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Optimization of Seed Germination of Traditional Aromatic Rice Lines of Rarh Bengal

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Abstract

The state of West Bengal has precious wealth of genetic diversity in aromatic rice, several varieties being reported in different areas of Rarh Bengal. Because of low germination percentage, causing low yield, many traditional aromatic varieties are being eliminating from the cultivation field. This article particularises the improvement of the germination of four traditional, non-basmati type landraces (Radhunipagal, Kataribhog, Kalonunia, and Lal-Badshabhog). To improve the germination rate, seeds of the four different varieties were pre-treated with different temperatures (pre-treated with hot water at 50°C, 60°C, and 70°C for 5 minutes), with nitrogenous substances (Thiourea-100 mM, and Potassium nitrate-100 mM solution) and phytohormones (IAA-100 mM, and GA-100 mM solution). A significant increase in germination percentage over the control was observed when treated with hot water (60°C and 50°C), 100 mM potassium nitrate, and 100 mM Thiourea. This research article provides information on enhancing the germination of both commercially and nutritionally important aromatic rice varieties, which is highly relevant for the mass production of effective and viable seed lots in agricultural fields.

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Keywords

Aromatic rice, Germination, Hotwater, Nitrogenous substances, Pre-treatment, Phytohormones.

Introduction

Aromatic rice is a gift from nature to the Indian subcontinent and all of humanity. In contrast to other rice classes, aromatic rice is in high demand and commands a higher premium price on the international market because of its wonderful aroma, variety of grains and nutrient-enriched, superb, flaky, soft texture when cooked. Aromatic rice has a special place in the Indian economy because of its flavour, scent, and high demand. India is currently exporting aromatic rice to the Middle East, the United States, the United Kingdom, Australia, Canada, and other nations, including South Africa, as a result of globalisation. Farmers are currently receiving competitive prices for these specific varieties (Bhowmik *et al.,* 2011). However, only 12 to 15 indigenous aromatic rice varieties (IARVs) are now being grown in dispersed, disorganised areas of West Bengal (Roy and Bandyopadhyay, 2014). West Bengal is also referred to as the "bowl of rice" as rice is the staple meal, and rice is grown over a large area of land in West Bengal.

As India is the birthplace of rice, it can produce a vast array of regional types and landraces, in addition to having the largest supply of traditional aromatic rice in the world. This region also has a diverse array of aromatic rice varieties due to its varied geographical dispersion (Sinha and Mishra, 2012). Among the aromatic rice, four non-basmati type landraces like Radhunipagal, Kataribhog, Kalonunia, and Lal-

Badshabhog have reportedly been cultivated traditionally in various parts of the Bankura district since ancient times (Sinha and Mishra, 2013). However, the local aromatic rice cultivars produce little and react poorly to fertiliser addition (Sinha and Mishra, 2013). Utilising organic manures can improve fertilizer usage efficiency, lower nitrogen supply costs, boost productivity in rainfed situations, and require less capital investment, particularly in unfavourable weather circumstances. Consequently, improving crop yield in relation to maintaining soil fertility requires the implementation of integrated nutrient management (INM) practices. Before all the above steps, seed showing and germination percentage of the shown seed are very much important, which play an important role in crop yield in the field. To improve this obstacle, an experiment was designed and executed to study any change in the germination percentage. This article talks about an experiment, which is related to germination percentage improvement by pre-treating with some factors, which is one of the key steps of rice farming, particularly traditional rice. Because we all know that one of the most crucial phases of any cultivation is germination, during which the embryonic axis lengthens after emerging from the seed coat and absorbing water in the right conditions (Bewley and Black, 1994). Seeds that are chosen for cultivation have low water content. A variety of environmental elements, including temperature, oxygen, nutrition, hormones, and light, are essential for seed germination (Baskin and Baskin, 2004; Yang *et al.,* 2020). The long-standing role of GA and ABA in the phytochrome-mediated seed germination response has been suggested by classical physiological research (Shinomura, 1997). Additionally, temperature is crucial; germination is delayed below 50°C.When the embryonic axis emerges through the seed coat and the germination process is finished, seeds are typically stored for incubation. The process of germination is based on the imbibition of seeds to go through the first reversible stage of germination, but not the embryonic axis emergence. Nowadays, some researchers are employing pre-treatments with various nitrogenous substances like nano urea (Mahakham *et al.,* 2017) and some other factors like hot water, phytohormones treatment to increase the rate of seed germination.

