

Overcoming physiological dormancy in common buckwheat (*Fagopyrum esculentum* Moench)

Vishwanath R Yalamalle

vishwanath_yal@yahoo.com

ICAR-IARI New Delhi <https://orcid.org/0000-0002-5534-0358>

Dunna Vijay

ICAR-IARI New Delhi <https://orcid.org/0000-0002-9001-2878>

Chawla Gautam

ICAR-IARI New Delhi <https://orcid.org/0000-0002-0411-7009>

Mishra Gyan Prakash

ICAR-IARI New Delhi <https://orcid.org/0000-0002-7023-2638>

Vijayakumar H P

ICAR-IARI New Delhi <https://orcid.org/0000-0001-6340-9899>

Dilshad Ahmad

ICAR-IARI New Delhi

Sandeep Kumar Lal

ICAR-IARI New Delhi <https://orcid.org/0000-0002-4077-9886>

Dinesh Chandra Joshi

ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora Uttarakhand- 263601 India

<https://orcid.org/0000-0002-4841-7281>

Rajendra Prasad Meena

ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora Uttarakhand- 263601 India

<https://orcid.org/0000-0002-3693-2278>

Research Article

Keywords: α -Amylase, Buckwheat, Dormancy, GA3, H2O2, KNO3, Seed Vigour

Posted Date: September 8th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3330021/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Genetic Resources and Crop Evolution on September 14th, 2023. See the published version at <https://doi.org/10.1007/s10722-023-01729-y>.

Abstract

The viability status of every accession stored in a seed bank is essential for effective germplasm conservation, and it is often assessed through germination tests. However, the presence of dormancy can impede this process. The dormancy release protocols for buckwheat seeds, which exhibit physiological dormancy, are not available. This study aims to identify effective dormancy-breaking protocols through the use of chemicals and phytohormones namely GA₃, KNO₃, and H₂O₂. Buckwheat seeds exhibited high dormancy with only 21% of seeds germinating. Seed treatment with 0.4% KNO₃ enhanced germination to 69%. Both KNO₃ and H₂O₂ treatments significantly reduced the time to 50% germination (t₅₀) (2.37 – 2.55 days) and mean germination time (MGT) (3.02 – 3.23 days). These treatments also enhanced the dormancy index (DI) (253.56 - 217.86). The seedling length was enhanced by 55% with 200 ppm GA₃. There was an enhancement in seed vigour indices by 305% and 260% in 20 mM H₂O₂ and 0.4% KNO₃ treatments respectively. Compared to the control, all the seed treatments enhanced the α-amylase activity. Both 0.4 % KNO₃ and 20 mM H₂O₂ treatments recorded the highest α-amylase activity. The result suggests that KNO₃ and H₂O₂ treatments reduced seed dormancy and improved seed germination and vigour by increasing the mobilization of seed reserves. Application of 0.4% KNO₃ or 20 mM H₂O₂ as a dormancy release mechanism can be incorporated during seed testing and germplasm evaluation of buckwheat seeds with physiological seed dormancy.

Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is a member of the Polygonaceae family, which includes around 800 species of herbaceous plants, shrubs, and trees within 30 to 40 genera that provide grain, edible stems, leaf vegetables, and several medicinal products. The fruit of the buckwheat is usually achene and may sometimes be enclosed in the perianth, forming a berry-like structure (Ellis et al. 1985). Among buckwheat species, common buckwheat (*Fagopyrum esculentum*) and Tartary (*Fagopyrum tataricum*) are commonly cultivated for grain consumption. Common buckwheat is more widely cultivated than Tartary buckwheat due to its large seed size, easy-to-hull seed coat, and absence of bitter taste. Buckwheat seeds are a good source of protein, lipids, dietary fiber, and minerals. They also contain flavonoids, fagopyrin, and buckwheat sterols. Due to its nutritional content, buckwheat has received increasing attention as a potential functional food (Jiang et al. 2007). The seeds of the buckwheat plant exhibit orthodox storage behaviour and possess physiological seed dormancy, it requires up to six months of after-ripening for breaking seed dormancy (Born and Corns 1958). In some parts of the world, buckwheat grows in the wild and is designated as difficult to control noxious weed (Born and Corns 1958). Seed dormancy is a well-known adaptive mechanism in plants growing in the wild, which allows them to delay germination until environmental conditions are favorable for growth and ensures the survival of the species (Willis et al. 2014). This mechanism is regulated by various physiological and molecular factors, including hormonal regulation, gene expression, and signalling pathways (Finch-Savage and Leubner-Metzger 2006).

Absciscic acid (ABA) is the primary hormone responsible for inducing dormancy in seeds, while gibberellic acid (GAs) and cytokinin promote germination. The balance of these hormones in the seed plays a crucial role in regulating seed dormancy. ABA prevents the mobilization of stored reserves by inhibiting the activity of hydrolytic enzymes, such as α -amylase, that are responsible for breaking down stored carbohydrates (Graeber et al. 2014). GAs promote germination by stimulating the synthesis of α -amylase and other hydrolytic enzymes, while CKs promote cell division (Kucera et al. 2005). Seed dormancy is also regulated by molecular mechanisms, including gene expression and signalling pathways. The genes involved in seed dormancy and germination have been extensively studied, and various signalling pathways, such as ABA, GA and Hydrogen peroxide (H_2O_2) signalling pathways, have been identified (Liu et al. 2010; Jeevan Kumar et al. 2015; Wojtyla et al. 2016).

Chemical treatments have been widely used to alleviate seed dormancy and promote germination in several crops. Among them, GA_3 , KNO_3 , and H_2O_2 are more common. GA_3 , a plant growth regulator has been shown to promote germination and break seed dormancy in a wide range of crops (Çırak et al. 2007; Finkelstein et al. 2008; Liu et al. 2010). The biosynthesis of GA occurs in the embryo, which facilitates the progression of germination (Yamaguchi et al. 2002). GA_3 stimulates the synthesis of α -amylase and other hydrolytic enzymes that are responsible for breaking down stored carbohydrates, promoting germination (Peng and Harberd 2002). Studies have shown that the dormancy release and germination are dependent on the balance of GA-ABA in both the embryo and the layers surrounding the seed (Debeaujon and Koornneef 2000). In wheat, upregulation of $GA20ox1$ and $GA3ox2$ genes during dormancy release is reported. The dormant varieties tend to accumulate less-active GA compared to non-dormant varieties (Liu et al. 2013). Gibberellic acid has been successfully used to overcome dormancy in several species including *Arabidopsis* (*Arabidopsis thaliana*) (Ogawa et al. 2003), wild rice (Timple et al. 2018), and tomato (*Solanum lycopersicum*) (Balaguera-López et al. 2009). Inorganic nitrates, such as KNO_3 , have been found to decrease the light sensitivity and light requirement of photoblastic seeds (Toole et al. 1955; Batak et al. 2002). When combined with red light fluences, nitrogenous compounds such as KNO_3 , nitroglycerine, and sodium nitroprusside were observed to enhance germination in *Arabidopsis thaliana* by up to 100%. The stimulative effect of potassium nitrate on seed germination was observed only in the presence of Phytochrome A (phyA) (Batak et al. 2002). KNO_3 is reported to increase the activity of hydrolytic enzymes, thus enhancing the mobilization of stored reserves (Nawel et al. 2011). Lara et al. (2014) reported enhanced seed germination in KNO_3 -primed seeds. KNO_3 enhanced the activity of nitrate reductases and nitric oxide (a source of ROS), which in turn increased the activity of Superoxide Dismutase (SOD) and Catalase (CAT) enzymes. KNO_3 has been reported to alleviate physiological seed dormancy in several crops, including wild rice (Timple et al. 2018), lettuce (*Lactuca sativa*) (Dong et al. 2012), *Arabidopsis* (Batak et al. 2002), and *Sisymbrium officinale* (Toorop 2015). H_2O_2 serves as a central signalling hub that regulates both seed dormancy and germination by simultaneously triggering the breakdown of absciscic acid (ABA) and the production of gibberellic acid (Kucera et al. 2005; Anand et al. 2019). Ishibashi et al. 2017 reported that the level of H_2O_2 in non-dormant barley seeds was higher than in dormant seeds. The researchers also recorded an enhanced activity of ABA-8'-hydroxylase, an ABA

catabolic enzyme, in the non-dormant seeds. H_2O_2 has been shown to promote germination by regulating the activity of various enzymes involved in seed germination, such as α -amylase (Nawaz et al. 2011). It has been reported to alleviate physiological dormancy in seeds of several crops, including wild rice (Timple et al. 2018); Wild almond (Zeinalabedini et al. 2009). In *Glehnia littoralis* the seed dormancy release was positively correlated with H_2O_2 accumulation (Shao et al. 2021). Stein et al. (2021) reported that treating cherry seeds with 880 mM H_2O_2 resulted in enhanced seed germination and reduced stratification time.

According to (Born and Corns 1958), the seed dormancy in buckwheat may have multiple factors contributing to it, and the seed coating did not hinder water imbibition, suggesting an absence of physical dormancy. Light treatments to the seed coat (partial removal), pre-chilling, and alternating temperatures promote the germination of dormant buckwheat seeds. Additionally, seed treatment with GA_3 only partially improved germination and was effective only in stored seeds. Several unsuccessful attempts have been made to overcome seed dormancy in buckwheat by use of the GA_3 (Born and Corns 1958), removal of the seed coat (Born and Corns 1958); blue light, dark, at 28°C (Singh and Mall 1977), acid scarification (Singh and Mall, 1977), ethyl alcohol (Singh and Mall 1977).

Seed testing, seed conservation, and crop production require information on germination, dormancy, and methods to enhance seed germination. The information on the successful alleviation of seed dormancy in buckwheat is limited. The present study attempts to address the issue of seed dormancy through the use of chemicals and phytohormones - GA_3 , KNO_3 , and H_2O_2 . Although seed dormancy has been previously reported in buckwheat, we did not find any seed dormancy treatments in the International Seed Testing Association (ISTA 2021) guidelines, which are followed by several genebanks including those in India. The results from the present study will be helpful in seed testing, germplasm conservation, and the seed growers of buckwheat.

Material and methods

Seed materials

Seed samples of buckwheat variety PRB-1 were procured from ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora Uttarakhand- 263601 India, 1600 m AMSL, latitude 29°35' N, longitude 79°39'E. The seeds were harvested in October 2022. The seeds were hermetically sealed and stored at 20 °C for three months before the experiment. The seed moisture was investigated following (ISTA 2021), using the high constant temperature oven method. Three replicates of 5 ± 0.5 g seed samples were dried at $130 \pm 2^\circ\text{C}$ for two hours and cooled in silica gel for 30 minutes. The seed moisture was expressed as a percentage on a fresh weight basis.

