinical trial.

Consent to Publish

All authors have read and approved the final manuscript and consent to its publication.

Consent to Participate

Not applicable. This study did not involve human participants or animals.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contribution

Nidhi Pandey: Conceptualization, methodology, investigation, data curation, literature review, manuscript writing – original draft preparation, and manuscript revision. Vaidurya Pratap Sahi and Prashant Rai: Supervision, conceptualization, experimental guidance, interpretation of results, manuscript review and editing. Sandeep Nalla: Experimental design, methodology development, and technical guidance. Nitish Kumar: Statistical analysis, data interpretation, and validation. Shobhit Raman Tiwari and Rajkaran Tripathi: Data collection, experimental execution, and maintenance of experimental records.All authors read and approved the final manuscript.

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Tables

TWO-WAY FACTORIAL ANALYSIS OF VARIANCE:

TableT1: Effect of Factor-1 (F1: smoke-water types) on germination and seedling growth parameters of buckwheat (Fagopyrum esculentum Moench). Values represent treatment means for Germination (%), Fresh Weight (g), Dry Weight (g), Vigour Index I (VI I), Vigour Index II (VI II), and Seedling Length (cm). AVERAGE indicates the overall mean across Factor-1 levels. SEM represents the standard error of the mean. C.D. (5%) denotes the critical difference at the 5% significance level. P-values indicate the significance of Factor-1 effects as determined by ANOVA; values ≤ 0.05 were considered statistically significant.

Factor-1						
	Germination	Fresh Weight	Dry Weight	VI 1	VI 2	Seedling length
F1: 1	80.57	1.12	0.07	841.50	6.06	9.96
F1: 2	48.91	1.07	0.07	517.66	3.42	10.42
F1: 3	64.57	1.05	0.08	709.40	5.41	9.91
AVERAGE	**64.68**	**1.08**	**0.07**	**689.52**	**4.96**	**10.10**
SEM	**1.28**	**0.02**	**0.00**	**26.05**	**0.18**	**0.24**
C.D. (5%) Factor-1	**3.67**	**0.06**	**0.01**	**74.45**	**0.51**	**0.70**
P-Value	**0.00**	**0.08**	**0.00**	**0.00**	**0.00**	**0.28**

Table T2: Effect of Factor-2 (F2: smoke-water concentration levels) on germination and seedling growth parameters of buckwheat (Fagopyrum esculentum Moench). Values represent treatment means for Germination (%), Fresh Weight (g), Dry Weight (g), Vigour Index I (VI I), Vigour Index II (VI II), and Seedling Length (cm). AVERAGE indicates the overall mean across Factor-2 levels. SEM denotes the standard error of the mean. C.D. (5%) represents the critical difference at the 5% level of significance. P-values indicate the significance of Factor-2 effects as determined by ANOVA; values ≤ 0.05 were considered statistically significant.

Factor-2						
	Germination	Fresh Weight	Dry Weight	VI 1	VI 2	Seedling length
F2: 1	12.00	0.43	0.04	84.27	0.48	7.02
F2: 2	80.67	1.26	0.09	949.95	7.64	11.75
F2: 3	60.33	1.27	0.07	680.25	4.15	11.07
F2: 4	69.00	1.16	0.07	784.65	4.22	11.09
F2: 5	76.22	1.30	0.08	847.34	6.53	11.37
F2: 6	64.67	1.09	0.07	523.03	4.38	7.77
F2: 7	89.89	1.06	0.08	957.17	7.33	10.61
AVERAGE	**64.68**	**1.08**	**0.07**	**689.52**	**4.96**	**10.10**
SEM	**1.96**	**0.03**	**0.00**	**39.79**	**0.27**	**0.37**
C.D. (5%) Factor-2	**5.61**	**0.09**	**0.01**	**113.72**	**0.78**	**1.06**
P-Value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Table T3: Interaction effect of Factor-1 (F1: smoke-water types) and Factor-2 (F2: concentration levels) on germination and seedling growth parameters of buckwheat (Fagopyrum esculentum Moench). Values represent treatment means for Germination (%), Fresh Weight (g), Dry Weight (g), Vigour Index I (VI I), Vigour Index II (VI II), and Seedling Length (cm) under each F1 × F2 combination. AVERAGE denotes the overall mean across treatments. SEM indicates the standard error of the mean. C.D. (5%) represents the critical difference at the 5% level for comparing interaction means. P-values indicate the significance of the interaction effect as determined by two-way ANOVA; values ≤ 0.05 were considered statistically significant. C.V. (%) denotes the coefficient of variation, reflecting experimental precision.