Materials and Methods

For this experiment, seed samples of four non-basmati type landraces like Radhunipagal, Kataribhog, Kalonunia, and Lal-Badshabhog were collected from the Repository of Amarkanan Rural Socio Environmental Welfare Society (ARSW Society), located in the village of Ranbahal at an elevation of 78 meters above sea level and between 86°36'E and 87°47'E. Seeds were sterilised using a 10% sodium hypochlorite solution and incubated for five minutes before being thoroughly rinsed with distilled water. Later, seed lots of all four landraces are separately treated with (a) 50°C, (b) 60°C, (c) 70°C, (d) IAA-100 mM, (e) GA-100 mM solution, (f) 100 mM Thiourea and (g) Potassium nitrate 100 mM solutions.

All temperature treatment was for 5 minutes, later they were then transferred to a previously prepared set-up of petri plates covered with two layers of Whatman No. 1 filter paper at 28±2°C and a 16-hr, 8-hr dark-light cycle.

Data Collection

Data collected day by day from seed sowing to the day when the germination percentage reaches up to maximum (90-100%). The following parameters were used to study during the experiment.

Germination Percentage: The germination percentage of seeds was calculated using the following formula (Pillai *et al.,* 2020).

Germination percentage (GP) = (TG/TS) × 100

Where TG = Total no. of germinated seeds and TS = Total no. of seeds sown.

Mean Germination Time (MGT): MGT indicates the average time (day), seeds take for germination. A lower MGT means faster germination.

Mean germination time (MGT) = (Gt × Dt)/G

Where Gt = no. of germinated seeds at day-t, Dt = no. of days at 't' from the day of sowing and G = total no. of germinated seeds (Pillai *et al.,* 2020).

Germination Index (GI): This value gives an idea about both how fast (Speed) and how many (total) seeds germinated. A higher GI means faster and more synchronised germination. It gives more weight to seeds that germinate earlier, making it a strong indicator of seed vigor (Pillai *et al.,* 2020).

$$GI=\sum (Gt/Dt)$$

Where G_t = Number of seeds germinated on day t.

D_t = Number of days since sowing.

Statistical Analysis

Analysis of variance (ANOVA) was used to test the significance of the treatment effects on seed germination to determine whether a significant difference (p =/< 0.05) occurred between treatments and the control. Germination percentage, heat map representation of the germination percentage, was generated using MS Excel 2021 and GraphPad Prism version 10.4.20for Windows (GraphPad Software, California, USA).

Results and Discussion

The pre-treatment of different factors increases the germination rate significantly (Table 2) in all landraces except Kataribhog, as it shows nearly 100% germination in the control condition. Radhunipagal shows an increased percentage of germination of 13.8% (Table 1) with 50°C, 18.94% with 60°C hot water and 41.4% (Table 1) when treated with 100 mM Thiourea. Kalonunia also show the improved rate of germination, which is 97.1% (Table 1) when treated with 60°C hot water, and 100 mM thiourea treatment shows an 8.4% (Table 1) increase in germination compared to the control. A massive increase in germination percentage was shown by Lal-Badshabhog, 714% (Table 1) with 50° C hot water treatment, 633% (Table 1) with IAA 100 mM treatment and 733% (Table 1) with 100mM Thiourea, respectively.

Germination is a key event in a plant's life cycle, which requires suitable environmental factors; among those factors, temperature, water, O_2, and growth hormones are crucial. In this experiment, temperature pre-treatment, treatment with external phytohormones and two nitrogenous substances have been used to enhance the germination rate. Along with natural treatment, there are a few common trending techniques to improve the germination, like hormonal treatment, treatment with nitrogenous substances.