Determination of 1000-seed weight

Eight replicates of 100 seeds were weighed and used to calculate the mean 1000-seed weight as per (ISTA 2021). The moisture content of the seed was 7.5% at the time of 1000 seed weight determination.

Determination of seed physical characteristics

The seed physical characteristics were measured digitally by using Image J software version 1.54b (<https://imagej.nih.gov/ij/index.html>). Seed length and seed width was calculated based on the average of 100 randomly selected seeds. The length-to-width ratio was calculated by dividing the length and width.

Scanning Electron Microscopy (SEM)

To study the surface characteristics of the seeds, the seeds were imaged using Scanning Electron Microscope (SEM). The seeds sample was cleaned using 70% ethanol and then air dried. To prevent charging of the surface, enhance the emission of secondary electrons, and provide a homogeneous surface for analysis the seeds were coated with a uniform, thin layer of gold-palladium alloy using a sputter coater (Emitech SC7620). After coating, the specimens were examined using SEM (Tescan Vega3) with a secondary electron detector at accelerating voltages of 30.0 kV, and images were taken at different magnifications with appropriate tilt angles. Seeds were uniformly scanned at the surface to reveal the seed coat ultrastructure.

Chemicals used in the study

All the chemicals used in the present study were analytical grade or above. GA_3 ($\geq 90\%$ purity) was procured from Sisco Research Laboratories Pvt. Ltd., India., H_2O_2 (30% w/v) was procured from Thermo Fisher Scientific India Pvt. Ltd., and KNO_3 (99.5% purity) was procured from Central Drug House Private Ltd., India.

Seed treatment and standard germination test

The treatment details and the physiological roles of chemicals used in the present study are summarized in Table 1. Standard germination testing was performed according to the (ISTA 2021) using three replicates of 50 seeds each. Seeds were placed on two layers of 15 cm Whatman no. 10 filter paper. The filter paper was dipped in chemical solutions per treatment (Table 1). Germination was performed by the top-of-the-paper method. Seeds were kept in a walk-in germinator at a constant temperature of 20°C in the presence of continuous cool white light 4000K. The final count was made on the 7th day after test initiation and only normal seedling were counted for calculating germination percentage.

Table 1

Treatment details and the physiological roles of phytohormones and chemicals used in the study

S.No	Chemicals	Concentrations	Physiological role	References
1.	GA ₃	100 ppm, 200 ppm, and 500 ppm	Stimulates the synthesis of α-amylase and other hydrolytic enzymes. Endosperm weakening and endosperm rupture. Induction of Class I β-1,3-glucanases	Kaneko et al. (2002); Müller et al. (2006); Leubner-Metzger et al. (1996)
2.	H ₂ O ₂	5 mM, 10 mM, and 20 mM	Triggers the breakdown of abscisic acid (ABA) and the production of gibberellic acid. Enhance the activity of α -amylase activity. Enhances the activity of ABA-8'-hydroxylase.	Kucera et al. (2005); Anand et al. (2019); Ishibashi et al. (2017)
3.	KNO ₃	0.1%, 0.2%, and 0.4%	Enhances the activity of hydrolytic enzymes. Enhanced Nitric Oxide (NO) and ROS production, enhanced Superoxide dismutase (SOD) and Catalase (CAT) production. Increase the light sensitivity of photoblastic seeds.	Nawel et al. (2011) Lara et al. (2014) Toole et al. (1955); Batak et al. (2002)

Seed Vigour Indices

Seed vigour was calculated as per (Abdul-Baki and Anderson 1973). On the 7th day, ten normal seedlings were chosen randomly, and their lengths were measured. The same seedlings were then dried at 50°C ± 1 till constant weight. Seedling vigour index-I was calculated as the product of the percentage of germination and the average length of the ten selected seedlings, measured in millimeters. Seedling vigour index-II, on the other hand, was calculated by multiplying the percentage of germination with the average weight of the ten selected seedlings, measured in milligrams.

Tetrazolium staining (TZ)

To confirm the viability of fresh ungerminated seeds (FUG) the FUG were subjected to a TZ test. In brief, at the end of the germination test i.e., 7th day, the seeds which were sound and remained ungerminated were subjected to the TZ test. These seeds were cut longitudinally through the fruit and seed coat, the seed coat and endosperm were removed to reveal the embryo. The embryos were soaked in 1% TZ solution at 30° C in the dark for 18 hours. After the staining period, the seeds were washed with water and assessed based on the intensity and consistency of tissue colour, following the criteria outlined in (ISTA

2003). The seeds were then categorized as viable or non-viable based on the staining pattern in vital parts of the embryo (ISTA 2003).

Germination metrics studies

The seeds having radicle protrusion (≥ 0.5 mm) were considered germinated for computing various germination parameters i.e. t_{50} , mean germination time (MGT), and speed of germination, which were derived using germinationmetrics (version 0.1.7.9000) an R package (Aravind et al. 2023). The area under the germination curve (AUGC) was calculated based on the germination curve fitting using a four-parameter hill function developed by El-Kassaby et al. (2008) in the germinationmetrics package. The dormancy index (DI) was determined by calculating the difference between the areas under the germination curves before and after dormancy-breaking treatments as per El-Kassaby et al. (2008). The speed of germination was calculated as per (Czabator 1962) with modification as suggested by (Evetts and Burnside 1972). The corrected germination speed was calculated by dividing the germination speed, estimated in terms of germination percentage, by the final germination percentage attained by the seed lot.

Estimation of α - amylase enzyme

The α -amylase enzyme was estimated as per (Li et al. 2000) with some modifications. In brief, seeds were subjected to different treatments (Table 1). After three days, the seed coat was removed, and 250 mg of seeds were used for extraction. The sample was homogenized in 5 ml of 10 mM CaCl_2 buffer and centrifuged at 12,000g; the supernatant was collected. One millilitre of the supernatant and one millilitre of 1% starch solution were added to a test tube and incubated at 37°C for 15 minutes. The reaction was stopped by adding two ml of Dinitrosalicylic acid reagent. The solution was heated in a boiling water bath for five minutes, after which one ml of 40% potassium tartrate was added. The absorbance was read at 540 nm, one unit is equivalent to the amount of enzyme liberating one mg maltose from starch at 37°C and expressed as mg maltose per gram fresh weight (mg maltose g^{-1} FW).

Statistical analysis

The data were analyzed using WASP - Web Agri Stat Package 2.0, which is hosted by the ICAR - Central Coastal Agricultural Research Institute (Anonymous 2023). The data was subjected to an analysis of variance, and means were separated using the least significant difference test with a significance level of $p \leq 0.05$. The percentage data were transformed using the arcsine function before analysis to normalize the variance. Grouping letters were assigned to treatment means using Fisher's least significant difference, with means that shared at least one common letter considered not statistically significant.

Result

Seed morphology and tetrazolium staining

The data on the seed morphological parameters are presented in (Table 2), Fig. 1. Buckwheat seeds were $53.10 \text{ mm} \pm 0.58 \text{ mm}$ long and $35.03 \pm 0.27 \text{ mm}$ wide, with a length/width ratio of 1.52 ± 0.17 . The seeds were oval, and the surface was light brown (Fig. 1A, 1B) with dark brown spots. The 1000 seed weight was $21.03 \pm 0.25 \text{ g}$. The SEM of buckwheat seed surface shows the absence of hairs or similar appendages on the seed coat. The seed coat did not have mucilage, which could affect the water uptake. Seed magnification at 500x shows that the testa exhibits a wavy-undulate wall pattern (Fig. 1D). TZ test shows that the endosperm did not show any red staining area indicating that the endosperm is devoid of any live tissue (Fig. 2). Endosperm covers the inner loop of the cotyledon. As shown in Fig. (2A, 2B) the cotyledon loops at the bottom, while the two cotyledons, which show a typical sinusoidal pattern, remain in tight contact. The cotyledon extends to the periphery along the seed coat.

Table 2

Physical characteristics of buckwheat seeds

	1000 seed weight (g)	Length (mm)	Width (mm)	Length-to-width ratio
Average	21.03 ± 0.25	53.10 ± 0.58	35.03 ± 0.27	1.52 ± 0.17
Range	0.73	2.92	1.40	0.90

The average value is expressed as the means ± standard deviation (n = 100)

Effects of seed treatments on seed germination (GP)

The effect of seed treatments on germination is presented in Table 3, Fig. 3. The seed treatments had significant ($p \leq 0.01$) effects on germination. The GP under all treatments increased compared with that of the control. Among treatments highest germination (69%) was recorded in 0.4% KNO_3 treated seeds (Fig. 3B), which was also statistically similar to 5mM H_2O_2 , 10mM H_2O_2 , 20mM H_2O_2 , and 0.2% KNO_3 treatments. All the KNO_3 and H_2O_2 treatments were effective in enhancing germination. Among GA_3 -treated seeds 200 ppm GA_3 had higher seed germination and germination declined in 500 ppm GA_3 treated seed. The lowest germination (21%) was recorded in the control which also had a high number of abnormal seedlings and FUG (Fig. 3A), Supplement-I.

Effects of seed treatments on time to 50% germination (t50)

The effect of seed treatments on the t50 is presented in Table 3. The seed treatments had significant ($p \leq 0.01$) effects on t50. The t50 under all treatments decreased in comparison to the control. Among treatments lowest t50 (2.37 days) was recorded in 0.2% KNO_3 treated seeds, which was also statistically similar to 5mM H_2O_2 , 10mM H_2O_2 , 20mM H_2O_2 , 0.1% KNO_3 and 0.4% KNO_3 treatments. All the KNO_3 and H_2O_2 treatments were effective in reducing the t50. The highest t50 (5.23 days) was recorded in 500 ppm GA_3 which was statistically similar to the control.

Effects of seed treatments on Mean Germination Time (MGT)

The effect of seed treatments on the MGT is presented in Table 3. The seed treatments had significant ($p \leq 0.01$) effects on MGT. The MGT under all treatments decreased in comparison to the control. Among treatments lowest MGT (3.02 days) was recorded in 0.2% KNO_3 treated seeds, which was also statistically similar to 5mM H_2O_2 , 10mM H_2O_2 , 20mM H_2O_2 , 0.1% KNO_3 , and 0.4% KNO_3 treatments. All the KNO_3 and H_2O_2 treatments were effective in reducing the MGT. The highest MGT (5.52 days) was recorded in 500 ppm GA_3 which was statistically similar to control.