Interaction (Factor-1 X Factor-2)

	Germination	Fresh Weight	Dry Weight	VI 1	VI 2	Seedling length
F1: 1 X F2: 1	12.00	0.29	0.04	77.84	0.48	6.49
F1: 1 X F2: 2	94.00	1.28	0.08	997.77	7.54	10.65
F1: 1 X F2: 3	92.33	1.48	0.05	1129.23	4.63	12.27
F1: 1 X F2: 4	90.33	1.29	0.04	1121.32	3.63	12.39
F1: 1 X F2: 5	92.00	1.47	0.11	948.41	10.11	10.28
F1: 1 X F2: 6	92.00	1.16	0.08	752.27	7.40	8.15
F1: 1 X F2: 7	91.33	0.85	0.09	863.67	8.61	9.49
F1: 3 X F2: 1	12.00	0.71	0.04	97.04	0.48	8.09
F1: 3 X F2: 2	55.00	1.20	0.09	644.07	4.92	11.63
F1: 3 X F2: 3	28.67	1.06	0.07	325.65	2.08	11.21
F1: 3 X F2: 4	41.67	1.12	0.09	431.47	3.82	10.21
F1: 3 X F2: 5	46.67	1.14	0.07	604.90	3.22	12.84
F1: 3 X F2: 6	76.67	1.16	0.05	632.17	3.78	8.16
F1: 3 X F2: 7	81.67	1.11	0.07	888.34	5.67	10.79
F1: 3 X F2: 1	12.00	0.29	0.04	77.92	0.48	6.49
F1: 3 X F2: 2	93.00	1.30	0.11	1208.03	10.48	12.97
F1: 3 X F2: 3	60.00	1.27	0.10	585.87	5.73	9.72
F1: 3 X F2: 4	75.00	1.06	0.07	801.17	5.22	10.66
F1: 3 X F2: 5	90.00	1.27	0.07	988.70	6.27	10.98
F1: 3 X F2: 6	25.33	0.94	0.08	184.63	1.96	7.00
F1: 3 X F2: 7	96.67	1.22	0.08	1119.49	7.72	11.56
AVERAGE	**64.68**	**1.08**	**0.07**	**689.52**	**4.96**	**10.10**
SEM	**3.40**	**0.05**	**0.00**	**68.91**	**0.47**	**0.64**
C.D. (5%) Factor-1 X Factor-2	**9.71**	**0.16**	**0.01**	**196.97**	**1.35**	**1.84**
P-Value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
C.V. %	**9.10**	**8.78**	**10.22**	**17.31**	**16.51**	**11.05**

CORRELATION ANALYSIS:

Table T4: Pearson's correlation matrix among seedling growth parameters in buckwheat (Fagopyrum esculentum Moench) genotype PRB1 under various smoke-water treatments. Values represent Pearson's correlation coefficients (r) calculated among germination percentage (GP), seedling length (SL), fresh weight (FW), dry weight (DW), seedling vigor index I (SVI-I), and seedling vigor index II (SVI-II). Correlation analysis was performed using pooled treatment data across smoke-water sources and concentrations. Significance levels are indicated as follows: ***** $p \leq 0.05$ (two-tailed), ****** $p \leq 0.01$ (two-tailed)

CORRELATION	Germination percentage	Fresh weight	Dry weight	VI I	VI II	Seedling length
Germination percentage	1	.845*	.950**	.966**	.949**	0.754
Fresh weight	.845*	1	.881**	.851*	.774*	.821*
Dry weight	.950**	.881**	1	.951**	.971**	.803*
VI I	.966**	.851*	.951**	1	.945**	.890**
VI II	.949**	.774*	.971**	.945**	1	.756*
Seedling length	0.754	.821*	.803*	.890**	.756*	1

