South African Journal of Botany 165 (2024) 91–100

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Seed dormancy and germination responses of cannabis landraces to various pre-treatments

Sabeliwe Langa[a,*], Lembe Samukelo Magwaza[b,c], Asanda Mditshwa[a], Samson Zeray Tesfay[a]

[a] Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg 3201, South Africa
[b] Department of Food Systems and Development, Faculty of Natural and Agricultural Sciences, PO Box 339, Bloemfontein 9300, Republic of South Africa
[c] Plant Science Laboratory, Cranfield University, Cranfield MK43 0AL, United Kingdom

ARTICLE INFO

Article History:
Received 1 October 2023
Revised 11 December 2023
Accepted 13 December 2023
Available online 22 December 2023

Edited by: Dr I. Demir

Keywords:
Cannabis
Dormancy breaking
Germination enhancement
Landrace

ABSTRACT

The current study evaluated the effectiveness of different pre-treatments for dormancy breaking and seed germination of five cannabis landraces. These landraces were collected from local growers in three regions of KwaZulu-Natal Province in South Africa, namely, Bergville (B), Hammersdale (H), and Ladysmith (L). Each genotype was assigned an alphabet and number based on where the seed material was collected. The five landraces were as follows, 'B1', 'K1', 'H1', 'L1', and 'L2'. The study was conducted using various pre-treatments, including potassium nitrate (KNO_3), hydrochloric acid (HCL), hot water (HW) at 70 °C, nitric acid (HNO_3), dry prechilling at 10 °C, gibberellic acid (GA_3), sulfuric acid (H_2SO_4), and water, which served as the control. Before the germination test, the seed viability of cannabis landraces was assessed using the tetrazolium chloride (TCC) test. Afterward, the seeds were germinated in Petri dishes with cotton pads moistened with distilled water for five (5) days. The study was later validated under tunnel conditions. The parameters such as germination percentage, germination rate, seedling length, and seed vigour index of the germinated seeds were then measured. The landraces, dormancy breaking treatments, and their interactions showed significant differences ($p < 0.001$) in germination percentage, germination rate index, seedling length, and seed vigour index under both laboratory and tunnel conditions. In summary, GA_3, KNO_3 and prechilling were the most effective pre-treatments to improve the germination and seedling growth of cannabis genotypes and breaking seed dormancy. Therefore, it was concluded that cannabis genotypes had physiological dormancy.

1. Introduction

"*Cannabis sativa* L., commonly known as hemp or marijuana (or dagga), is an annual dioecious and dicotyledonous plant belonging to the *Cannabaceae* family" (Flores-Sanchez et al., 2009; Chaohua et al., 2016; Lata et al., 2016). Cannabis is useful in "several agro-industrial fields, namely agriculture, textile, bio-composite, papermaking, automotive, construction, biofuel, functional food, oil, cosmetics, and personal care, as well as in the pharmaceutical industry" (Salentijn et al., 2015; Chaohua et al., 2016; Grulichova et al., 2017; Zuk-Golaszewska and Golaszewska, 2018). The multi-industrial application of cannabis owes to its oily seeds, long and durable fibers, and high content of cannabinoids, terpenes, and phenolic compounds (Grulichova et al., 2017; Zuk-Golaszewska and Golaszewska, 2018; Smith et al., 2019). With the recent discovery of medicinal cannabis, there have been re-regulations on the cultivation of cannabis since it was banned worldwide due to the intoxicating effects of its compound, delta-8-tetrahydrocannabinol (Thomas, 2012).

Traditionally, cannabis usually grows in disturbed areas such as the "borders of fields, on rubbish heaps near or habitations in farmyards, vacant lots, and in disturbed areas of pastures" (Small, 2015). If cultivated, it requires a low level of irrigation and fertilization, particularly after its establishment (Forapani et al., 2001; Ascrizzi et al., 2019). However, seed germination involves complex physiological processes that respond to environmental signals such as water potential, light, and other factors (Bano et al., 2021). Many seeds still fail to germinate after being processed and placed in favorable growing conditions (Önol and Yildirim, 2021). Seed germination and performance also vary from plant to plant, resulting in different kinds of dormancy (Önol and Yildirim, 2021).

The cause of dormancy varies among plant species, but it is categorized into physical, morphological, physiological, morpho-physiological or a combination of physical and physiological dormancy, and thus, the seed must undergo the necessary changes for the germination to start (Golmohammadzadeh et al., 2015; Chahtane et al., 2017; Soltani et al., 2018). "Under natural conditions, necessary changes

* Corresponding author.
E-mail address: sabeliwelanga@gmail.com (S. Langa).

https://doi.org/10.1016/j.sajb.2023.12.021

occur gradually as a result of varying combinations of aeration, moisture, temperature, and light". Dormancy could also be released using different methods, including phytohormones or chemical treatments (Önol and Yildirim, 2021). The standard procedures for breaking dormancy include prechilling, scarification, and treatments with gibberellic acid (GA_3) or nitric acid (KNO_3), as well as soaking seeds in hot water, or heat shock (Majd et al., 2013; Golmohammadzadeh et al., 2015; Labbafi et al., 2018). Scarification can be done mechanically or chemically (Utami et al., 2021). Mechanical scarification includes seeds shaken with an abrasive material such as sand or scratched with a knife (Utami et al., 2021). Under chemical scarification, seeds are soaked in chemical materials such as H_2SO_4, KNO_3, and HCl (Utami et al., 2021).