Effect of temperature pre-treatment (50°C, 60°C, 70°C)

Temperature pre-treatment, also known as thermal priming done by exposing seeds to relevant temperatures to overcome dormancy and stimulate germination. In this experiment, pretreatment timing was 5 minutes for each. Among different pretreatment temperatures, 60°C shows an effective result in all the varieties except Kataribhog compared to the control treatment (Table 1). Also, the germination index (Table 1) is higher when applying a temperature pre-treatment, which means that temperature pre-treatment somehow, stimulates germination by breaking physical barriers like the seed coat and helps in activation of metabolic processes.

Thus, it found temperature is a critical factor in the germination of rice seed, like other seeds, viz. *Tagetes minuta* and *Cymbopogon martini* (Kumar *et al.,* 2010). Temperature is a key component in regulating plant growth and development, and its impact on seed germination is complicated since it influences each stage of the process differently and is not independent of other variables (Mayer and Poljakoff-Mayber, 1989).

Chemical treatments

Treatment with- IAA (100 mM) and GA (100 mM)

IAA is a naturally occurring plant hormone responsible for cell division and cell elongation; also, its treatment is associated with the acceleration of germination and improving seed Vigor (Mahmood ur Rehman *et al.,* 2024). Treatment with IAA reduces the complete germination time to 11 days in some species (Mandal *et al.,* 2025). Likewise, IAA-100 mM shows a little increase in germination percentage in Kalonunia and Lal-Badshabhog.

Alongside, GA plays an important role in breaking dormancy and promoting germination (Kaur *et al.,* 2020). GA_3 enhances germination by stimulating the synthesis of the enzyme alpha amylase that degrades stored reserve food, providing energy to the growing embryo. As reported, GA treatment shows 100% germination on *Aesca japonica* and a significant germination increase in *Prunus avium.*

Treatment with- Thiourea (100mM) and KNO3 (100mM)

Among the pre-sowing treatments, treatment with nitrogenous substances is one of the common ones. Nowadays, nano-urea is used to promote germination. Thiourea is usually known to break seed dormancy by acting as a reducing agent. Cetinbas and Koyuncu (2006) reported that treatment with thiourea shows asignificant germination increase in *Prunus avium*. Another study with 150 ppm thiourea shows significant germination compared to KNO_3 and GA treatment (Junaid *et al.,* 2023).

Table.1 showing GP – Germination percentage, GI – Germination index, GMT – Germination Mean Time percentage (with SE ±, Table 1) of four landraces under pre-treatment with different factors.