Figures

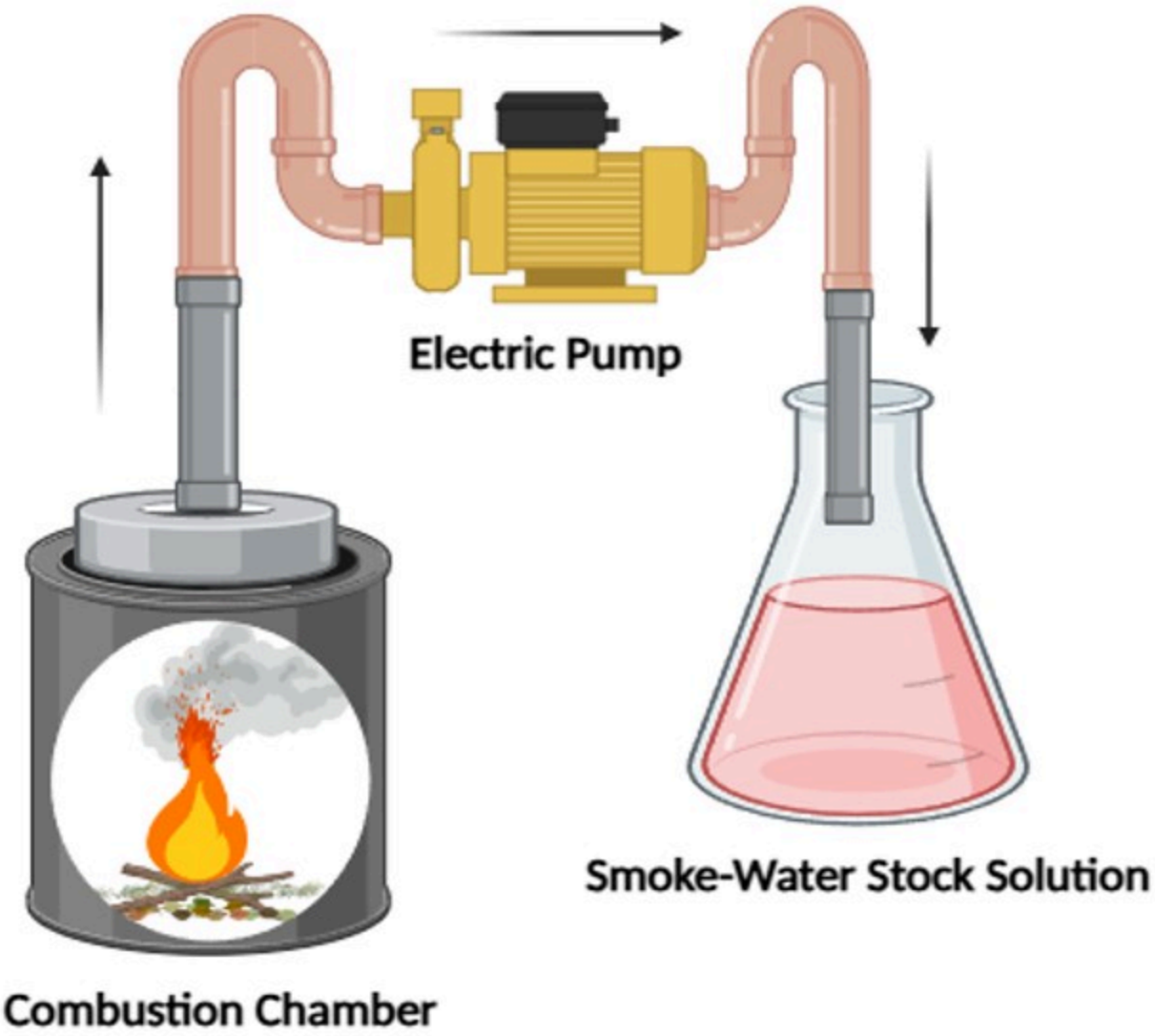


Figure 1

Schematic representation of the smoke-generation and trapping system used for preparing bioactive smoke-water stock solution.