Pre-soaking the seeds in GA_3 or potassium nitrate (KNO_3) is known to alleviates both the physical and physiological dormancy (Tapfumaneyi et al., 2023). Prechilling simulates cold winter conditions for seeds exhibiting physiological dormancy, which leads to increased synthesis of GA_3 in the embryo and thereby aiding seed germination (Golmohammadzadeh et al., 2015). On the other hand, hydrochloric acid (HCL), sulphuric acid (H_2SO_4), nitric acid (HNO_3) and hot water (HW) are known to break physical dormancy by thinning the seed coat, which may harm the seed and damage the embryo (Gunes et al., 2013; Jaganathan et al., 2019; Jin et al., 2023). However, the results often vary as "induction of germination in dormant seeds may depend on the plant species, environmental conditions, growth form, seed origin and type of dormancy" (Golmohammadzadeh et al., 2015; Kildisheva et al., 2020; Islam et al., 2021; Flores-Sánchez et al., 2022).

Like any other crop, cannabis also has certain residual dormancy even though it is known to grow easily in any environment (Jovičić et al., 2019; Elias et al., 2020). In fact, it is suggested that wild cannabis can stay dormant but viable in soils for up to 10 years (Jovičić et al., 2019). Physiological dormancy is prevalent in cannabis because of the indeterminate growth habit of the female inflorescence, resulting in varied maturation times for seeds on the inflorescence (Önol and Yildirim, 2021). Elias et al. (2020) studied dormancy breaking through wet prechilling at 10 °C in 'Merlot' and 'Berry Blossom' hemp varieties. They were able to break the seed dormancy within 5 days and achieved higher germination compared to the control treatment. In another study, the germination of 'Ferimon', 'Han NW', and 'Morpeth' hemp varieties exposed to wet prechilling at 4 °C for 5 days, gibberellic acid (GA_3) at 500 and 1000 mg·L^{-1} and chlorine dioxide (ClO_2) at 500 and 1000 mg·L^{-1} was reduced compared to control (Islam et al., 2021). However, wet prechilling at 4 °C enhanced shoot and root growth of 'Ferimon', 'Han NW', and 'Morpeth' hemp varieties compared to the control (Islam et al., 2021).

However, studies on seed dormancy and dormancy breaking techniques are still lacking in South African cannabis genotypes. It is also evident from the previous studies that there is limited information on the existence of dormancy breaking techniques that promote both seed germination and seedling growth in cannabis. Furthermore, hemp seeds are characterized by hard outer shell (pericarp, also known as hull) (Schultz et al., 2020). It is known that a hard seed coat (i.e., shell) may act as a physical barrier to the emergence of radicles and leaves, leading to physical dormancy (Elias et al., 2020; Kildisheva et al., 2020). Understanding that the seed coat exerts such germination-restrictive action, therefore, there is a need for determining the influence of cannabis seed hardness on seed germination (Chaves et al., 2017). Therefore, there is a need to assess different methods for breaking dormancy and determine how different cannabis genotypes respond to these techniques in order to enhance germination and seedling development and reduce the duration of seed dormancy. Thus, the aim of the study is to evaluate the effect of various pre-treatments in breaking dormancy of cannabis seeds and determine the most effective pre-treatment for germination and seedling growth.

2. Materials and methods

2.1. Plant material

Seeds of five (5) cannabis landraces were used in the current study. The landraces were collected from cannabis growers in different regions of KwaZulu-Natal, namely, Ladysmith (Ugwayi wesiZulu and Iswazi), Bergville (Bergville Natal and Ugwayi wesiZulu), and Hammersdale in Durban (Durban poison) These seeds were kept at room temperature until use at the Horticultural laboratory in Agricultural Science Campus (with latitude: −29.636311 and longitude: 30.409060) at the University of KwaZulu-Natal Pietermaritzburg, South Africa. The collected landraces were given pseudonyms, as presented in Table 1. The cannabis seeds were sorted according to colour. Dark-coloured seeds were associated with maturity whereas greenish-coloured seeds were associated with immaturity (Miraji et al., 2021). Therefore, dark-coloured seeds were used for the experiment. Seeds showing defects were removed from the seed lot (Muszyński and Gladyszewska, 2008).

2.2. Experimental design

The experiment was set under laboratory conditions and was later validated under tunnel conditions. Each experiment was laid out in a randomized complete block design with a 5 × 8 factorial treatment design and replicated three times. Each experiment resulted in a total of 120 experimental units (90 mm petri dishes for the laboratory and polystyrene seedling trays for the tunnel experiment). The treatments were as follows: seed dormancy breaking consisted of eight (8) levels (control, sulphuric acid, potassium nitrate, hydrochloric acid, gibberellic acid, nitric acid, hot water, and prechilling); and landraces consisted of five (5) levels.

The techniques for seed dormancy breaking were adopted from Tiwari et al. (2018) with minor modifications. The selected seed dormancy-breaking treatments were applied as described below.

Hydrochloric acid: Cannabis seeds were immersed in HCL 32 % AR (Minema Chemicals (Pty) Ltd, 34 Ridge Rd, Laser Park; Johannesburg, South Africa) for 1 h under a fume hood. After soaking in HCL, the seeds were rinsed three times with distilled water. This procedure was followed by soaking seeds in distilled water for 24 h at room

Table 1
The pseudonyms of the cannabis landraces collected from different regions of KwaZulu-Natal.

	Geographic information						
Location	Latitude:	Longitude:	Elevation	Average rainfall	Average temperature	Landrace name	Pseudonym:
Ladysmith	−28.57533	29.85948	1009.53 m	108.69 mm	23.31 °C	Ugwayi wesiZulu	'L1'
						Iswazi	'L2'
Bergville	−28.667679	29.034111	1137.86 m	109.36 mm	23.45 °C	Natal	'B1'
	−28.677544	29.125553				Ugwayi wesiZulu	'K1'
Hammersdale	−29.870903	30.633519	597 m	110.34 mm	23.66 °C	Durban Poison	'H1'

temperature, which were air-dried thereafter. The study followed this procedure for all the applied treatments.

Sulphuric acid: Cannabis seeds were immersed in H_2SO_4 98 % AR (Minema Chemicals (Pty) Ltd, 34 Ridge Rd, Laser Park; Johannesburg, South Africa) for 1 h under a fume hood.