Table 3

Effect of seed dormancy breaking treatments on seed germination parameters in buckwheat

Treatments	Germination	Time to 50% germination (t50) (days)	Mean germination time (MGT) (days)	Speed of germination	Area under Germination the curve (AUGC)
GA ₃ 100 ppm	48 (43.84) ± 8.00 ^{cd}	2.98 ± 0.26 ^c	3.76 ± 0.12 ^c	0.28 ± 0.01 ^c	276.40 ± 19.14 ^c
GA ₃ 200 ppm	53 (46.91) ± 2.31 ^{bc}	3.27 ± 1.44 ^b	4.01 ± 0.30 ^c	0.27 ± 0.02 ^c	245.47 ± 11.54 ^c
GA ₃ 500 ppm	36 (36.76) ± 8.72 ^d	5.23 ± 0.16 ^a	5.52 ± 0.17 ^a	0.18 ± 0.01 ^d	118.05 ± 3.50 ^d
H ₂ O ₂ 5 mM	64 (53.17) ± 6.00 ^{ab}	2.43 ± 0.17 ^d	3.07 ± 0.29 ^d	0.36 ± 0.03 ^{ab}	352.30 ± 30.91 ^{ab}
H ₂ O ₂ 10 mM	62 (52.04) ± 9.17 ^{ab}	2.49 ± 0.11 ^d	3.21 ± 0.29 ^d	0.35 ± 0.02 ^{ab}	319.30 ± 7.55 ^b
H ₂ O ₂ 20 mM	67 (55.15) ± 2.31 ^a	2.52 ± 0.09 ^d	3.09 ± 0.13 ^d	0.35 ± 0.01 ^{ab}	341.35 ± 27.45 ^{ab}
KNO ₃ 0.1%	65 (54.07) ± 9.24 ^{ab}	2.45 ± 0.14 ^d	3.05 ± 0.14 ^d	0.36 ± 0.02 ^{ab}	353.34 ± 4.70 ^a
KNO ₃ 0.2%	65 (53.70) ± 11.02 ^{ab}	2.37 ± 0.09 ^d	3.02 ± 0.08 ^d	0.36 ± 0.01 ^a	355.00 ± 35.36 ^a
KNO ₃ 0.4%	69 (56.41) ± 4.16 ^a	2.55 ± 0.06 ^d	3.23 ± 0.14 ^d	0.33 ± 0.01 ^b	354.13 ± 14.95 ^a
Control %	21 (27.47) ± 3.06 ^e	5.11 ± 0.05 ^a	5.17 ± 0.12 ^b	0.21 ± 0.01 ^d	101.44 ± 10.85 ^d
Mean	55.13 (47.95)	3.14	3.71	0.31	281.68
CD (0.05)	7.34	0.27	0.33	0.03	33.60
CD (0.01)	10.00	0.36	0.46	0.04	45.82

Each value is expressed as the means ± standard deviation (n = 3). Different superscript letters showed significant differences ($p \leq 0.05$). The values in the parenthesis are arc sine transformed values

Effects of seed treatments on the Speed of Germination

The effect of seed treatments on the speed of germination is shown in Table 3. The seed treatments had significant ($p \leq 0.01$) effects on the speed of germination. The speed of germination under all treatments

increased compared with that of control. Among treatments highest speed of germination was recorded in 0.2% KNO₃ (0.36) treated seeds which were also statistically similar to 0.1% KNO₃, 5mM H₂O₂, 10mM H₂O₂, and 20mM H₂O₂ treated seeds. Conversely, the lowest speed of germination (0.18) was recorded in the 500 ppm GA₃ which was statistically similar to control.

Effects of seed treatments on the Area Under Germination Curve (AUGC)

The effect of seed treatments on the AUGC is shown in Table 3 and Fig. 5. The seed treatments had significant ($p \leq 0.01$) effects on the AUGC. The AUGC under all treatments increased compared with that of the control. Among treatments highest AUGC was recorded in 0.2% KNO₃ (355.00), 0.4% KNO₃, and 0.1% KNO₃ treated seeds which were also statistically similar to 5 mM H₂O₂, and 20 mM H₂O₂ treated seeds. Conversely, the lowest AUGC was recorded in control (101.44) which was statistically similar to 500 ppm GA₃ treatment.

Effects of seed treatments on the Dormancy Index (DI)

The effect of seed treatments on the DI is shown in Fig. 7. The seed treatments had significant ($p \leq 0.01$) effects on the DI. All the KNO₃ and H₂O₂ treatments had significantly higher DI. Among treatments, highest DI was recorded in 0.2% KNO₃ (253.56), 0.4% KNO₃, 0.1% KNO₃, 5mM H₂O₂, 10mM H₂O₂, and 20mM H₂O₂ treated seeds. Conversely, the lowest DI (16.61) was recorded in the 500 ppm GA₃ treatment.

Effects of seed treatments on seedling length (SL)

The SL after seed treatments is presented in Table 4, Fig. 6. The seed treatments had significant ($p \leq 0.05$) effects on SL. The SL under all treatments increased compared with that of the control. The highest SL (15.87 cm) was recorded in 200 ppm GA₃ seed treatment which was statistically similar to 10mM H₂O₂. Conversely, the lowest seedling length was recorded in the control, which was statistically similar to the 500 ppm GA₃ treatment.

Effects of seed treatments on Seedling Dry Weight (SDW)

The seedling dry weight (SDW) after seed treatments is presented in Table 4. The seed treatments had significant ($p \leq 0.05$) effects on SDW. The SDW under all treatments increased compared with that of the control. The highest SDW was recorded in 10mM H₂O₂ (10.86 mg) seed treatment which was statistically similar to 100 ppm GA₃, 200 ppm GA₃, 20mM H₂O₂, and 0.4% KNO₃ seed treatment. Conversely, the lowest SDW was recorded in the control (9.17 mg), 5mM H₂O₂, and 500 ppm GA₃, which was statistically similar to 0.1% KNO₃, 0.2% KNO₃, 0.4% KNO₃, and 20 mM H₂O₂ treatment.

Table 4
Effect of seed dormancy breaking treatments on seed vigour parameters in buckwheat

Treatments	Seedling length (cm)	Seedling dry weight (mg)	Vigour index-I	Vigour index-II	Speed of germination
GA ₃ 100 ppm	11.38 ± 1.10 ^{bc}	10.29 ± 0.81 ^{ab}	551.79 ± 139.98 ^{bc}	497.52 ± 118.54 ^c	1.46 ± 0.09 ^c
GA ₃ 200 ppm	15.87 ± 1 ^a	10.28 ± 0.19 ^{ab}	846.83 ± 72.81 ^a	548.29 ± 20.18 ^{bc}	1.33 ± 0.02 ^c
GA ₃ 500 ppm	10.49 ± 1 ^c	9.18 ± 0.10 ^c	372.87 ± 69.14 ^{cd}	330.95 ± 82.47 ^d	0.82 ± 0.04 ^d
H ₂ O ₂ 5 mM	11.71 ± 2.79 ^{bc}	9.17 ± 0.36 ^c	758.09 ± 227.68 ^a	587.65 ± 73.63 ^{abc}	1.99 ± 0.23 ^{ab}
H ₂ O ₂ 10 mM	14.3 ± 3.04 ^{ab}	10.86 ± 0.84 ^a	876.44 ± 149.67 ^a	674.50 ± 125.75 ^{ab}	1.82 ± 0.02 ^b
H ₂ O ₂ 20 mM	13.41 ± 0.73 ^{bc}	10.23 ± 0.89 ^{abc}	901.96 ± 32.39 ^a	688.36 ± 51.29 ^{ab}	1.91 ± 0.15 ^{ab}
KNO ₃ 0.1%	12.64 ± 0.45 ^{bc}	9.67 ± 0.49 ^{bc}	824.14 ± 102.54 ^a	628.53 ± 54.62 ^{abc}	2.05 ± 0.07 ^a
KNO ₃ 0.2%	11.67 ± 0.29 ^{bc}	9.73 ± 0.28 ^{bc}	753.52 ± 120.64 ^{ab}	630.57 ± 122.98 ^{abc}	2.03 ± 0.19 ^a
KNO ₃ 0.4%	12.71 ± 0.89 ^{bc}	10.14 ± 0.87 ^{abc}	881.79 ± 89.94 ^a	703.87 ± 82.87 ^a	1.93 ± 0.13 ^{ab}
Control %	10.21 ± 2.42 ^c	9.17 ± 0.88 ^c	222.33 ± 81.66 ^d	195.26 ± 31.13 ^d	0.67 ± 0.06 ^d
Mean	12.44	9.87	698.98	548.50	0.31
CD (0.05)	2.83	1.10	204.95	195.83	0.206
CD (0.01)	-	-	279.52	143.59	0.281

Each value is expressed as the means ± standard deviation (n = 3). Different superscript letters showed significant differences ($p \leq 0.05$).

Effects of seed treatments on Vigour Index-I

The effect of seed treatments on vigour index-I is presented in Table 2. The seed treatments had significant ($p \leq 0.01$) effects on vigour index-I. The vigour index-I under all treatments increased compared with that of the control. Among treatments highest vigour index-I was recorded in 0.4% KNO₃ (901.96) treated seeds, which was statistically similar to 5mM H₂O₂, 10mM H₂O₂, 20mM H₂O₂, 0.2%

KNO₃, and 0.1% KNO₃. Conversely, the lowest vigour index-I was recorded in the control (222.33) which was statistically similar to 500 ppm GA₃.

Effects of seed treatments on vigour index-II

The effect of seed treatments on vigour index-II is presented in Table 4. The seed treatments had significant ($p \leq 0.01$) effects on vigour index-II. The vigour index-II under all treatments increased compared with that of the control. Among treatments highest vigour index-II was recorded in, 0.4% KNO₃ (703.87), which was also statistically similar to 0.1% KNO₃, 0.2% KNO₃, 5 mM H₂O₂, 10mM H₂O₂, and 20mM H₂O₂ treated seeds. Conversely, the lowest vigour index-II was recorded in the control (195.26) which was statistically similar to 500 ppm GA₃.