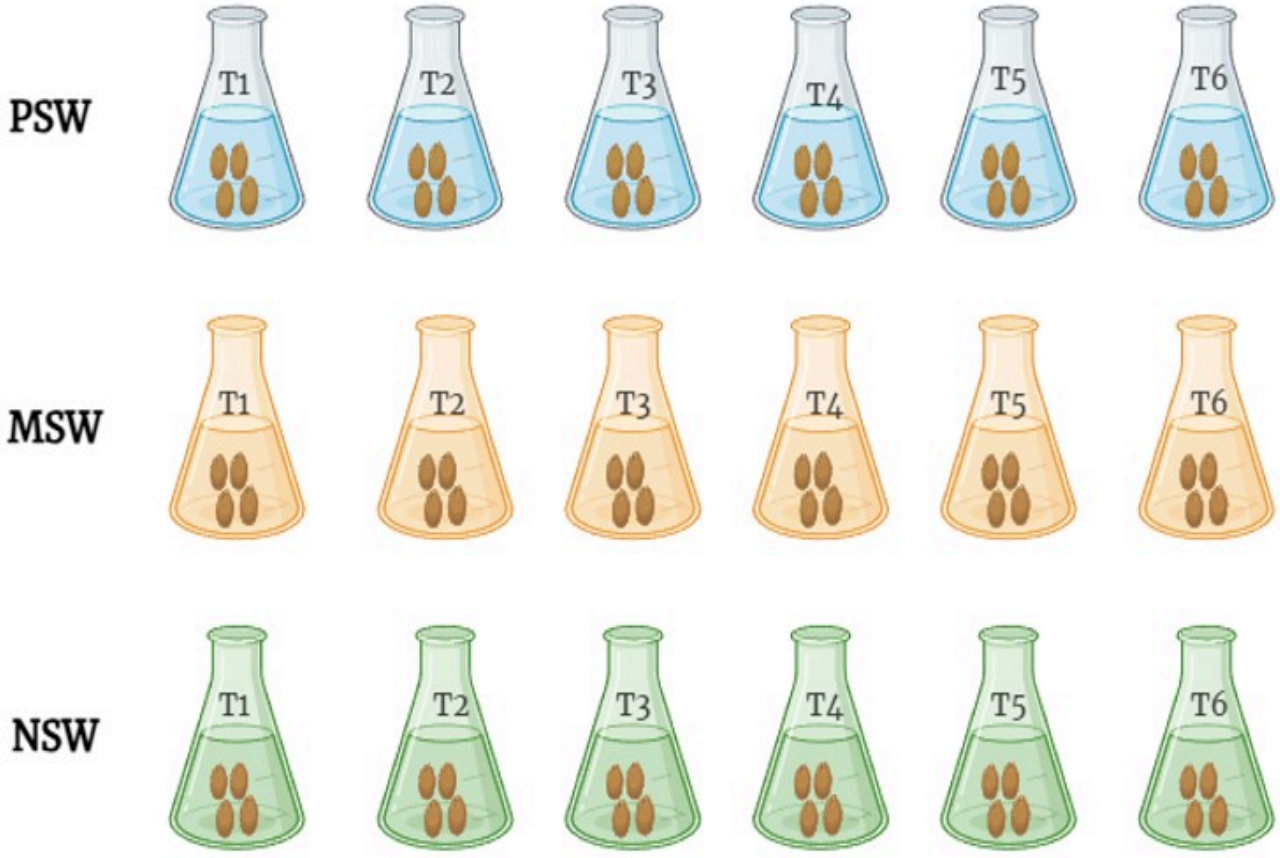


Figure 2

Schematic overview of buckwheat seed treatments using three biomass-derived smoke-water types: paddy straw smoke-water (PSW), mango leaf smoke-water (MSW), and neem leaf smoke-water (NSW). Seeds were exposed to graded concentrations (0.1%, 0.5%, 2%, 5%, 8%, 10%).

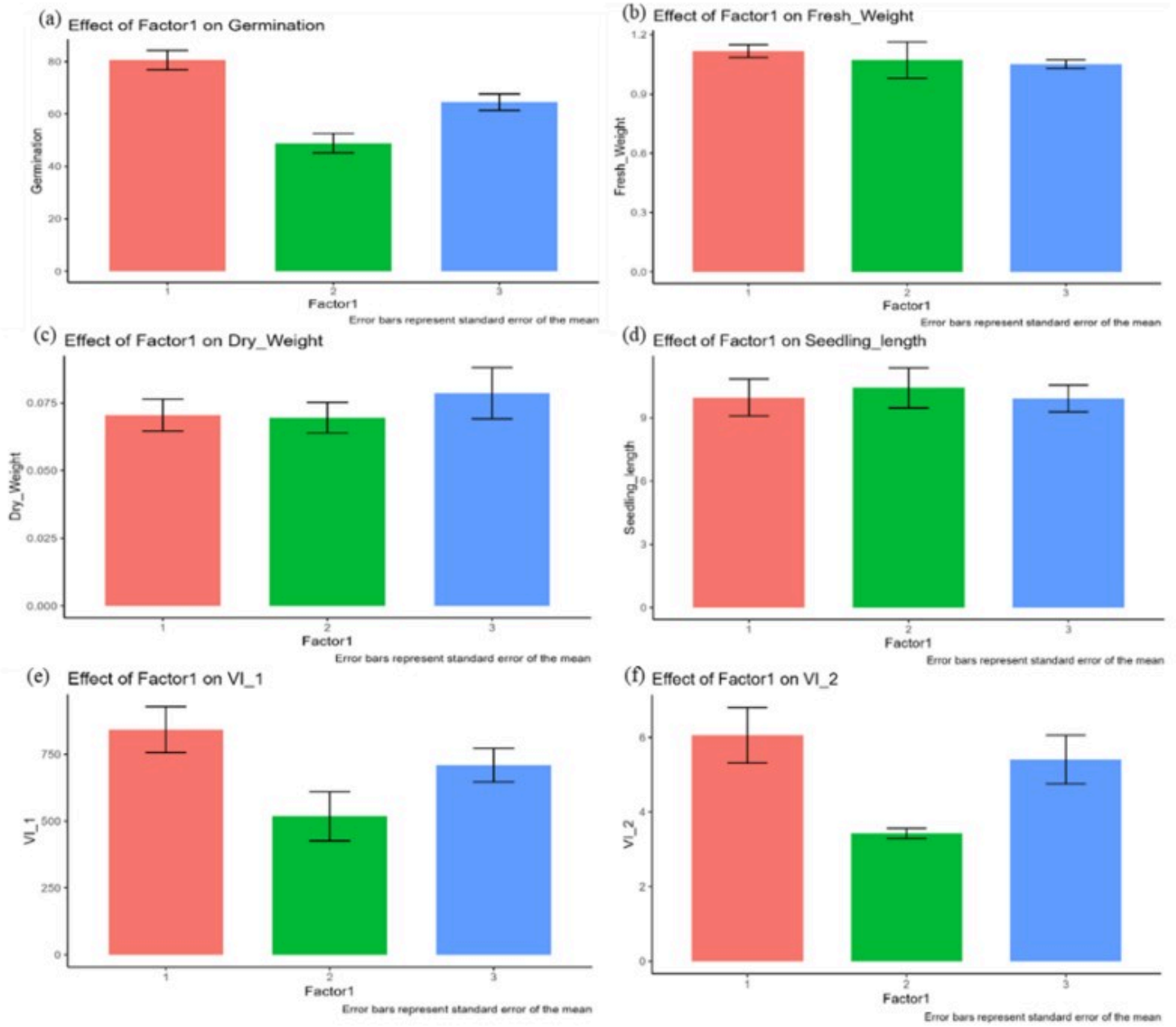


Figure 3

ANOVA: Effect of Factor-1 (smoke-water types) on seed germination and seedling development parameters of buckwheat (Fagopyrum esculentum Moench). (a) Germination (%), (b) Fresh Weight (g), (c) Dry Weight (g), (d) Seedling Length (cm), (e) Vigour Index I (VI I), and (f) Vigour Index II (VI II) across smoke-water treatments. Bars show the average values, and the error bars indicate ± standard error of the mean (SEM). Statistical significance of Factor-1 effects was determined by two-way ANOVA at $P \leq 0.05$.