Hot water treatment: Seeds in the dry state were placed in test tubes sealed with aluminum foil and placed in a water bath set at 70 °C for 1 h. Thereafter, seeds were soaked in distilled water for 24 h at room temperature and then air dried.

Nitric acid: Seeds were immersed in HNO_3 60 % AR (Minema Chemicals (Pty) Ltd, 34 Ridge Rd, Laser Park; Johannesburg, South Africa) for 1 h under a fume hood.

Gibberellic acid: Seeds were immersed in a gibberellic acid (GA_3) (Merck KGaA, 64271 Darmstadt, Germany) solution for 24 h at room temperature. The gibberellic acid solution was prepared by adding 10 mg of GA_3 into 1 litre of distilled water.

Potassium nitrate: Seeds were immersed in a 0.2 % potassium nitrate (KNO_3) (Merck KGaA, 64271 Darmstadt, Germany) solution for 1 h under a fume hood.

Prechilling: Prechilling in the current study is referred to as dry prechilling. The current study adopted the prechilling technique by Elias et al. (2020) with some modifications. The seeds in the dry state, instead of wet, were stored in a sealed zipper bag and kept in a cold room set at 10 ± 0.5 °C for 5 days. Thereafter, seeds were soaked in distilled water for 24 h at room temperature and then air dried.

Water: The untreated seeds, which served as control, were soaked in distilled water for 24 h before germination, and then air-dried.

2.3. Seed viability test

Seeds were subjected to the viability test using 2,3,5-triphenyltetrazolium chloride (TCC) (Sigma Aldrich, Merck KGaA, 64271 Darmstadt, Germany) and the procedure was adopted from Mandizvo and Odindo (2019). TCC is a rapid and effective method for evaluating viability or physiological quality in seeds (Paiva et al., 2017). It is a water-soluble compound which promotes changes in colour when in contact with living seed tissues due to the activity of dehydrogenase enzymes (Virgens et al., 2019). Seed samples were preconditioned by soaking in distilled water for 18 h, at 25 °C in an incubator, and then excised with a scalpel blade through the embryo. The excised seeds were immersed in a 1 % tetrazolium salt solution (TCC) for 12 h at 25 °C at room temperature. The experiment was replicated three times, and each replicate consisted of 30 seeds. Seed viability evaluation was done by placing the seeds in categories as viable and non-viable according to the coloration of the embryonic axis. Viable seeds were characterized by embryo showing uniform shiny pink color, and non-viable seeds were characterized by a totally white color (Paiva et al., 2017). Only the percentage of viable seeds was recorded.

2.4. Germination test

Thirty (30) seeds of each cannabis genotype were placed on cotton pads moistened with distilled water in 90 mm Petri dishes or in a seedling tray containing lawn dressing compost obtained from Duzi Turf, New England Rd, Pietermaritzburg, South Africa. Lawn dressing compost is characterized as a finely sifted black organic weed-free medium. It is manufactured of a mixture of organic waste and sewage sludge, with a composition of 85 % humus and 15 % 2–8 mm larger particles. Each seed was planted in a seedling tray hole and the germinating seeds were irrigated regularly to ensure adequate moisture. The experiment was replicated three times for consistency. The seeded petri dishes were then transferred into a germination chamber (Snijders Scientific ECD01E Climaste Chamber, Tilburg, Holland) maintained at a temperature of 30/25 °C and light cycle of 16/8 h. The germination progress was monitored daily, and the number of germinated seeds was recorded for each replicate of 30 seeds for 5 days under laboratory conditions and 14 days under tunnel conditions. A seed was considered to have germinated when the emerging radical elongated to a length of 2 mm or more. The germination percentage (GP) was calculated according to Khalaki et al. (2019) using Eq. (1) and was expressed in percentages (%).

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad (1)$$

The germination rate index (GRI) was calculated according to Awasthi et al. (2016) using Eq. (2) and was expressed as a percentage per day (%/day).

$$GRI = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \ldots + \frac{\text{Number of germinated seeds}}{\text{Day of final count}} \quad (2)$$

2.5. Seedling growth test

After the germination test, the seedling length of germinated seeds was measured on the final day of germination using a ruler and expressed in millimetres. The seedling length was calculated as an average for all the germinated seeds for each landrace and treatment. The seedling vigour index (SVI) was calculated from the final germination percentage and seedling length according to Awasthi et al. (2016) using Eq. (3).

$$SVI = \frac{\text{Final germinated percentage} \times \text{Seedling length}}{100} \quad (3)$$

2.6. Statistical analysis

The data was analyzed using GenStat®, 20.1 Edition (VSN International, Hamel Hampstead, UK, 2020) at the 5 % significance level. Tukey's test with GenStat® was used to separate the means of significantly different variables.

3. Results

3.1. Seed viability

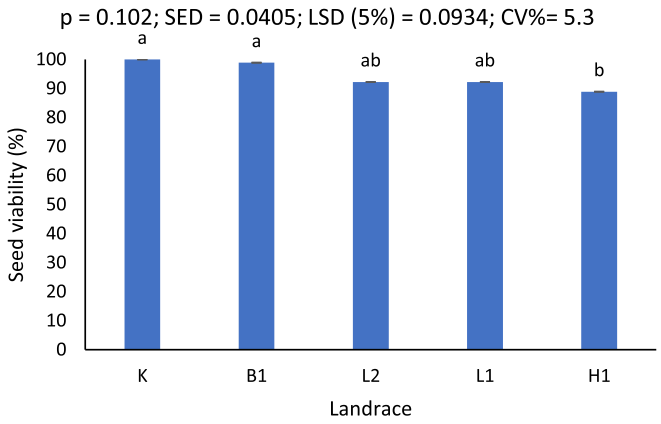
The results showed no significant differences ($p = 0.102$) amongst landraces for seed viability as assessed using tetrazolium chloride (TCC). The results showed that all seeds of landrace 'K1' were stained red (completely or partially stained), indicating 100 % seed viability, as shown in Fig. 1. Landrace 'B1' recorded a seed viability mean of 98.89 %, while 'L2' and 'L1' recorded a seed viability mean of 92.22 %. Landrace 'H1' recorded the lowest seed viability mean of 88.89 %.