Effects of seed treatments on α -Amylase activity

The effect of seed treatments on α -Amylase activity is presented in Fig. 8. The seed treatments had significant ($p \leq 0.01$) effects on α -Amylase activity. The α -Amylase activity under all treatments increased compared with that of the control. Among treatments highest α -Amylase activity was recorded in 0.4% KNO₃ (0.42 mg maltose g⁻¹ FW), which was also statistically similar to 20 mM H₂O₂ seed treatment. Conversely, the lowest α -Amylase activity was recorded in the control (0.02 mg maltose g⁻¹ FW).

Discussion

Buckwheat (*Fagopyrum esculentum* Moench) is included in the International Seed Testing Association (ISTA 2021) rules for seed germination. Though, seed dormancy in buckwheat has been reported as early as 1949 (Everson 1949), ISTA has not prescribed any guidelines for dormancy removal in buckwheat. ISTA guidelines are followed internationally in various labs including National Bureau of Plant Genetic Resources, India for seed testing and dormancy breaking (Pandey et al. 2019; ISTA 2021). According to ISTA, when 5% or more fresh ungerminated seeds are present, their potential to germinate must be determined by methods such as dissection, tetrazolium, or excised embryos (ISTA 2003, 2021). Compared to the TZ test, chemical seed treatments are simple and economical ways to determine seed viability status; moreover, TZ tests require special training and experience (Landis and South 1997). The information on the dormancy status and methods of breaking seed dormancy is essential during the initial screening to assess the viability of seeds prior to germplasm storage.

The productivity and quality of several crops are often influenced by factors such as seed dormancy, which can lead to uneven germination and reduced yield (Lamichhane et al. 2018). Seed dormancy is a natural adaptation of several species governed by physical and physiological mechanisms that prevent seed germination under unfavourable environmental conditions. Physiological seed dormancy is caused by various physiological mechanisms within the embryo itself relieved by various environmental factors

such as temperature, light, chemicals and after ripening. Physiological dormancy is common in many plant species and is particularly prevalent in angiosperms (Baskin and Baskin 2014), including buckwheat (Born and Corns 1958; Metzger 1992).

In the present study, the effects of GA_3 , KNO_3 , and H_2O_2 on the dormancy and seed vigour parameters of buckwheat seeds was studied. These chemicals are known to alleviate seed dormancy and enhance germination in several species (Toole et al. 1955; Leubner-Metzger et al. 1996; Kaneko et al. 2002; Batak et al. 2002; Kucera et al. 2005; Müller et al. 2006; Ishibashi et al. 2017; Anand et al. 2019). Seeds were treated with different concentrations of GA_3 , KNO_3 , and H_2O_2 (Table 1). In control, 21% germination was recorded, whereas, all the dormancy-breaking treatments enhanced the germination which varied from (48% – 69%). Suzuki et al. (2020) studied the germination of buckwheat in freshly harvested varieties 'Hitachiakisoba' and 'Harunoibuki' seeds, which recorded 21.1% and 37.4% germination respectively. In the present study, the viability status of the ungerminated seed was confirmed using the tetrazolium test, in the control more than 75% FUG were viable, (Fig. 4A and 4B). This confirmed that FUGs were still viable and could germinate if appropriate dormancy-breaking treatment was given. All the H_2O_2 and KNO_3 treatments had higher germination. It is interesting to note that control group not only had high FUG, but also high abnormal seedlings, which were drastically reduced in KNO_3 and H_2O_2 treatments. The enhancement in the standard germination may also be attributed to the reduced number of abnormal seedlings after treatments (Supplement-I). Though GA_3 did enhance germination, the germination enhancement was not as high as the former. The performance of GA_3 -treated seed peaked at 200 ppm and declined at 500 ppm, indicating the toxic effect at higher concentrations. Lee et al. (2022) reported the growth of *Penthorum chinense* radicles was negatively affected by GA_3 at all concentrations, probably due to higher concentrations of GA_3 . Born and Corns 1958 reported that treating *Fagopyrum tataricum* seeds with 10 – 1,000 ppm GA_3 did not significantly reduce seed dormancy. The GA_3 500 ppm also had low seedling length, seedling dry weight, vigour index-I, vigour index-II, and speed of germination. GA_3 when applied in appropriate concentration can promote cell elongation, enhance seed coat, and endosperm weakening (Kwon et al 2020). Previous studies in buckwheat have shown that GA_3 seed treatments are partially effective in breaking seed dormancy, and the effect was more pronounced in stored seeds (Born and Corns 1958). In the present study, the buckwheat seeds were stored for three months prior to treatment, which suggests that seed dormancy persisted beyond three months of storage. This explains the reasons for the ineffectiveness of GA_3 in effectively breaking seed dormancy in buckwheat. GA is not effective in some seeds with intermediate physiological dormancy and deep physiological dormancy (Lee et al. 2022).

Seed vigour parameters like t_{50} , MGT, speed of germination, and AUGC are indicators of seed quality, in terms of faster germination and early seedling establishment (Baalbaki et al. 2018). Seed germination is generally expressed as a percentage and represents the population as a single unit. However, the rate of germination can exhibit variation among individuals within populations with different stages of dormancy (Foley 2001; Lacerda et al. 2004; Baskin and Baskin 2014; Talská et al. 2020). Measurement of the germination rate at the individual seed provides valuable insight into the start, uniformity, and speed

of germination in a seed lot (Soltani et al. 2015). The germination of two lots having the same final germination but different speed, and uniformity of emergence cannot be compared. In the present study, all the H₂O₂ and KNO₃ treatments had significantly lower t₅₀ and MGT (Table 3). The t₅₀ shows the time to 50% germination of the total seed. MGT measures the mean germination time and does not consider a fixed germination percentage, lower the MGT, the faster a population of seeds germinates. Higher speed of germination and AUGC reflect the better performance of a seed lot or the response to a particular dormancy-breaking treatment (Renzi et al. 2020). In the present study, all the treatments of H₂O₂ and KNO₃ (except 0.4%) had significantly ($p \leq 0.01$) higher speed of germination (Table 3). The AUGC is a graphical demonstration of a predicted germination curve. The average germination values are plotted over the curve representing maximum germination, time to 50 percent germination, and area under the germination curve (AUGC). Seeds with higher speed of germination, high total germination, and low t₅₀ have higher AUGC. In the present study all the KNO₃ treatments, 5 mM H₂O₂ and 10 mM H₂O₂ treatments had significantly ($p \leq 0.01$) higher AUGC. Compared to the control 0.2% KNO₃ treatment had more than three times higher AUGC. According to Baalbaki et al. (2018) among various vigour parameters studied AUGC was most effective in representing the minor difference in vigour of lettuce seeds. Seed priming with KNO₃ has been previously reported to reduce germination time and improved germination rate in tomato (Lara et al. 2014), and *Gladiolus alatus* (Salman et al. 2012).

Seed vigour is an important seed quality parameter, manifested in the form of field emergence and crop performance. Seed lots with the same germination, but seeds vigour perform differently in the field. Seeds with high vigour have faster and uniform emergence across diverse environmental conditions (Lamichhane et al. 2018). Seedling length and seedling dry weight are important vigour parameters, which are also used to derive vigour indices - vigour index-I and vigour index-II (Abdul-Baki and Anderson 1973; Finch-Savage and Bassel 2016). From the grower's perspective, the effective dormancy-breaking treatment should not only enhance seed germination but must also enhance seed vigour. In the present study, all the seed dormancy breaking treatments were effective in enhancing the seed vigour except the 500 ppm GA₃ seed treatment. 200 ppm GA₃ seed treatment was effective in enhancing seedling length, seedling dry weight, and vigour index-I (Table 4), whereas 100 ppm GA₃ was effective in enhancing seedling dry weight. GA is known to enhance cell elongation, weakening of endosperm, and expansion of embryo cells and enhance α -amylase (Miransari and Smith 2014). Compared to other GA treatments the superior performance of 200 GA₃ ppm treatment could also attribute to significantly higher α -amylase production (Fig. 8), which could have helped in the faster mobilization of carbohydrates for developing seedlings. Seed treatment with H₂O₂ was effective in enhancing seedling dry weight (except H₂O₂ 5 mM), vigour index-I and vigour index-II, and speed of germination (except H₂O₂ 10mM). H₂O₂ is a ROS that also acts as a signalling molecule it has diverse roles in seed germination and dormancy breaking. It has been reported to enhance α -amylase activity, contributes to endosperm and seed coat weakening, ABA catabolism, and enhance GA production (Kucera et al. 2005; Ishibashi et al. 2017; Anand et al. 2019). Seed vigour enhancement due to H₂O₂ treatments has been reported previously in several crops (Wahid et al. 2008; Kindiger 2019). All the KNO₃ seed treatments were effective in increasing the vigour index-I and

vigour index-II, and the speed of germination, in addition, 0.4% KNO_3 was also effective in enhancing seedling dry weight (Table 3). The main effect of KNO_3 is through nitrate compound. Nitrates are known to counter the effect of ABA, enhancing both ABA catabolism and the production of hydrolytic enzymes. In the present study, among KNO_3 treatments, 0.4% KNO_3 was the most effective, enhancing most of the vigour parameters, it also had had highest α -amylase activity (Fig. 8). Developing seeds requires energy which is obtained from stored carbohydrates, α -amylase helps in the breakdown of complex sugars into monosaccharides, which is utilized by the germinating seeds. The enhanced vigour in KNO_3 treatment could be attributed to the improved availability of nutrients for developing seedlings. In peas seed treatment with KNO_3 increased GA1 and decreased ABA in both seedlings and cotyledons, resulting in a decline in the ABA/GA ratio (Hernández et al. 2021). In capsicum seed treatment with 3% KNO_3 enhanced the seedling length, fresh weight, and seed vigour (Amjad et al. 2007).