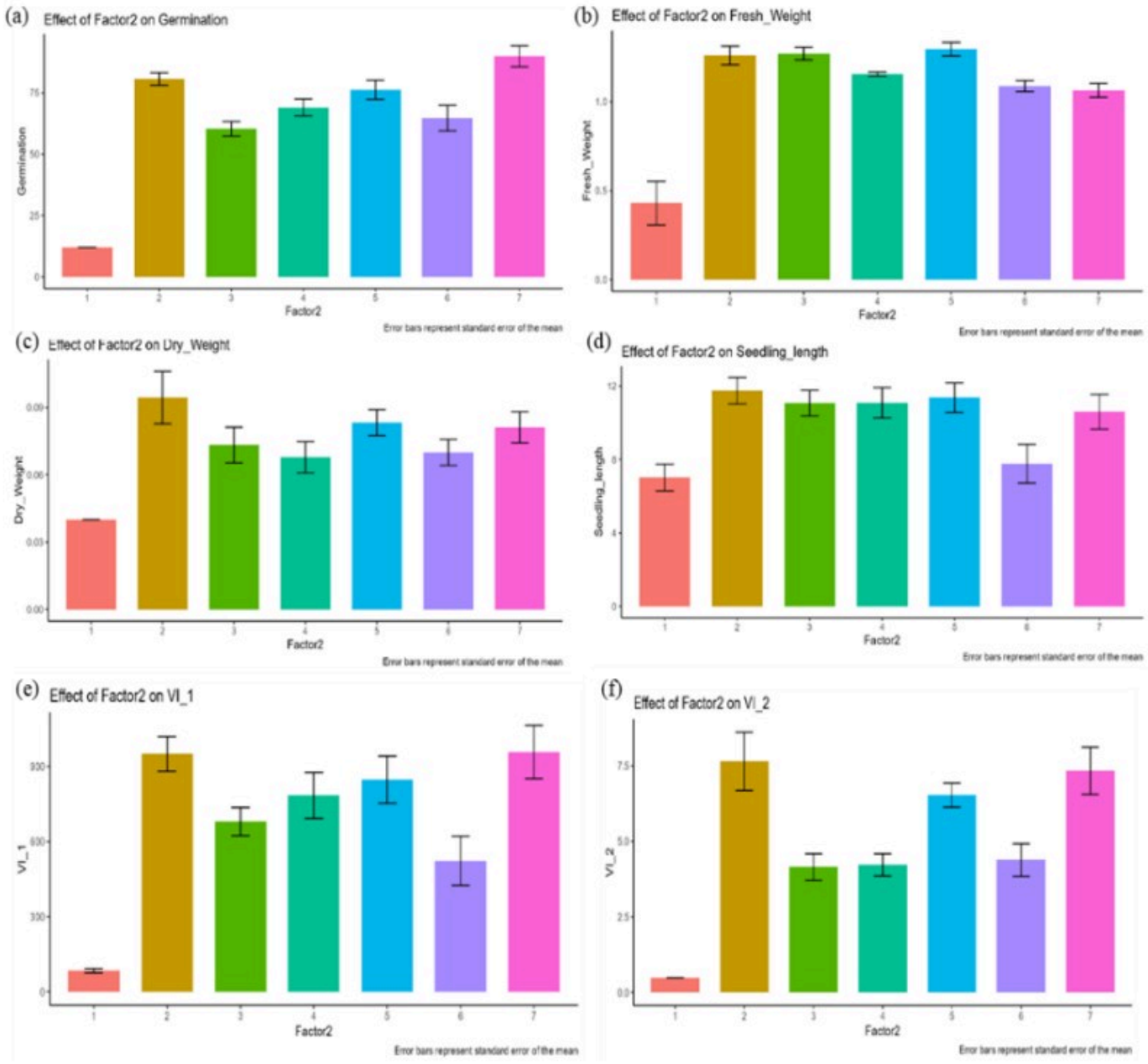


Figure 4

ANOVA: Effect of Factor-2 (smoke-water concentration levels) on seed germination and overall seedling growth parameters of buckwheat (Fagopyrum esculentum Moench). (a) Germination (%), (b) Fresh Weight (g), (c) Dry Weight (g), (d) Seedling Length (cm), (e) Vigour Index I (VI I), and (F) Vigour Index II (VI II) across concentration treatments. Bars represent mean values, and error bars indicate ± standard error of the mean (SEM). Statistical significance of Factor-2 effects was determined by two-way ANOVA at $P \leq 0.05$.