3.2. Germination and seedling growth under laboratory conditions

The landraces, pre-treatments, and their interactions showed significant differences ($p < 0.001$) in germination percentage, germination rate index, seedling length and seed vigour index under laboratory conditions, as shown in Table 2.

3.2.1. Seed germination

The results revealed that there were no significant differences between control and treatments such GA_3, KNO_3, H_2SO_4 and prechilling in all the cannabis genotypes. In landrace 'B1', the germination recorded under H_2SO_4, KNO_3, and GA_3 was higher compared to the control, as shown in Fig. 2. H_2SO_4, KNO_3, and GA_3 recorded germination percentages of 92.22, 91.11, and 88.89 %, respectively, and control recorded 82.22 %. Similarly, H_2SO_4, KNO_3, and GA_3 recorded higher germination percentages in landrace 'L2' and 'L1' compared to control. In landrace 'L2', H_2SO_4, GA_3, and KNO_3 recorded germination



*Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

Fig. 1. Seed viability of cannabis landraces before dormancy-breaking treatments ($p = 0.102$).

Table 2
Analysis of variance (ANOVA) table for seed germination percentage (GP), germination rate index (GRI), seedling length (SL), and seed vigour index (SVI) of cannabis landraces subjected to various dormancy breaking pre-treatments under laboratory conditions.

		Mean square			
Source	Df	GP	GRI	SL	SVI
Landrace	4	0.1864**	1.2587**	220.06**	0.0269**
Treatment	7	1.7199**	4.6427**	5479.68**	0.3432**
Landrace*Treatment	28	0.0341**	0.0178**	39.78**	0.0043**

ns - not significant ($p > 0.05$); *significant ($p < 0.05$); **highly significant ($p < 0.001$).

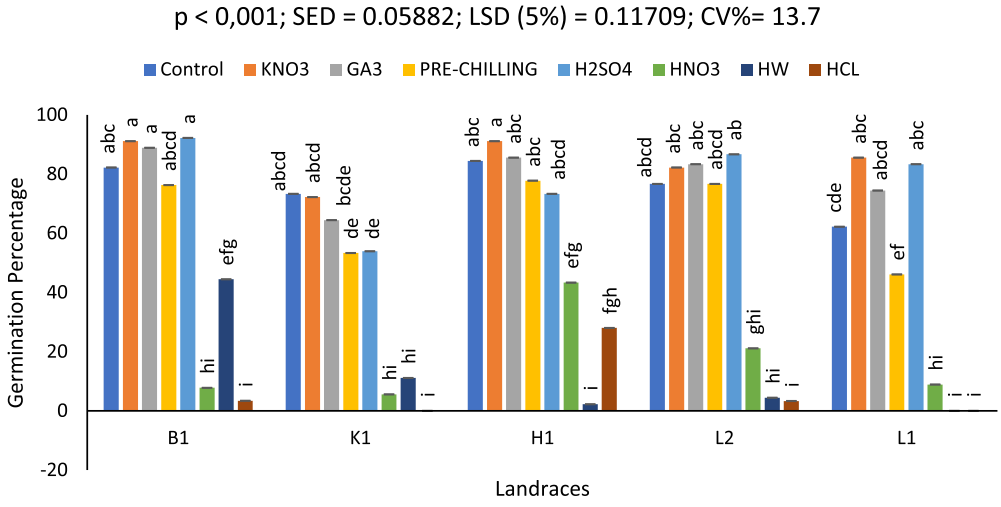
of 86.67, 83.33, and 82.22 %, respectively. In landrace 'L1', KNO_3, H_2SO_4, and GA_3 recorded a germination of 85.56, 83.33, and 74.44 %, respectively. In landrace 'K1', none of the applied pre-treatments recorded germination higher than 73.33 % recorded under control. Instead, 'K1' germination was completely inhibited under HCL. In landrace 'H1', KNO_3 and GA_3 recorded higher germination of 91.11 and 85.56 % compared to control. HCL and HW had completely inhibited the germination of landrace 'L1'.

3.2.2. Germination rate index

The results revealed that H_2SO_4 recorded the highest GRI in cannabis landraces, except 'H1' as control recorded the highest, as shown in Fig. 3.