Embryonic ABA is vital for inducing and maintaining seed dormancy, which inhibits the transition from embryonic to germination growth (Rodríguez-Gacio et al. 2009) various seed dormancy-breaking chemicals act at various levels countering the effect of ABA and enhance hydrolytic enzymes like α -amylase, thus promoting dormancy breakage (Ishibashi et al. 2017). In the present study compared to the control, all the treatments enhanced the α -amylase. The highest α -amylase was recorded in 0.4% KNO_3 and 20 mM H_2O_2 . KNO_3 has been reported previously to enhance the activity of α -amylase thus facilitating the mobilization of stored reserves for developing seedlings (Basra et al. 2006). Lara et al 2014 treated tomato seeds with KNO_3 , which enhanced the production of nitric oxide (a potential ROS), the enhancement in germination was attributed to ROS production. ROS accumulation has been accepted as an essential step in the initiation of germination and dormancy breaking in seeds (Bailly 2019). The enhancement of germination and seedling vigour in KNO_3 treated seed may be attributed to enhancement in the α -amylase and production of ROS. ROS can trigger mRNA and protein oxidation, which act upon the negative regulators of germination such as ABA. At the nuclear level, ROS activates the ABA catabolism genes and promotes GA synthesis, thus affecting the ABA-GA balance and resulting in dormancy release. ROS accumulation during imbibition also results in cell wall loosening and cell elongation thus facilitating radicle emergence (Kucera et al. 2005; Ishibashi et al. 2017; Anand et al. 2019; Bailly 2019). The enhancement of α -amylase due to KNO_3 treatment has been previously reported in several crops (Basra et al. 2006; Nawaz et al. 2011). Compared to the control GA_3 did enhance the α -amylase. Though GA_3 200 ppm had considerably higher α -amylase content, the dormancy alleviation was not effective as other treatments. This suggests that in buckwheat apart from α -amylase synthesis, other mechanisms may be involved in dormancy release. The process of dormancy breaking is a complex process involving various hormones, ROS, and crosstalk between themselves (Verma et al. 2016), which might be triggered by KNO_3 and H_2O_2 , though a detailed study is required to draw a full conclusion.

Conclusion

Buckwheat seeds possess a physiological nondeep dormancy, which can be broken by treating with 0.4% KNO₃ or 20 mM H₂O₂. These treatments significantly enhanced seed germination, seed vigour, and α-amylase activity. Consideration should be given to the effect of different germinating temperatures on seed dormancy in buckwheat. The finding from the present study will serve as a valuable reference for seed testing and those involved in the conservation of buckwheat germplasm.

Declarations

Acknowledgment

The authors are thankful to Mr. Gaurav Thakran for assisting in obtaining SEM images.

Data availability

All data are available from the corresponding author on reasonable request. The variety PRB-1 is multiplied and maintained by ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora Uttarakhand- 263601 India.

Author contributions

YVR, DV, GC, VHP, and AD contributed to the study conception and design. YVR and DV performed the data analysis. YVR wrote the first draft of the manuscript. LSK and MGP acquired funds and supervised the analyses. JDC and MRP supplied the seed material. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

Ethics declarations

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

References

1. Abdul-Baki AA, Anderson JD (1973) Vigor Determination in Soybean Seed by Multiple Criteria 1. Crop Sci 13:630–633. <https://doi.org/10.2135/cropsci1973.0011183X001300060013x>
2. Amjad M, Ziaf K, Iqbal Q, et al (2007) Effect of seed priming on seed vigour and salt tolerance in hot pepper. Pak J Agri Sci 44:408–414
3. Anand A, Kumari A, Thakur M, Koul A (2019) Hydrogen peroxide signaling integrates with phytohormones during the germination of magnetoprimered tomato seeds. Sci Rep 9:8814. <https://doi.org/10.1038/s41598-019-45102-5>
4. Anonymous (2023) WASP - Web Agri Stat Package 2.0. <https://ccari.icar.gov.in/wasp2.0/index.php>. Accessed 17 Apr 2023

5. Aravind J, Vimala Devi S, Radhamani J, et al (2023) Germinationmetrics: Seed germination indices and curve fitting. R package version 0.1.7.9000. <https://github.com/aravind-j/germinationmetrics><https://cran.r-project.org/package=germinationmetrics>
6. Baalbaki R, Chaharsoughi, SA, Le, V, Koonse, S 2018 Radicle length in agar is a reliable predictor of seed vigor. *Seed Technol J* 39:(1/2), 155–171. <http://www.jstor.org/stable/45135885>
7. Bailly C (2019) The signalling role of ROS in the regulation of seed germination and dormancy. *Biochem J* 476:3019–3032. <https://doi.org/10.1042/BCJ20190159>
8. Balaguera-López HE, Cárdenas-Hernández JF, Álvarez-Herrera JG (2009) Effect of gibberellic acid (Ga3) on seed germination and growth of tomato (*Solanum lycopersicum* L.). *Acta Horti* 141–148. <https://doi.org/10.17660/ActaHortic.2009.821.15>
9. Baskin CC, Baskin CJM (2014) *Seeds: ecology, biogeography, and evolution of dormancy and germination*, Second Edition. Elsevier
10. Basra SMA, Farooq M, Tabassum R, Ahmed N (2006) Evaluation of seed vigour enhancement techniques on physiological and biochemical basis in coarse rice (*Oryza sativa* L.). *Seed Sci Technol* 34:719–728. <https://doi.org/10.15258/sst.2006.34.3.18>
11. Batak I, Dević M, Gibal Z, et al (2002) The effects of potassium nitrate and NO-donors on phytochrome A- and phytochrome B-specific induced germination of *Arabidopsis thaliana* seeds. *Seed Sci Res* 12:253–259. <https://doi.org/10.1079/SSR2002118>
12. Born WH Vanden, Corns WG (1958) Studies on seed dormancy, plant development, and chemical control of tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.): I. seed dormancy. *Can J Plant Sci* 38:357–366. <https://doi.org/10.4141/cjps58-055>
13. Çırak C, Kevseroğlu K, Ayan AK (2007) Breaking of seed dormancy in a Turkish endemic hypericum species: *Hypericum aviculariifolium* subsp. *depilatum* var. *depilatum* by light and some pre-soaking treatments. *J Arid Environ* 68:159–164. <https://doi.org/10.1016/j.jaridenv.2006.03.027>
14. Czabator FJ (1962) Germination Value: An index combining speed and completeness of pine seed germination. *For Sci* 8:386–396. <https://doi.org/10.1093/forestscience/8.4.386>
15. Debeaujon I, Koornneef M (2000) Gibberellin requirement for arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiol* 122:415–424. <https://doi.org/10.1104/pp.122.2.415>
16. Dong T, Tong J, Xiao L, et al (2012) Nitrate, abscisic acid and gibberellin interactions on the thermoinhibition of lettuce seed germination. *Plant Growth Regul* 66:191–202. <https://doi.org/10.1007/s10725-011-9643-5>
17. El-Kassaby YA, Moss I, Kolotelo D, Stoehr M (2008) Seed germination: Mathematical representation and parameters extraction. *For Sci* 54:220–227. <https://doi.org/10.1093/forestscience/54.2.220>
18. Ellis RH, Hong TD, Roberts EH (1985) *Handbook of seed technology for genebanks*. Volume II. Compendium of specific germination information and test recommendations. Chapter 58. Polygonaceae. International Board for Plant Genetic Resources, Rome

19. Everson L (1949) Preliminary studies to establish laboratory methods for the germination of weed seed. *Proceedings of the Association of Official Seed Analysts* 39:84–89
20. Evetts LL, Burnside OC (1972) Germination and seedling development of common milkweed and other species. *Weed Sci* 20:371–378. <https://doi.org/10.1017/S004317450003589X>
21. Finch-Savage WE, Bassel GW (2016) Seed vigour and crop establishment: extending performance beyond adaptation. *J Exp Bot* 67:567–591. <https://doi.org/10.1093/jxb/erv490>
22. Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. *New Phytologist* 171:501–523. <https://doi.org/10.1111/j.1469-8137.2006.01787.x>
23. Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. *Annu Rev Plant Biol* 59:387–415. <https://doi.org/10.1146/annurev.arplant.59.032607.092740>
24. Foley ME (2001) Seed dormancy: an update on terminology, physiological genetics, and quantitative trait loci regulating germinability. *Weed Sci* 49:305–317. [https://doi.org/10.1614/0043-1745\(2001\)049\[0305:SDAUOT\]2.0.CO;2](https://doi.org/10.1614/0043-1745(2001)049[0305:SDAUOT]2.0.CO;2)
25. Ghodrati V, Rousta MJ (2012) Effect of salinity and priming with GA3 on the germination and growth of corn (*Zea mays* L.) under saline conditions. *Int j agric crop sci* 4: 882-885
26. Graeber K, Linkies A, Steinbrecher T, et al (2014) Delay of germination 1 mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. *Proc Natl Acad Sci U S A* 111. <https://doi.org/10.1073/pnas.1403851111>
27. Hernández JA, Díaz-Vivancos P, Acosta-Motos JR, Barba-Espín G (2021) Potassium nitrate treatment is associated with modulation of seed water uptake, antioxidative metabolism and phytohormone levels of pea seedlings. *Seeds* 1:5–15. <https://doi.org/10.3390/seeds1010002>
28. Ishibashi Y, Aoki N, Kasa S, et al (2017) The Interrelationship between abscisic acid and reactive oxygen species plays a key role in barley seed dormancy and germination. *Front Plant Sci* 8: <https://doi.org/10.3389/fpls.2017.00275>
29. ISTA (2021) International Rules for Seed Testing. Bassersdorf, Switzerland <https://doi.org/10.15258/istarules.2021.F>
30. ISTA (2003) Working Sheets on Tetrazolium Testing, Volume I - Agricultural, Vegetable and Horticultural Species, I. Bassersdorf, Switzerland
31. Jeevan Kumar SP, Rajendra Prasad S, Banerjee R, Thammineni C (2015) Seed birth to death: dual functions of reactive oxygen species in seed physiology. *Ann Bot* 116:663–668. <https://doi.org/10.1093/aob/mcv098>
32. Jiang P, Burczynski F, Campbell C, et al (2007) Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum*, and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Res Int* 40:356–364. <https://doi.org/10.1016/j.foodres.2006.10.009>
33. Kaneko M, Itoh H, Ueguchi-Tanaka M, et al (2002) The α -Amylase Induction in Endosperm during Rice Seed Germination Is Caused by Gibberellin Synthesized in Epithelium. *Plant Physiol* 128:1264–1270. <https://doi.org/10.1104/pp.010785>