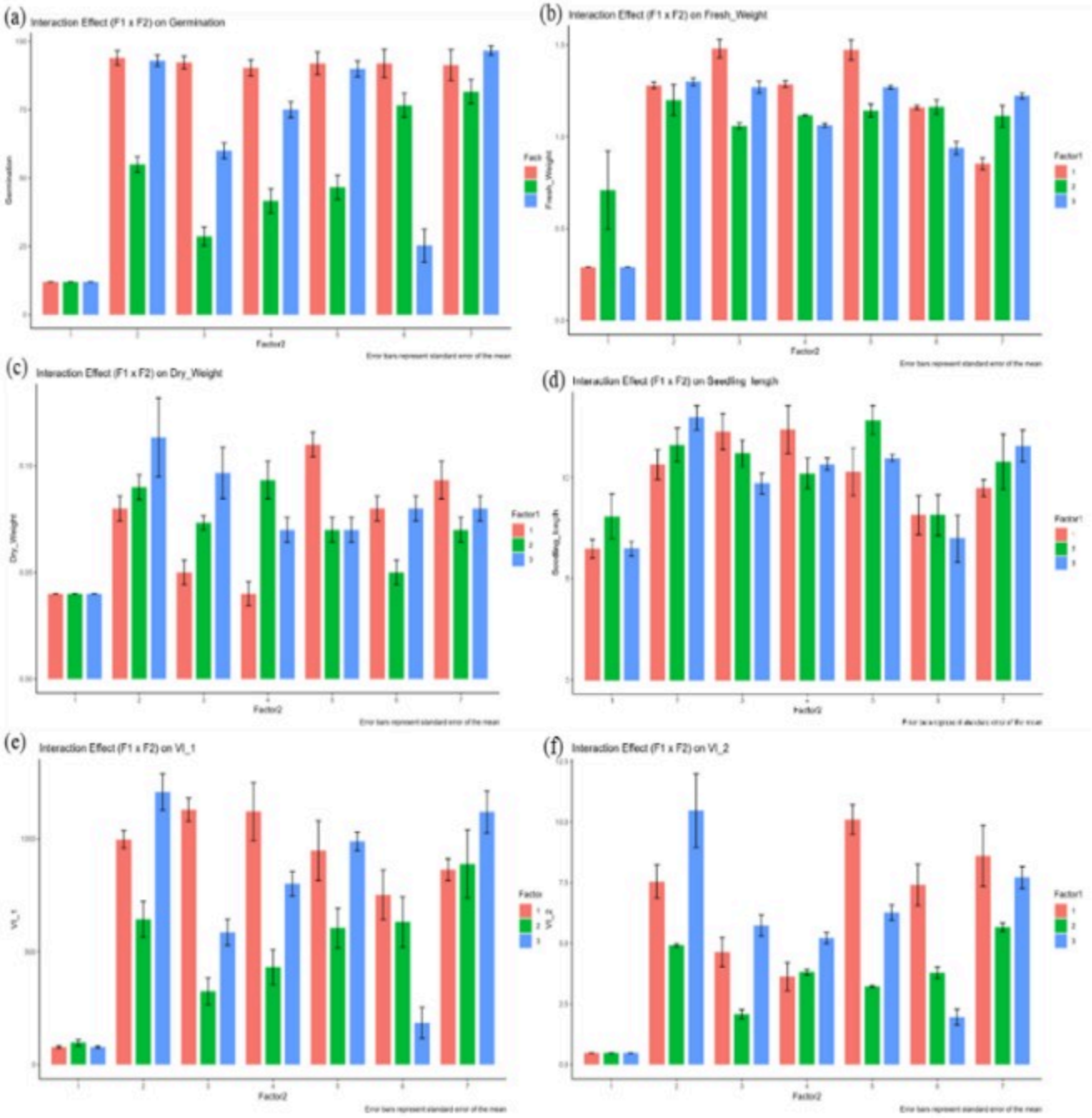


Figure 5

ANOVA: Interaction effect of Factor-1 (smoke-water types) and Factor-2 (concentration levels) on germination and seedling growth parameters of buckwheat (Fagopyrum esculentum Moench). (a) Germination (%), (b) Fresh Weight (g), (c) Dry Weight (g), (d) Seedling Length (cm), (e) Vigour Index I (VI I), and (F) Vigour Index II (VI II) under different F1 × F2 treatment combinations. Bars represent mean values, and error bars indicate ± standard error of the mean (SEM). Statistical significance of interaction effects was determined by two-way ANOVA at $P \leq 0.05$.