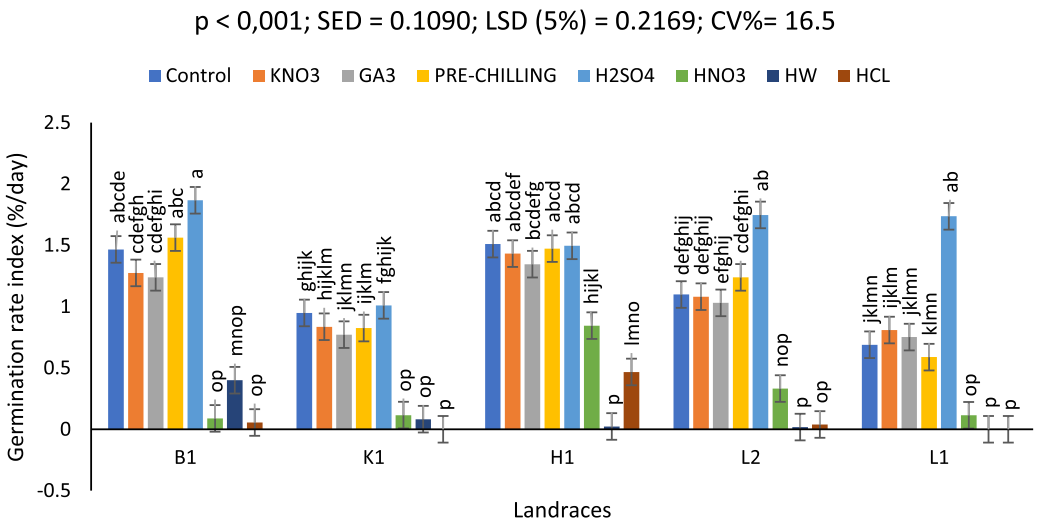
3.2.3. Seedling length

The results revealed that control recorded higher seedling length means compared with the applied pre-treatments. Control recorded higher seedling length means of 51.74, 48.18, 40.50, and 33.88 mm for landrace 'B1', 'K1', 'L2', and 'L1', respectively. The lowest seedling length means of 1.56, 1.00, and 1.50 mm for landrace 'B1', 'K1', and 'L1' were recorded under HNO_3 treatment, respectively. For landrace 'L2', the lowest seedling length mean of 1.00 mm was recorded under HCL treatment, as shown in Fig. 4. Compared to the control, prechilling and KNO_3 treatment recorded higher seedling length means of 42.46 and 40.42 mm for landrace 'H1', respectively. The lowest



*Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

Fig. 2. Effect of eight different seed dormancy-breaking treatments on germination percentage among five (5) cannabis landraces under laboratory conditions ($p < 0.001$).



* Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

Fig. 3. Effect of eight different seed dormancy-breaking treatments on germination rate index among five (5) cannabis landraces under laboratory conditions ($p < 0.001$).

seedling length mean of 1.33 mm for landrace 'H1' was recorded under HW treatment.

3.2.4. Seed vigour index

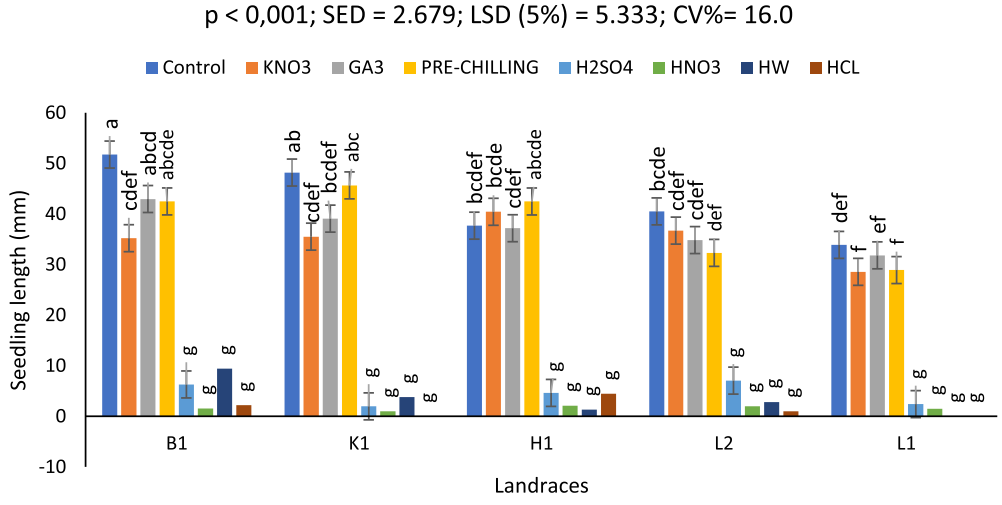
The results revealed that control recorded higher SVI compared with the applied dormancy breaking treatments. Control recorded higher SVI of 42.53, 35.21, and 31.20 for landrace 'B1', 'K1', and 'L2', respectively. The lowest SVI means of 0.12 and 0.06 were recorded under HNO_3 treatment in landrace 'B1' and 'K1', respectively. The lowest SVI mean for 'L2' of 0.10 was recorded under HCL treatment, as shown in Fig. 5. Compared to the control, KNO_3 and prechilling treatment recorded higher SVI means of 36.89 and 32.73 for landrace 'H1', respectively. For landrace 'L1', KNO_3 and GA_3 treatment recorded higher SVI means compared to the control of 24.47 and 23.64, respectively. The lowest SVI means of 0.04 and 0.00 were recorded for landrace 'H1' and 'L1', respectively, under HW treatment.

3.3. Germination and seedling growth under tunnel conditions

The landraces, pre-treatments, and their interactions showed significant differences ($p < 0.001$) in germination percentage, germination rate index, seedling length, and seed vigour index under tunnel conditions, as shown in Table 3.

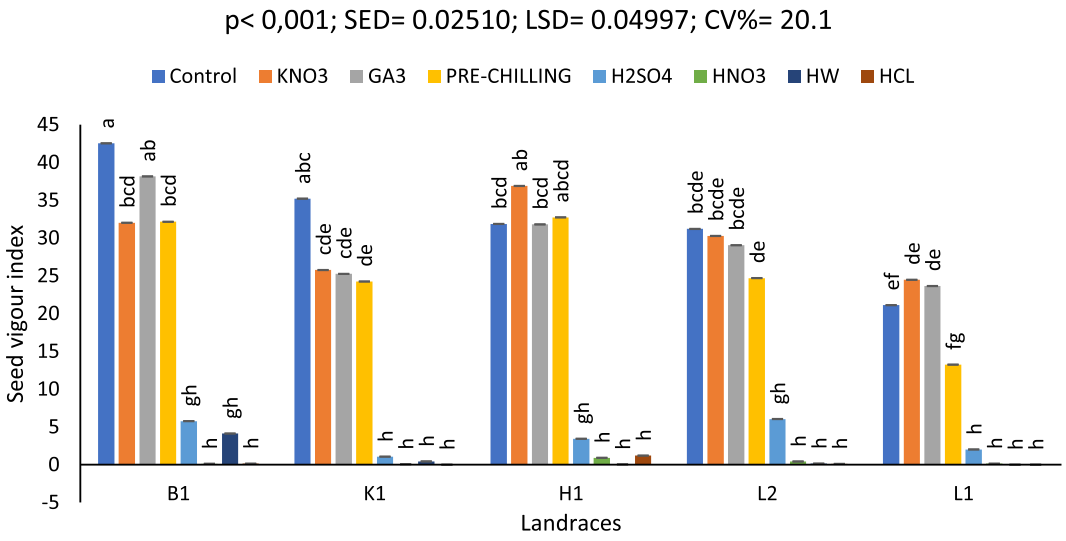
3.3.1. Seed germination

The results revealed that the applied seed dormancy breaking treatments such as HCL, HNO_3 and H_2SO_4 completely inhibited the germination of all the cannabis genotypes and, therefore, their analyzed data was excluded. HW also hindered the germination of all the cannabis genotypes compared to control, with complete inhibition in 'H1' and 'L2', as shown in Fig. 6. Compared to control, prechilling treatment recorded significantly higher germination of 65.56 % in 'H1', The results also reveal that there was



* Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

Fig. 4. Effect of eight different seed dormancy-breaking treatments on the radicle length among five (5) cannabis landraces under laboratory conditions ($p < 0.001$).



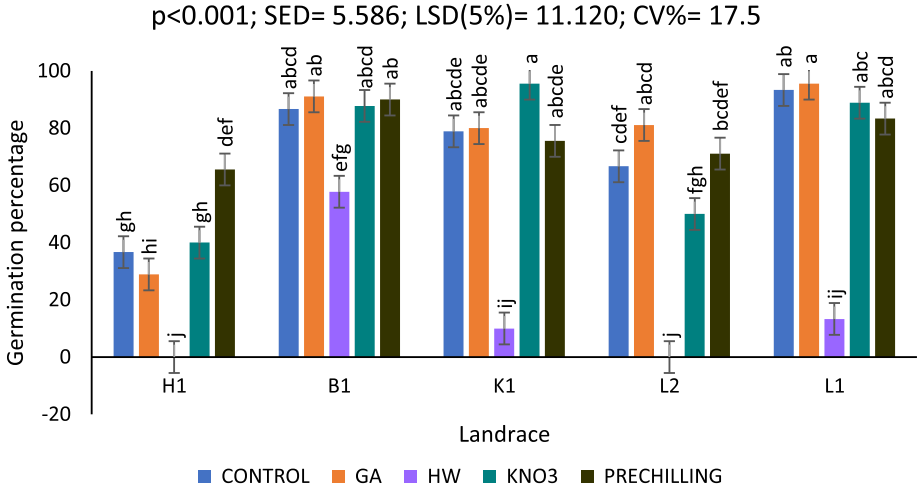
* Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

Fig. 5. Effect of eight different seed dormancy-breaking treatments on seed vigour index among five (5) cannabis landraces under laboratory conditions ($p < 0.001$).

Table 3
Analysis of variance (ANOVA) table for seed germination percentage (GP), germination rate index (GRI), seedling length (SL), and seed vigour index (SVI) of cannabis landraces subjected to various dormancy breaking pre-treatments under tunnel conditions.

				Mean square		
Source	df	Mean square for GP	df	GRI	SL	SVI
Landrace	4	3435.74**	4	1687.08**	3387.3**	11,019.6**
Treatment	7	21,623.90**	4	3943.95**	39,655.0**	32,001.3**
Landrace*Treatment	28	16,667.41**	16	214.97**	1133.1**	1218.4**

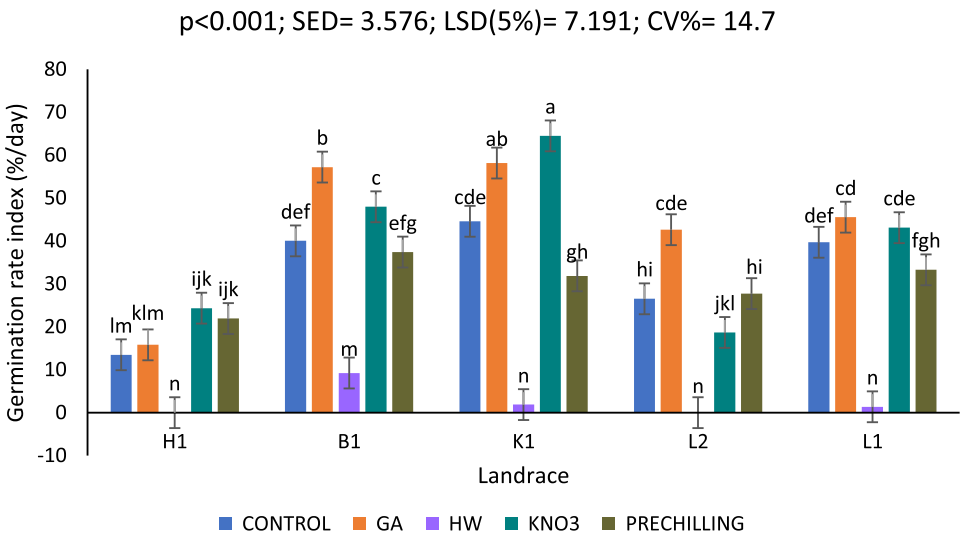
ns - not significant ($p > 0.05$); *significant ($p < 0.05$); **highly significant ($p < 0.001$).



*Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

Fig. 6. Effect of eight different seed dormancy-breaking treatments on germination percentage among five (5) cannabis landrace under tunnel conditions ($p < 0.001$).

no significant difference between control and treatments such GA_3, KNO_3 and prechilling in 'B1', 'K1', 'L2', and 'L1'. Similarly, there was no significant difference between control and treatments such GA_3 and KNO_3. However, KNO_3 recorded higher germination of 40 % in 'H1' compared to 36.67 % of control. GA_3, KNO_3 and prechilling recorded higher germination of 91.11, 87.78 and 90.00 % in 'B1' compared to 86.67 % of control. GA_3 and KNO_3 recorded higher germination of 80.00 and 95.56 % in 'K1' compared to 78.89 % of control. GA_3 and KNO_3 also recorded higher germination of 81.11 and 71.11 % in 'L2' compared to 66.67 % of control. GA_3 also recorded higher germination of 95.56 % in 'L1' compared to 93.33 % of control.



*Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

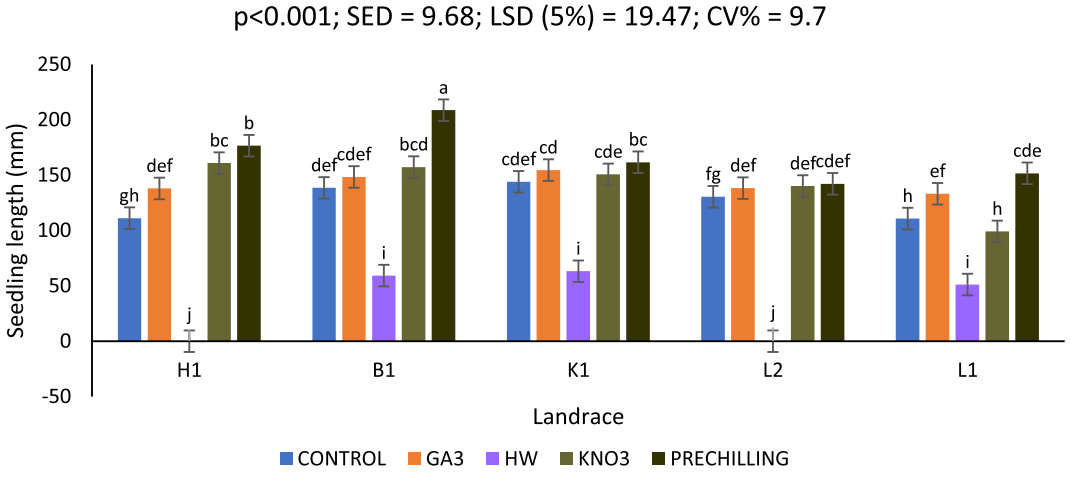
Fig. 7. Effect of eight different seed dormancy-breaking treatments on germination rate index among five (5) cannabis landraces under tunnel conditions ($p < 0.001$).

3.3.2. Germination rate index

The results revealed that the germination rate index varied in genotypes with the applied seed dormancy breaking treatments. However, the germination rate index recorded for all cannabis genotypes was significantly lower under HW compared to control, as shown in Fig. 7. GA_3, KNO_3 and prechilling recorded a higher germination rate index in 'H1' of 15.8, 24.35 and 21.93 %/day, respectively, compared to 13.49 %/day recorded for control. GA_3 and KNO_3 recorded a higher germination rate index of 57.22 and 47.97 %/day in 'B1', respectively, compared to 40.03 %/day recorded for control. GA_3 and KNO_3 also recorded a higher germination rate index of 58.16 and 64.49 %/day in 'K1', respectively, compared to 44.59 %/day recorded for control. Furthermore, GA_3 and KNO_3 also recorded a higher germination rate index of 45.55 and 43.10 %/day in 'L1', respectively, compared to 39.70 %/day recorded for control. GA_3 and prechilling recorded a higher germination rate index of 42.63 and 27.74 %/day in 'L2', respectively, compared to 26.53 %/day recorded for control.

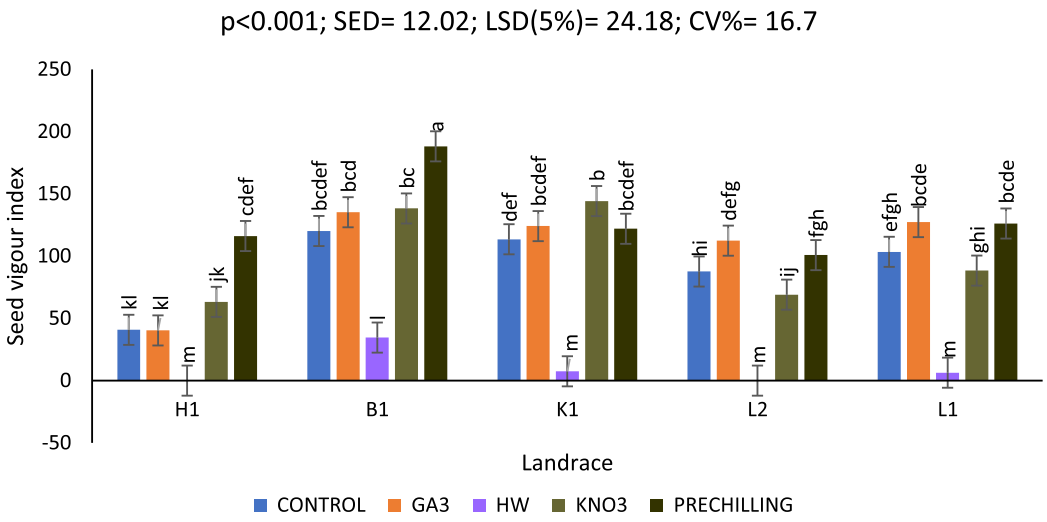
3.3.3. Seedling length

The results revealed that seedling under GA_3, KNO_3 and prechilling recorded longer length in 'H1', 'B1', 'K1' and 'L2' compared to control, as shown in Fig. 8. GA_3, KNO_3 and prechilling recorded a seedling length of 138.0, 160.9 and 176.6 mm in 'H1', respectively, and control recorded 111.2 mm. GA_3, KNO_3 and prechilling recorded a seedling length of 148.4, 157.3 and 208.7 mm in 'B1', respectively, and control recorded 138.5 mm. GA_3, KNO_3 and prechilling recorded a seedling length of 154.6, 150.7 and 161.7 mm in 'K1', respectively, and control recorded 144.0 mm. GA_3, KNO_3 and prechilling recorded a seedling length of 138.3, 140.2 and 142.2 mm in 'L2', respectively, and control recorded 130.5 mm. The results also revealed that



*Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

Fig. 8. Effect of eight different seed dormancy-breaking treatments on the radicle length among five (5) cannabis landraces under tunnel conditions ($p < 0.001$).