34. Kindiger B (2019) Enhancing Germination of *Bromus Auleticus* Seeds Using a 15% Hydrogen Peroxide Treatment. *Seed Sci Technol* 47:103–106. <https://doi.org/10.15258/sst.2019.47.1.11>
35. Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. *Seed Sci Res* 15:281–307. <https://doi.org/10.1079/SSR2005218>
36. Kwon HJ Shin SL, Kim YR, Kim SY (2020) Effects of temperature, gibberellic acid, and KNO₃ treatments on seed germination of the wild plant *Maesa japonica*. *Seed Sci. Technol* 1: 65–72. <https://doi.org/10.15258/sst.2020.48.1.09>
37. Lacerda DR, Lemos Filho JP, Goulart MF, et al (2004) Seed-dormancy variation in natural populations of two tropical leguminous tree species: *Senna multijuga* (Caesalpinoideae) and *Plathymenia reticulata* (Mimosoideae). *Seed Sci Res* 14:127–135. <https://doi.org/10.1079/SSR2004162>
38. Lamichhane JR, Debaeke P, Steinberg C, et al (2018) Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. *Plant Soil* 432:1–28. <https://doi.org/10.1007/s11104-018-3780-9>
39. Landis TD, South DB 1997 National Proceedings: Forest and Conservation Nursery Associations, 1996. US Department of Agriculture, Forest Service, Pacific Northwest Research Station.
40. Lara TS, Lira JMS, Rodrigues AC, et al (2014) Potassium nitrate priming affects the activity of nitrate reductase and antioxidant enzymes in tomato germination. *J Agric Sci* 6: <https://doi.org/10.5539/jas.v6n2p72>
41. Lee MH, Song CH, Park CH, et al (2022) Effect of gibberellic acid treatment and alternating temperature on breaking physiological dormancy and germination in *Penthorum chinense* Pursh (Penthoraceae). *Seed Sci Technol* 50:207–219. <https://doi.org/10.15258/sst.2022.50.2.04>
42. Leubner-Metzger G, Fründt C, Meins F (1996) Effects of gibberellins, darkness and osmotica on endosperm rupture and class I β -1,3-glucanase induction in tobacco seed germination. *Planta* 199:282–288. <https://doi.org/10.1007/BF00196570>
43. Li HS, Sun Q, Zhao SJ, Zhang WH (2000) Principles and techniques of plant physiological biochemical experiment. Higher Education, Beijing
44. Liu A, Gao F, Kanno Y, et al (2013) Regulation of Wheat seed dormancy by after-ripening is mediated by specific transcriptional switches that induce changes in seed hormone metabolism and signaling. *PLoS One* 8:e56570. <https://doi.org/10.1371/journal.pone.0056570>
45. Liu Y, Ye N, Liu R, et al (2010) H₂O₂ mediates the regulation of ABA catabolism and GA biosynthesis in Arabidopsis seed dormancy and germination. *J Exp Bot* 61:2979–2990. <https://doi.org/10.1093/jxb/erq125>
46. Metzger JD (1991) The physiological basis of achene dormancy in *Polygonum convolvulus* (Polygonaceae). *Amer. J. Bot.* 79: 882–886. <https://doi.org/10.1002/j.1537-2197.1992.tb13669.x>
47. Miransari M, Smith DL (2014) Plant hormones and seed germination. *Environ Exp Bot* 99:110–121. <https://doi.org/10.1016/j.envexpbot.2013.11.005>
48. Müller K, Tintelnot S, Leubner-Metzger G (2006) Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and

- endosperm rupture of cress and *Arabidopsis thaliana*. *Plant Cell Physiol* 47:864–877.
<https://doi.org/10.1093/pcp/pcj059>
49. Nawaz A, Amjad M, Aslam Pervez M, Afzal I (2011) Effect of halopriming on germination and seedling vigor of tomato. *Afr J Agric Res* 6:3551–3559. <https://doi.org/10.5897/AJAR11.064>
 50. Nawel N, Rym K, Hela M, et al (2011) The effect of osmopriming on germination, seedling growth and phosphatase activities of lettuce under saline condition. *Afr J Biotechnol* 10:14366–14372.
<https://doi.org/10.5897/AJB11.1204>
 51. Ogawa M, Hanada A, Yamauchi Y, et al (2003) Gibberellin biosynthesis and response during *Arabidopsis* seed germination. *Plant Cell* 15:1591–1604. <https://doi.org/10.1105/tpc.011650>
 52. Pandey CD, Koul KA, Devi VS et al. 2019 Training manual International Training Programme on Management Of plant genetic resources for officers from Directorate of Seed Testing and Certification Ministry of Agriculture, Baghdad, Republic of Iraq (15 -20 July 2019).
<http://www.nbpgr.ernet.in/Downloadfile.aspx?EntryId=8767>
 53. Peng J, Harberd NP (2002) The role of GA-mediated signalling in the control of seed germination. *Curr Opin Plant Biol* 5:376–381. [https://doi.org/10.1016/S1369-5266\(02\)00279-0](https://doi.org/10.1016/S1369-5266(02)00279-0)
 54. Renzi JP, Duchoslav M, Brus J, et al (2020) Physical dormancy release in *Medicago truncatula* seeds is related to environmental variations. *Plants* 9:503. <https://doi.org/10.3390/plants9040503>
 55. Rodríguez-Gacio Mdel C, Matilla-Vázquez MA, Matilla AJ 2009 Seed dormancy and ABA signaling: the breakthrough goes on. *Plant Signal Behav.* 4 (11):1035 - 49.
<https://doi.org/10.4161/psb.4.11.9902>
 56. Salman M, Ishfaq AH, Syed Zia Ul H, et al (2012) Evaluation of seed priming on germination of *Gladiolus alatus*. *Afr J Biotechnol* 11. <https://doi.org/10.5897/ajb12.1158>
 57. Shao C, Wang G, Ding X, et al (2021) Physiological and biochemical characteristics of cold stratification to overcome morphophysiological dormancy in *Glehnia littoralis* seed. *Seed Sci Technol* 49:19–24. <https://doi.org/10.15258/sst.2021.49.1.03>
 58. Singh VP, Mall SL (1977) Seed germination studies in *Fagopyrum esculentum* Moench. I. Role of light and temperature. *Proc Indian National Sci Acad* 37–43
 59. Soltani E, Ghaderi-Far F, Baskin CC, Baskin JM (2015) Problems with using mean germination time to calculate rate of seed germination. *Aust J Bot* 63:631. <https://doi.org/10.1071/BT15133>
 60. Stein M, Serban C, McCord P (2021) Exogenous ethylene precursors and hydrogen peroxide aid in early seed dormancy release in sweet cherry. *J Am Soc Hortic Sci* 146:50–55.
<https://doi.org/10.21273/JASHS04952-20>
 61. Suzuki T, Hara T, Hara T, Katsu K (2020) Effect of storage temperature on occurrence of secondary dormancy in buckwheat seeds. *Seed Sci Technol* 48:257–267.
<https://doi.org/10.15258/sst.2020.48.2.13>
 62. Talská R, Machalová J, Smýkal P, Hron K (2020) A comparison of seed germination coefficients using functional regression. *Appl Plant Sci* 8: <https://doi.org/10.1002/aps3.11366>

63. Timple SE, Hay FR, Mercado MaFO, et al (2018) Response of intact seeds of wild rice (*Oryza*) species to dry heat treatment and dormancy-breaking chemicals. *Seed Sci Technol* 46:157–173. <https://doi.org/10.15258/sst.2018.46.1.16>
64. Toole EH, Toole VK, Borthwick HA, Hendricks SB (1955) Photocontrol of *Lepidium* seed germination. *Plant Physiol* 30:15–21. <https://doi.org/10.1104/pp.30.1.15>
65. Toorop PE (2015) Nitrate controls testa rupture and water content during release of physiological dormancy in seeds of *Sisymbrium officinale* (L.) Scop. *Seed Sci Res* 25:138–146. <https://doi.org/10.1017/S0960258514000397>
66. Verma V, Ravindran P, Kumar PP (2016) Plant hormone-mediated regulation of stress responses. *BMC Plant Biol* 16:86. <https://doi.org/10.1186/s12870-016-0771-y>
67. Wahid A, Sehar S, Perveen M, et al (2008) Seed pretreatment with hydrogen peroxide improves heat tolerance in maize at germination and seedling growth stages. *Seed Sci Technol* 36:633–645. <https://doi.org/10.15258/sst.2008.36.3.13>
68. William H, Born V, Corns WG (1958) Studies on seed dormancy, plant development, and chemical control of Tartary buckwheat (*Fagopyrum tatartcum* (L.) Gaertn.) I. Seed Dormancy. *Can J Plant Sci* 38:357–366
69. Willis CG, Baskin CC, Baskin JM, et al (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytologist* 203:300–309. <https://doi.org/10.1111/nph.12782>
70. Wojtyla Ł, Lechowska K, Kubala S, Garnczarska M (2016) Different modes of hydrogen peroxide action during seed germination. *Front Plant Sci* 7. <https://doi.org/10.3389/fpls.2016.00066>
71. Yamaguchi S, Kamiya Y, Sun T (2002) Distinct cell-specific expression patterns of early and late gibberellin biosynthetic genes during *Arabidopsis* seed germination. *The Plant Journal* 28:443–453. <https://doi.org/10.1046/j.1365-313X.2001.01168.x>
72. Zeinalabedini M, Majourhat K, Khayam-Nekoui M, et al (2009) Breaking seed dormancy in long-term stored seeds from Iranian wild almond species. *Seed Sci Technol* 37:267–275. <https://doi.org/10.15258/sst.2009.37.2.01>