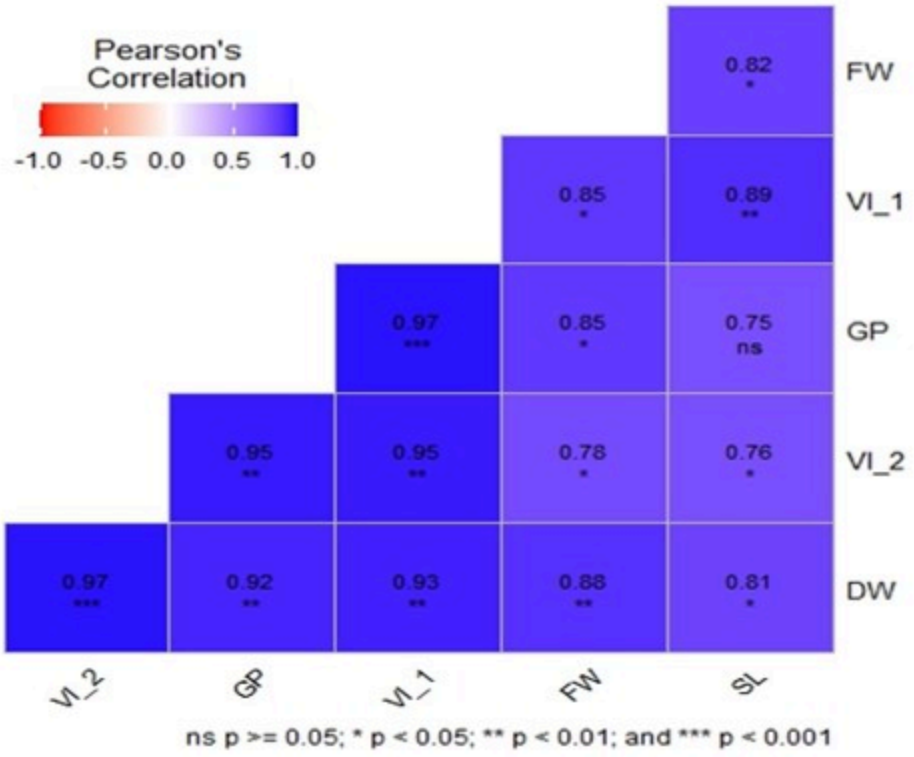


Figure 6

Pearson's correlation heatmap illustrating interrelationships among germination, seedling growth, and vigour traits in buckwheat (Fagopyrum esculentum Moench) genotype PRB1 under smoke-water treatments. Heatmap representation of Pearson's correlation coefficients (r) among germination percentage, seedling length, fresh weight, dry weight, seedling vigour index I, and seedling vigour index II across all smoke-water concentrations. Colour gradients indicate the direction and strength of correlations (blue: negative; red: positive), with intensity proportional to the magnitude of r. Statistical significance was determined using two-tailed tests and is denoted as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), ns = not significant)