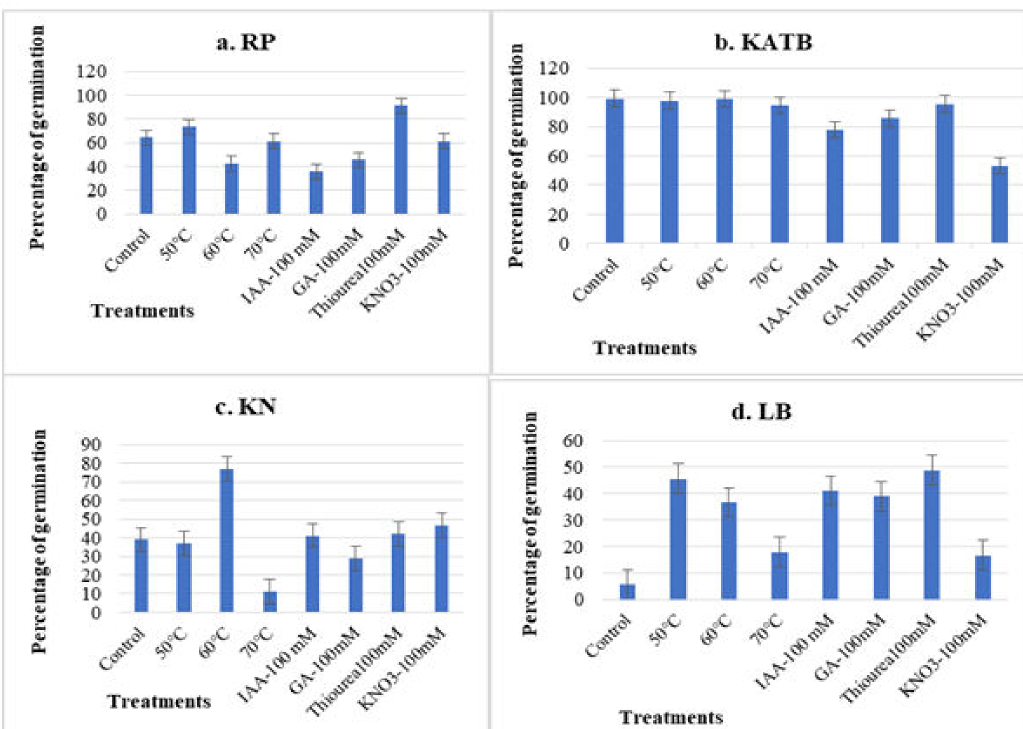
Landraces	Treatment	GP	GI	GMT
RADHUNIPAGAL	Control	64.4 ± 0.33	45.8 ± 1.9	2.6 ± 0.1
	50°C	73.3 ± 0.57	51.0 ± 1.3	2.8 ±.09
	60°C	76.6 ± 0.33	51.5 ± 1.5	2.8 ± 0.1
	70°C	61.1 ± 0.33	38.1 ± 0.8	2.0 ± 0.04
	IAA-100 mM	35.6 ± 0.33	24.6 ± 1.3	1.3 ± 0.08
	GA-100 mM	45.6 ± 0.33	20.5 ± 0.5	1.7 ± 0.04
	Thiourea 100 mM	91.1 ± 0.33	66.6 ± 1.6	3.7 ± 0.1
	KNO3-100 mM	61.1 ± 0.66	44.6 ± 0.8	2.5 ± 0.05
KATARIBHOG	Control	99.3 ± 1.15	84.8 ± 2.4	4.8 ± 0.10
	50°C	97.8 ± 0.33	80.6 ± 1.7	4.5 ± 0.10
	60°C	98.9 ± 0.33	88.0 ± 5.5	4.9 ± 0.34
	70°C	94.4 ± 0.33	80.8 ± 1.1	4.5 ± 0.06
	IAA-100 mM	77.8 ± 0.89	50.6 ± 3.4	2.8 ± 0.24
	GA-100 mM	85.6 ± 0.89	53.1 ± 3.7	3.0 ± 0.21
	Thiourea 100 mM	95.6 ± 0.89	70.6 ± 3.1	3.8 ± 0.23
	KNO3-100 mM	53.0 ± 0.0	38.3 ± 1.7	2.2 ± 0.10
KALONUNIA	Control	38.9 ± 0.33	10.8 ± 0.17	2.4 ± 0.05
	50°C	36.7 ± 1.15	6.9 ± 1.39	1.7 ± 0.27
	60°C	76.7 ± 0.57	14.5 ± 2.91	3.5 ± 0.41
	70°C	11.1 ± 0.33	2.2 ± 0.50	0.5 ± 0.07
	IAA-100 mM	41.1 ± 0.33	9.2 ± 0.61	2.1 ± 0.30
	GA-100 mM	28.9 ± 0.33	4.5 ± 0.35	1.2 ± 0.27
	Thiourea 100 mM	42.2 ± 0.33	9.7 ± 1.43	2.2 ± 0.24
	KNO3-100 mM	46.7 ± 0.57	11.2 ± 0.43	2.5 ± 0.09
Lal-Badshabhog	Control	5.6 ± 0.33	1.3 ± 0.34	0.3 ± 0.09
	50°C	45.6 ± 0.89	12.4 ± 1.08	2.8 ± 0.14
	60°C	36.7 ± 0.57	6.0 ± 1.7	1.6 ± 0.62
	70°C	17.8 ± 0.89	4.1 ± 0.89	0.9 ± 0.17
	IAA-100 mM	41.1 ± 0.33	9.0 ± 2.9	2.1 ± 0.44
	GA-100 mM	38.9 ± 0.33	11.3 ± 1.70	2.4 ± 0.25
	Thiourea 100 mM	48.9 ± 0.89	13.1 ± 0.20	2.9 ± 0.12
	KNO3-100 mM	16.7 ± 1.15	3.6 ± 0.60	0.8 ± 0.13

Table.2 Two-factor ANOVA, where the p-value is significant ($p < 0.05$).