Supplement

Supplement-I is not available with this version

Figures

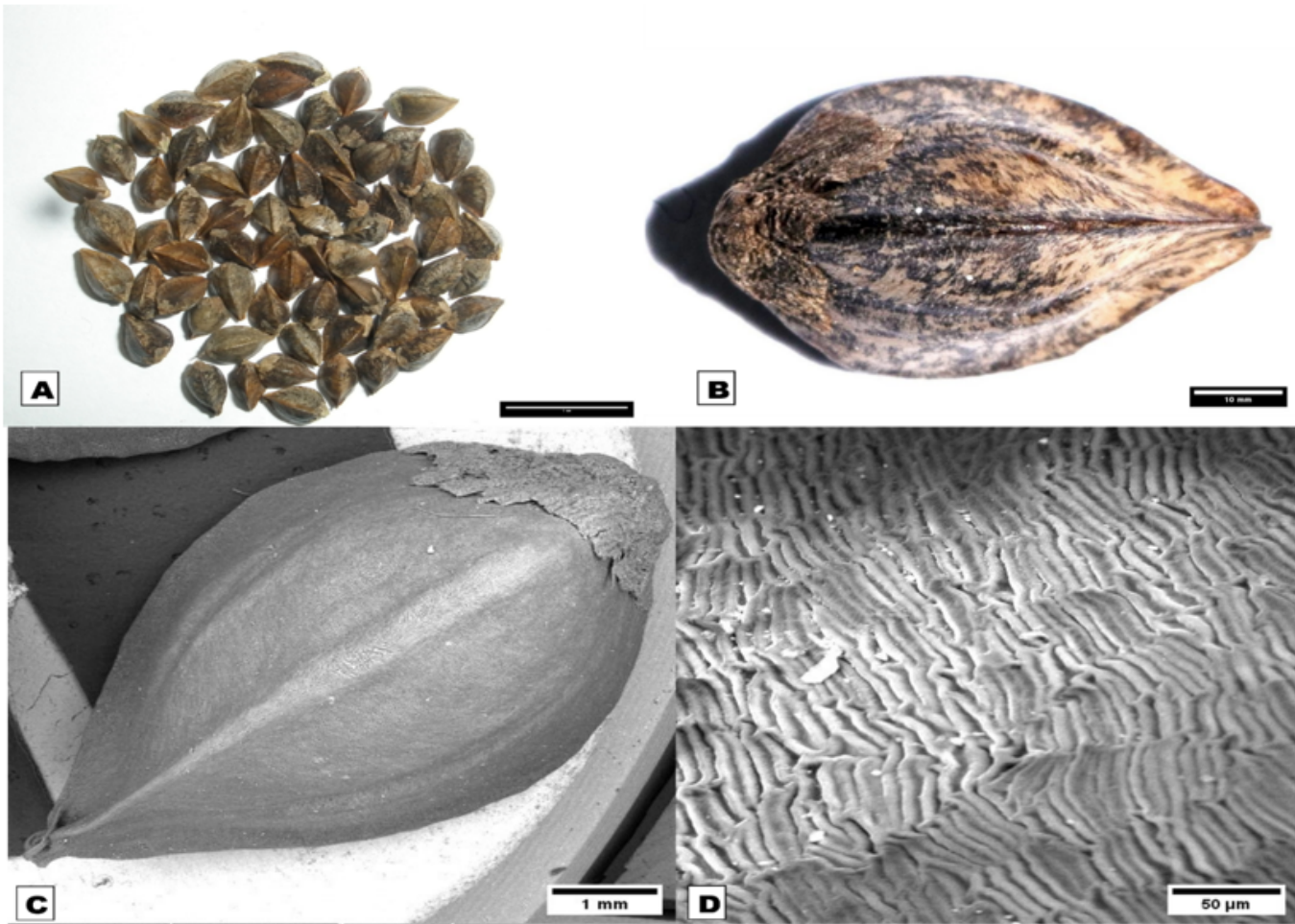


Figure 1

A: Buckwheat seed, scale bar = 1 cm; **B:** Close-up, scale bar = 10 mm. **C:** SEM of buckwheat seed (24x), scale bar = 1 mm **D:** SEM revealing microstructure of buckwheat seed coat (500x), scale bar = 50 μm

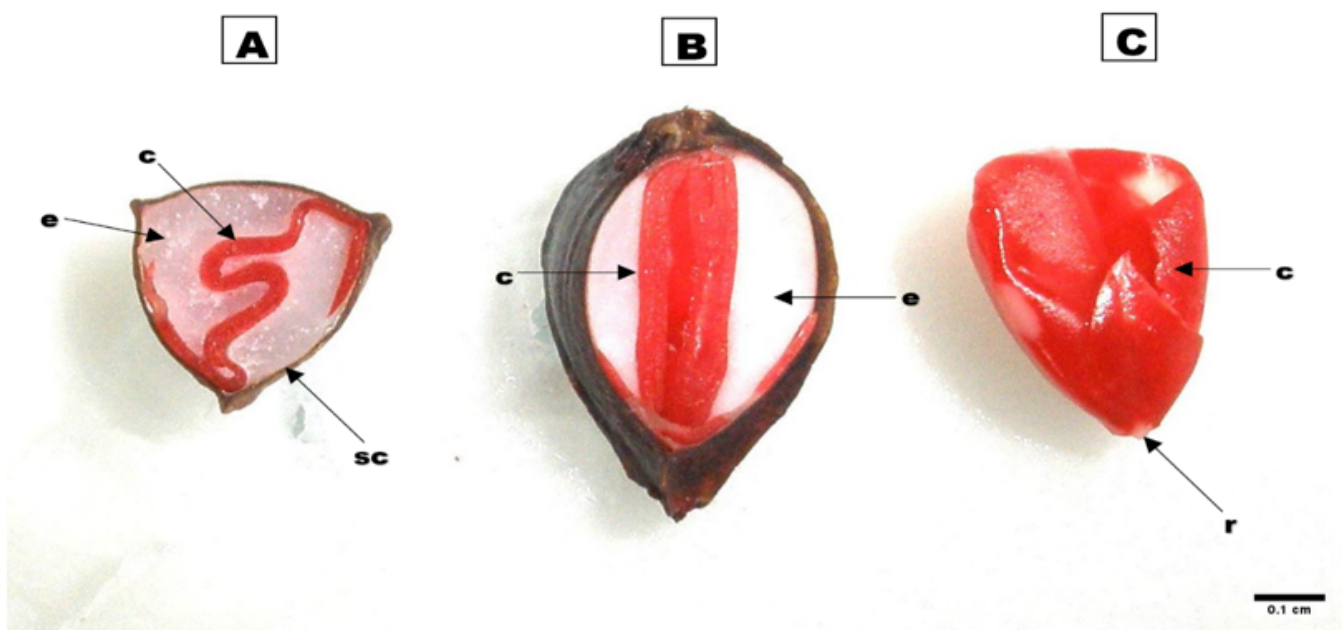


Figure 2

A: Horizontal section of buckwheat seed; B: Longitudinal section of buckwheat seed; C: Excised embryo. c - embryo, e - endosperm, sc - seed coat, r - radicle. Scale bar = 0.1 cm

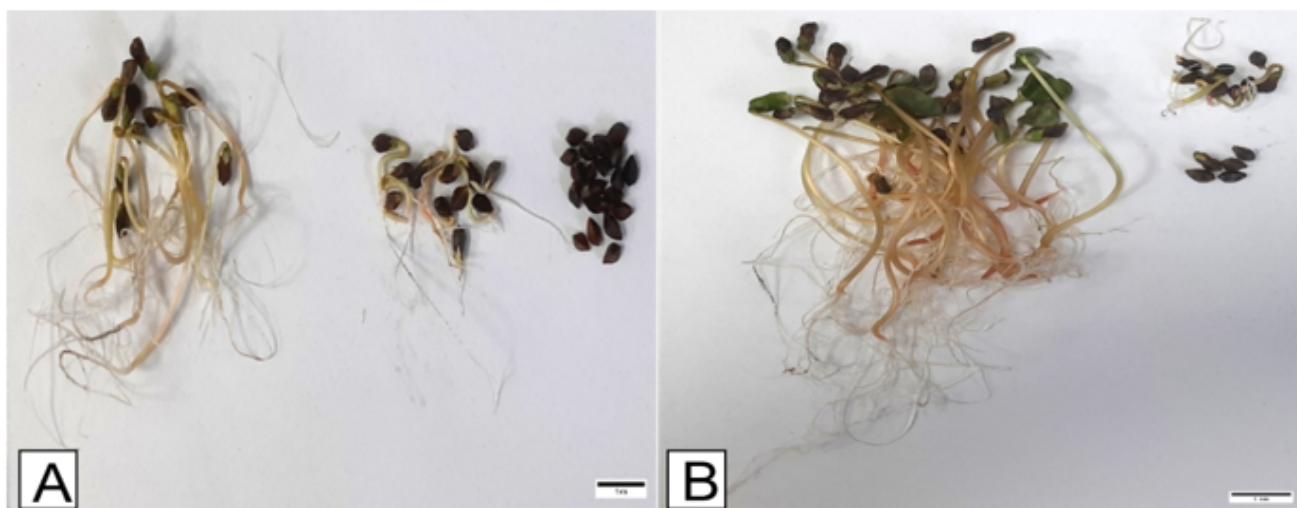


Figure 3

Normal seedlings, abnormal seedlings, and fresh ungerminated seeds in buckwheat A: Control and B: 0.4% KNO₃ treated seeds. Scale bar = 1 cm



Figure 4

2, 3, 5 triphenyl tetrazolium chloride (TTZ) stained seeds of buckwheat variety PRB-1 in control, depicting (A) Viable and (B) Non-viable seeds as per (ISTA, 2003). Scale bar = 1 cm

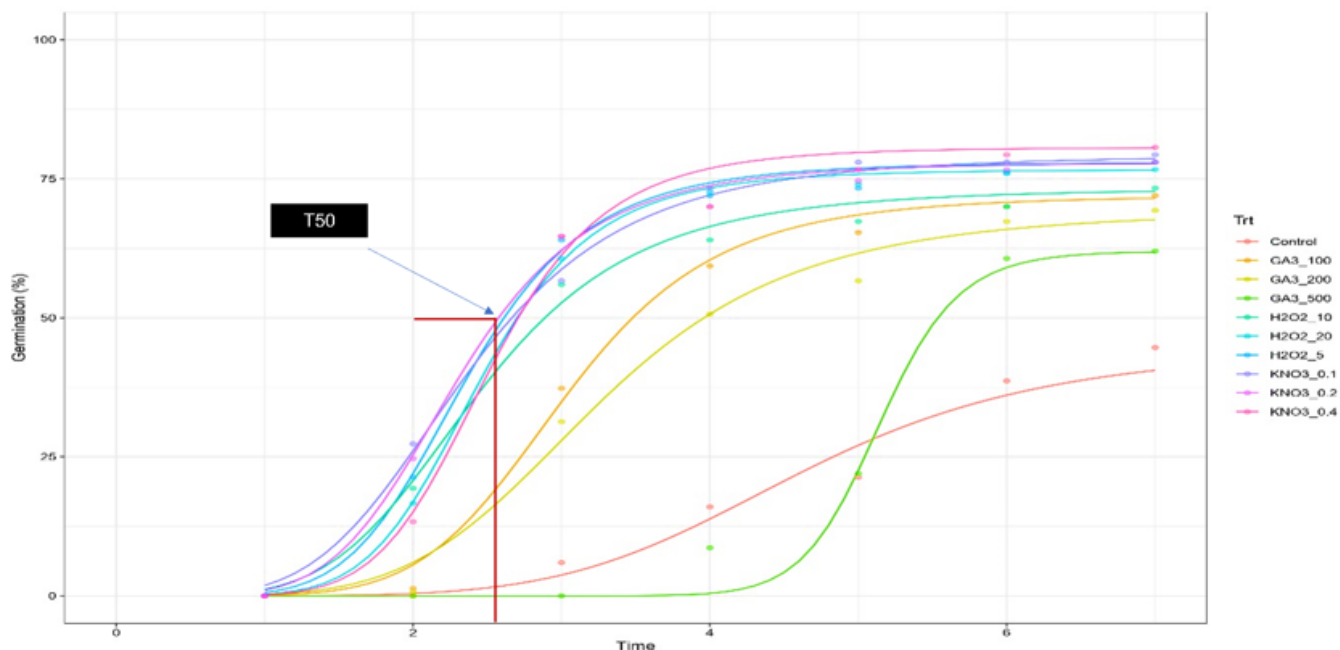


Figure 5

Graphical demonstration of a predicted germination curve of buckwheat. The average germination values are plotted over the curve representing maximum germination, time to 50 percent germination, and area under the germination curve (AUGC). AUGC is the area below the curve between time ($t = 0$ days and $t = 7$ days). The t_{50} for treatment KNO_3 0.2 % having the lowest t_{50} (2.37 days) is highlighted