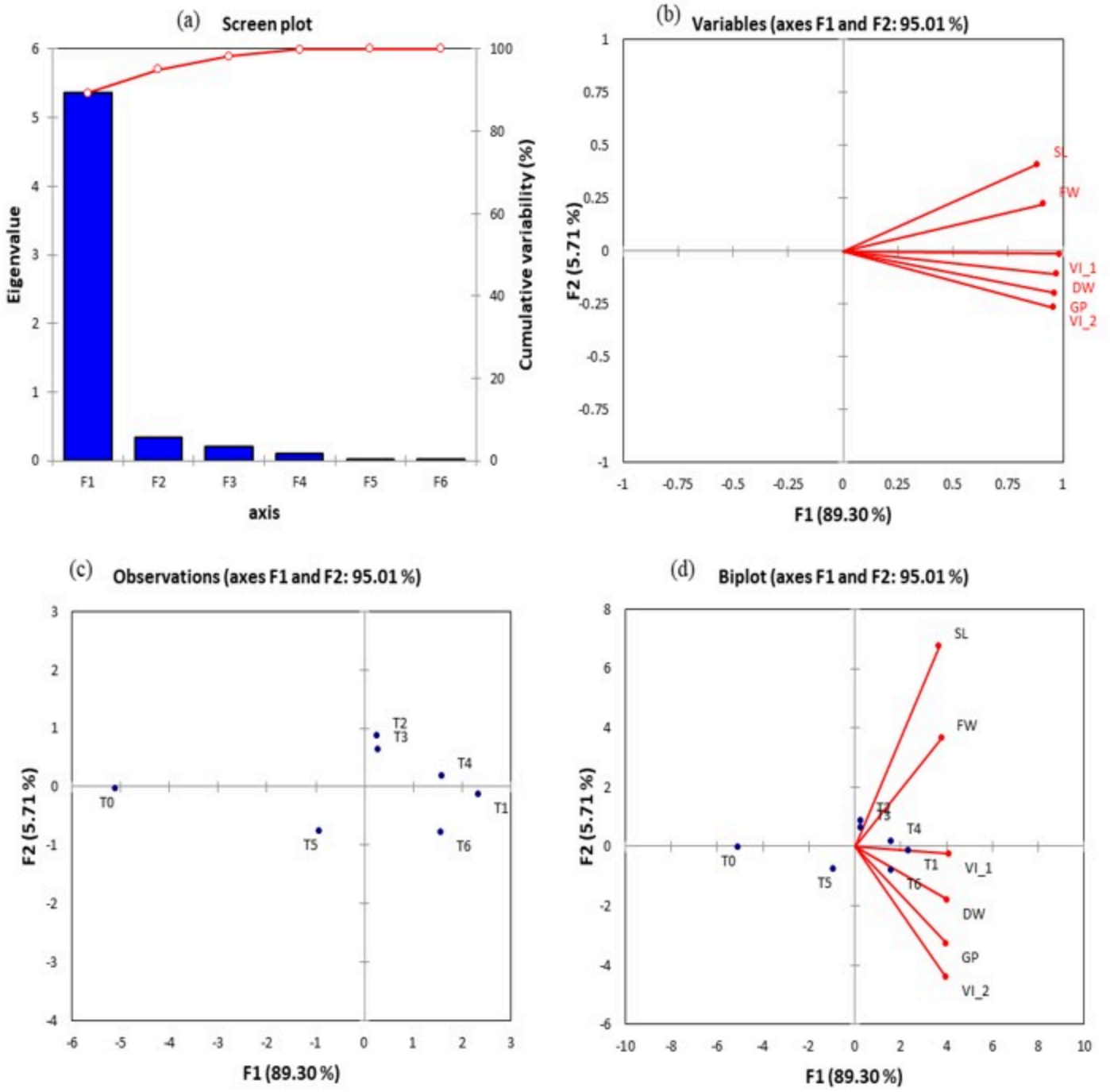


Figure 7

Principal component analysis (PCA) of seedling growth traits in buckwheat (Fagopyrum esculentum Moench) under smoke-water treatments. (a) Screen plot showing the proportion of total variance explained by successive principal components, indicating the relative contribution of each component to overall trait variability. (b) Loading plot illustrating the magnitude and direction of individual seedling traits contributing to PC1 and PC2, highlighting the most influential variables driving multivariate differentiation. (c) Score plot displaying the distribution and clustering pattern of genotypes and/or smoke-water treatments based on composite trait performance. (d) PCA biplot integrating trait loadings

and treatment/genotype scores, depicting the relationships among traits and their collective influence on the separation and grouping of treatments under smoke-water application.

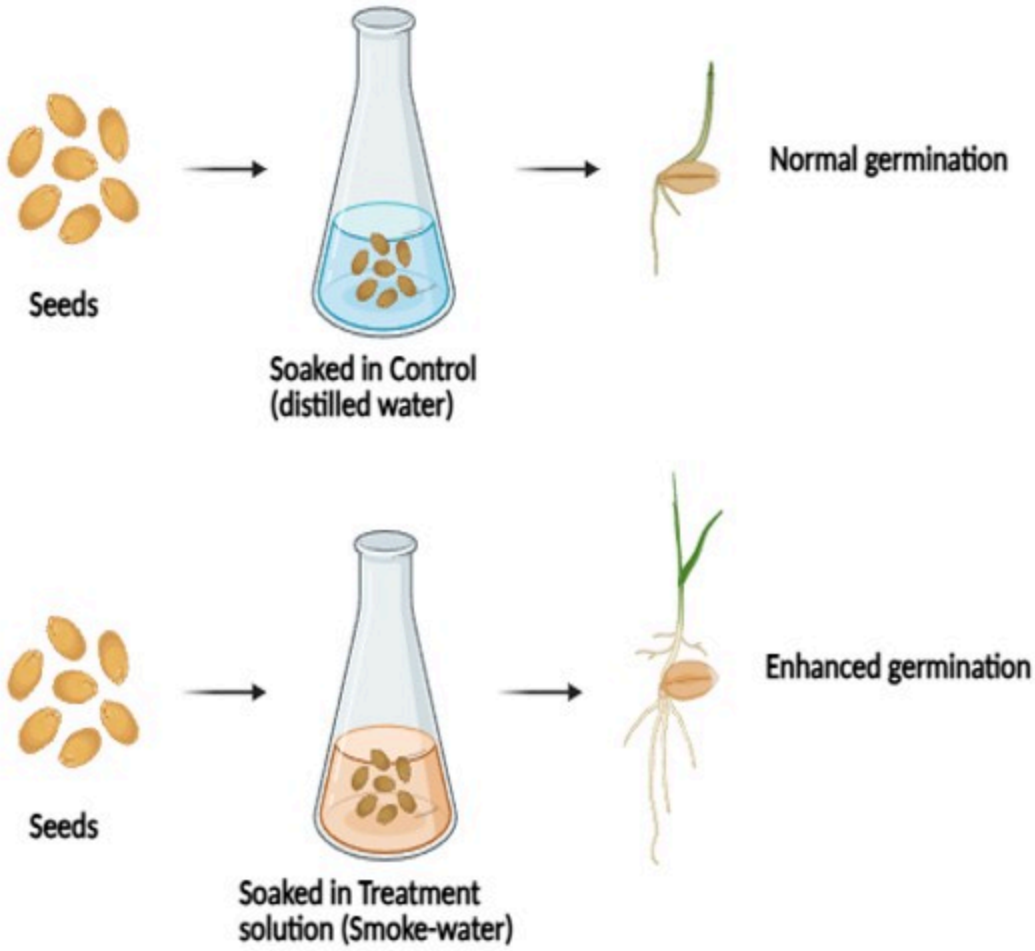


Figure 8

Schematic illustration of the effect of smoke-water treatment on seed germination in buckwheat (Fagopyrum esculentum Moench). Seeds soaked in distilled water (control) exhibited normal germination, whereas seeds treated with smoke-water solution showed enhanced germination and improved early seedling growth, indicating the stimulatory effect of smoke-derived bioactive compounds.