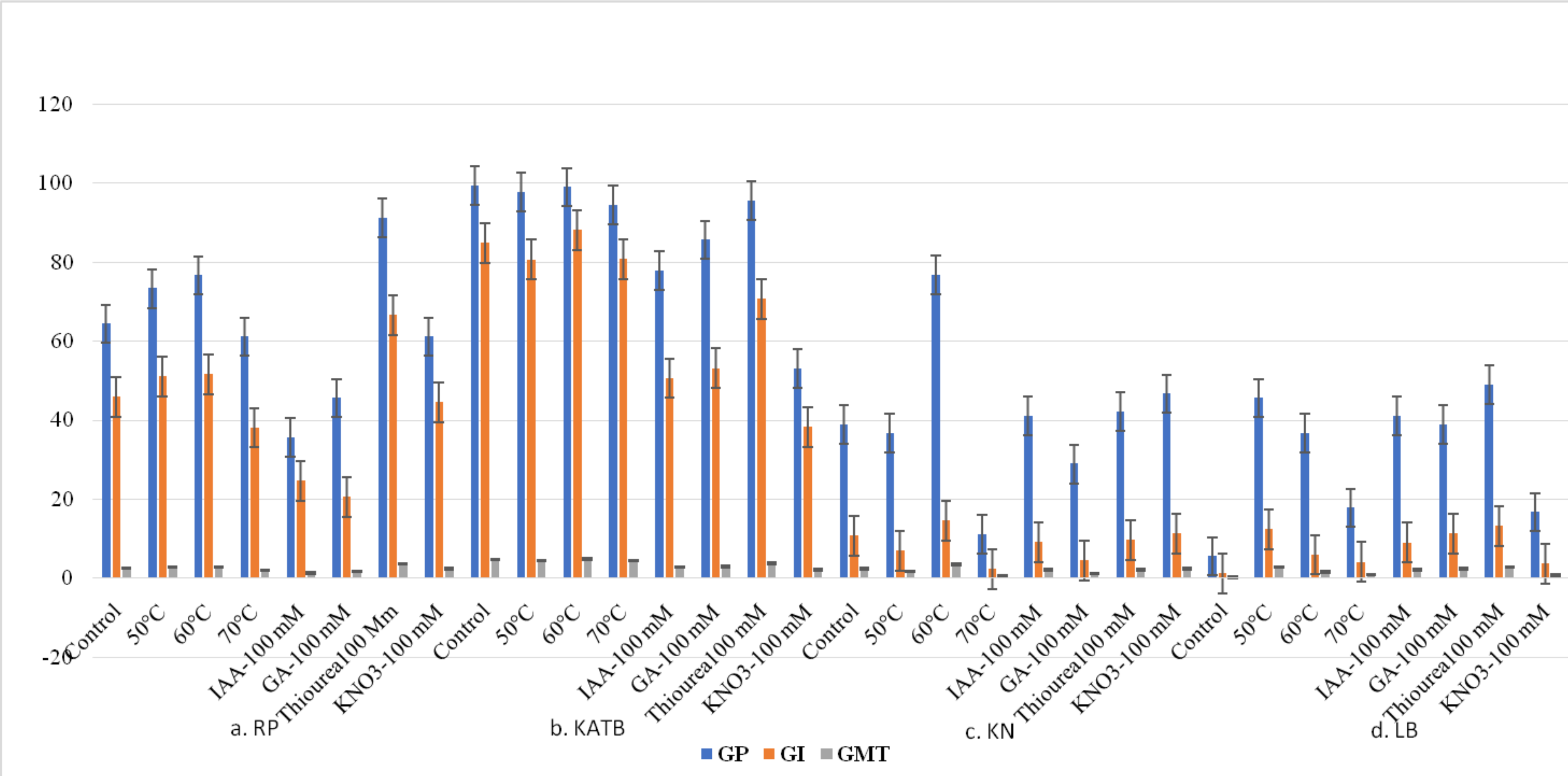
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	915.0729167	7	130.7247024	111.0581542	2.58987E-33	2.15642397
Columns	4052.864583	3	1350.954861	1147.713864	1.46367E-55	2.748190888
Interaction	1221.71875	21	58.17708333	49.42477876	1.17516E-31	1.723277736
Within	75.33333333	64	1.177083333			
Total	6264.989583	95				