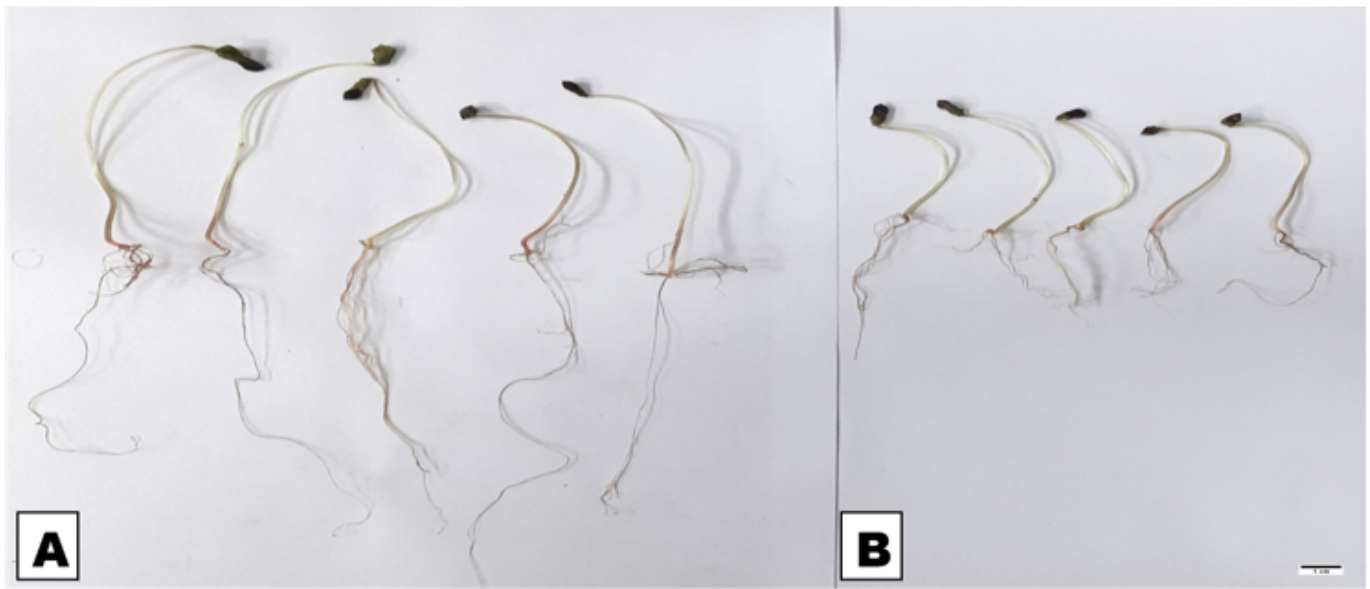


Figure 6

Seedling length A: GA₃ 200 ppm treatment and B: Control. Scale bar = 1 cm

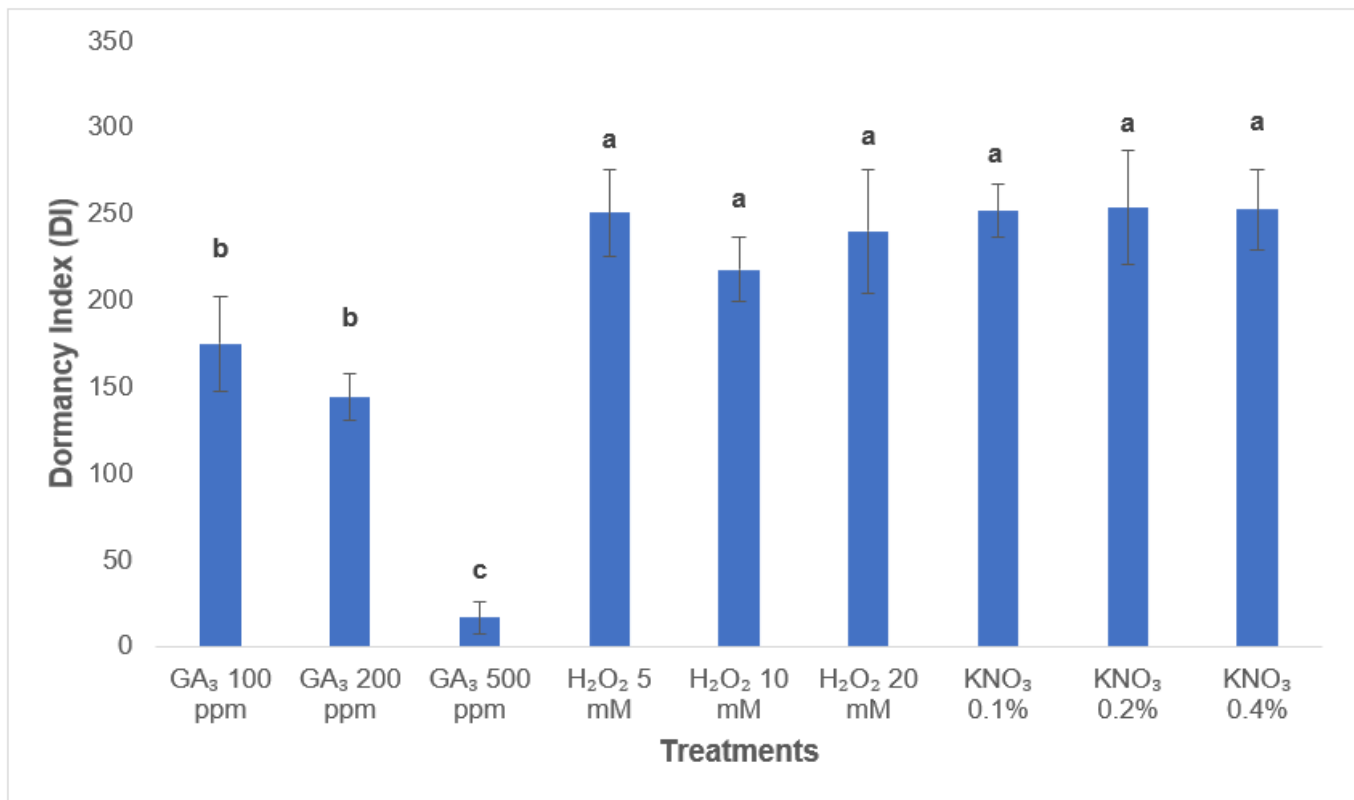


Figure 7

Graphical demonstration of dormancy Index (DI) representing the difference between the areas under the germination curves (AUGC) before and after seed treatments. Each value is expressed as the means $\pm$ standard deviation ($n = 3$). Significant differences ($p \leq 0.05$) in the AUGC between different treatments are indicated by different letters at the same time.

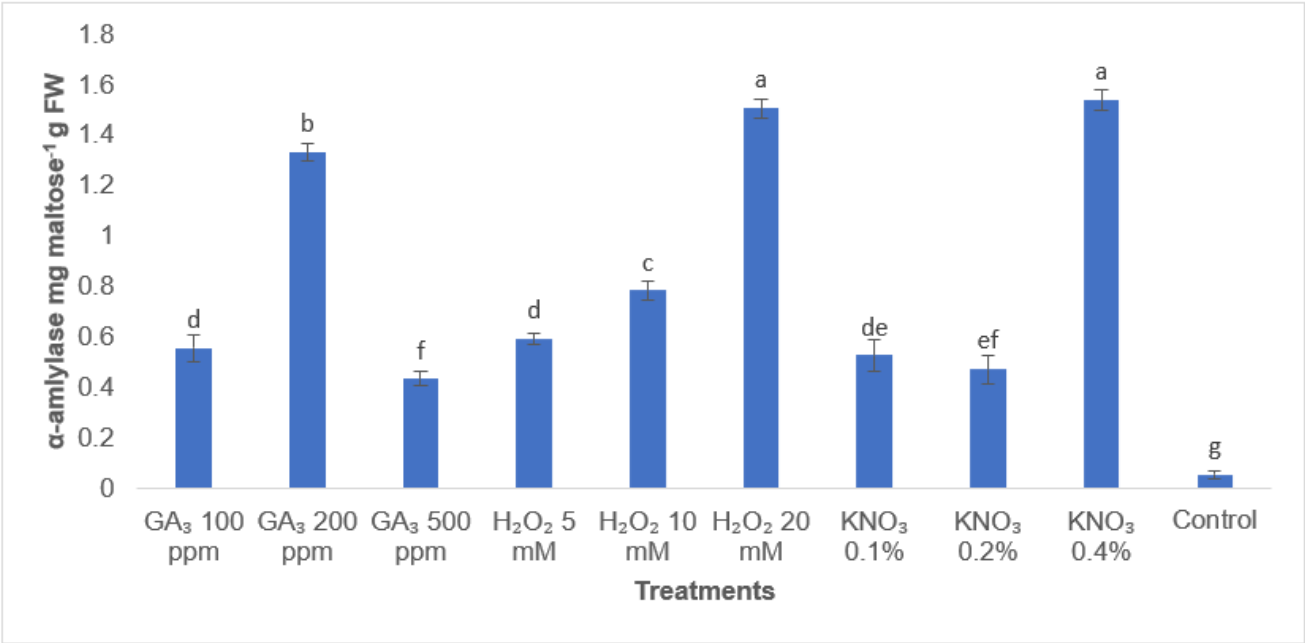


Figure 8

α-amylase activity in response to dormancy breaking treatment in buckwheat. Significant differences ($p \leq 0.05$) in α-amylase activity between different treatments are indicated by different letters at the same time. Each value was expressed as the means $\pm$ standard deviation ($n = 3$). FW- fresh weight