Figure.1 Radhunipagal- Small grain, whitish, scented rice; 2. Kataribhog- Large grain, whitish, scented rice; 3. Kalonunia- Large grain, whitish, scented rice; 4. Lal-Badshabhog- Large grain, whitish, scented rice.

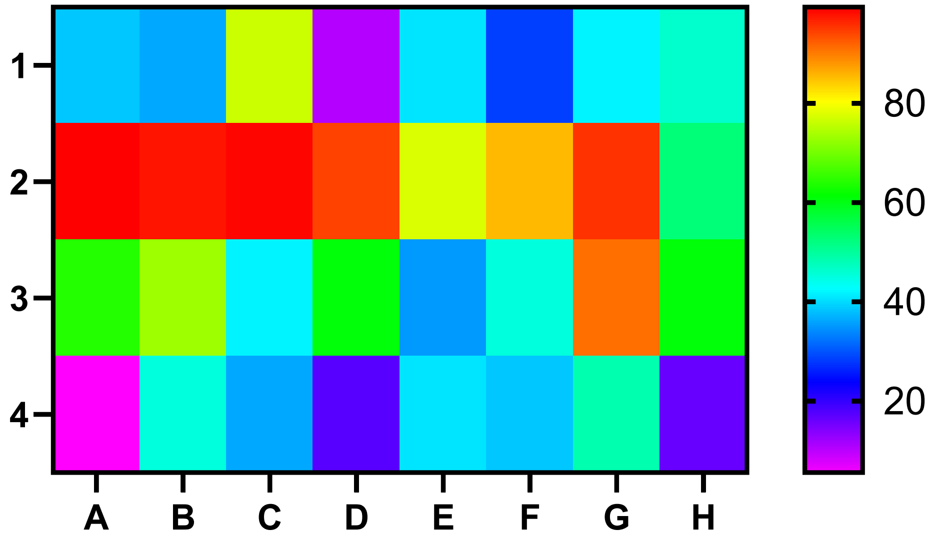
Graph.1 (a–d) Graph showing the percentage of seed germination of four landraces under pre-treatment with different factors (a). Radhunipagal (RP), (b). Kataribhog (KATB) (c). Kalonunia (KN), (d). Lal –Badshabhog (LB).



Graph.2 A comparative presentation of Germination percentage, Germination index, and Germination mean time of four landraces. (a) Radhunipagal (RP), (b). Kataribhog (KATB) (c). Kalonunia (KN), (d). Lal –Badshabhog (LB).



Heatmap.1 Heatmap represents the germination percentage of four different landraces (1. Radhunipagal, 2. Kataribhog, 3. Kalonunia, 4. Lal-Badshabhog) with different treatment (A. Control, B. 50°C, C. 60°C, D. 70°C, E. IAA 100mM, F. GA 100mM, G. Thiourea 100mM, H. KNO_3 100mM)



Also, KNO_3 improve germination in *Sorbus pohuashanensis* when treated with a specific concentration (Lei *et al.,* 2013). In this experiment, thiourea shows an effective percentage of germination increase in all landraces (Table 1). Alongside KNO_3, play both roles, as a nutrient and a stimulator for germination. Like thiourea, KNO_3 show a positive effect on germination induction (Kwon *et al.,* 2020).

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Conflicts of interest

The authors declare that they have no potential conflicts of interest.

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