*Columns followed by the same letter are not significantly different, while columns with different footnote are significantly different according to Fisher's test (P<0.05). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation.

Fig. 9. Effect of eight different seed dormancy-breaking treatments on seed vigour index among five (5) cannabis landraces under tunnel conditions ($p < 0.001$).

seedlings under GA_3 and prechilling had longer length of 133.2 and 151.7 mm in 'L1', respectively, compared to 110.8 mm recorded for control.

3.3.4. Seed vigour index

The results revealed that seed vigour index was higher in 'B1' and 'K1' under GA_3, KNO_3 and prechilling compared to control, as shown in Fig. 9. GA_3, KNO_3 and prechilling recorded a higher seed vigour index of 135.23, 138.28 and 188.18 in 'B1', respectively, and control recorded 120.17. GA_3, KNO_3 and prechilling recorded a higher seed vigour index of 124.11, 144.21 and 121.96 in 'K1', respectively, and control recorded 113.53. The results also revealed that seed vigour index was higher in 'L2' and 'L1' under GA_3 and prechilling compared to control. GA_3 and prechilling recorded a seed vigour index of 112.41 and 100.81 in 'L2', respectively, and control recorded 87.63. GA_3 and prechilling recorded a seed vigour index of 127.33 and 126.19 in 'L1', respectively, and control recorded 103.44. Furthermore, the results revealed that KNO_3 and prechilling a higher seed vigour index of 63.24 and 116.13 in 'H1', respectively, compared to

4. Discussion

Generally, germination percentage, germination rate index, seedling length and seed vigour index varied among cannabis genotypes with the applied treatments under both laboratory and tunnel conditions. Amongst the cannabis genotypes, 'B1' generally had higher germination percentage, seedling length and seed vigour under both tunnel and laboratory conditions. On the other hand, 'H1' and 'L1' generally had poor germination performance compared to other genotypes under tunnel and laboratory conditions, respectively. The results revealed some degree of correlation in germination and seedling growth performance with the viability of the seeds of the cannabis genotypes. This follows that 'B1' had second highest seed viability percentage, while 'H1' had the lowest and 'L1' had the second lowest seed viability percentage. These findings were expected as it is known that viable seed indicates its capability to germinate and produce a normal seedling when it is not dormant (Quintana et al., 2023).

Under the laboratory conditions, H_2SO_4, KNO_3, and GA_3 generally proved highly effective in breaking the seed dormancy of cannabis landraces, while prechilling, HNO_3, HCL, and HW proved ineffective. These results were then validated under tunnel conditions which simulated field conditions. Under the tunnel conditions, KNO_3, GA_3 and prechilling proved highly effective in breaking the seed dormancy of cannabis genotypes, while H_2SO_4, HNO_3, HCL, and HW proved ineffective. Notably, prechilling performance proved effective in breaking the seed dormancy of cannabis landraces under tunnel conditions but was ineffective under laboratory conditions. Specifically, prechilling was generally most effective treatment in enhancing seed germination and seedling length under tunnel conditions and ultimately, seed vigour index. Such findings about the cannabis genotypes and applied treatments were expected as previous studies suggests that these germination and seedling growth parameters varies with environmental conditions, in response to pre-treatments (Golmohammadzadeh et al., 2015; Kildisheva et al., 2020; Islam et al., 2021; Flores-Sánchez et al., 2022). The tunnel findings corroborate with Elias et al. (2020), who achieved dormancy breaking through wet prechilling at 10 °C in 'Merlot' and 'Berry Blossom' hemp varieties, although the tunnel study used dry prechilling. Whereas the laboratory findings are in contradiction with both Elias et al. (2020) and findings of the current study. It is worth highlighting that dry prechilling is associated with not providing enough moisture like wet prechilling does to activate the hydrolytic enzymes in preparation of seeds for germination once they are moved to favourable conditions (Amini et al., 2015).

Furthermore, the seedling length and seed vigour index was generally higher under control compared to the applied pre-treatments under laboratory conditions. The findings of the laboratory study are in contrast with the findings of Aliloo and Darabinejad (2013), who reported that prechilling, KNO_3 and GA_3 pre-treatments recorded higher seedling length and seed vigour in *Heliotropium europaeum* compared to control. However, Aliloo and Darabinejad (2013) used 1.5 % KNO_3 for 1 h and GA_3 at a concentration of 250 mg per litre for 12 h. Thus, besides pre-treatment concentration and duration of seed contact, the findings of the laboratory study on the seedling length and seed vigour lower than control were associated with the fact that species response to pre-treatments may vary with the growth stage (Golmohammadzadeh et al., 2015; Kildisheva et al., 2020; Islam et al., 2021). However, the tunnel results revealed that prechilling, KNO_3 and GA_3 generally improved the seedling length and see vigour index of the cannabis landraces compared to control. Thus, the findings of the current study under tunnel conditions agree with the findings by Aliloo and Darabinejad (2013), however, in contradiction with the

laboratory findings as prechilling, GA_3 and KNO_3 improved seedling length and seed vigour index in cannabis genotypes. The differing findings between the laboratory and tunnel studies may indicate that the cannabis thrived under tunnel conditions because it simulated the actual growth environment of the cannabis genotypes.

The complete germination inhibition of H_2SO_4, HNO_3, HCL in all cannabis genotypes under tunnel conditions was associated with high concentrations which could have been corrosive and extended seed soaking duration resulting into seed and embryo damage; and ultimately inhibiting germination (Maesaroh and Demirbağ, 2020). H_2SO_4 having been effective in breaking seed dormancy through germination and germination rate index under laboratory conditions revealed that it oxidized the seed coats, resulting in softening and rupture of the seed coats (Zare et al., 2011; Mensah and Ekeke, 2016). Thereafter, the germination process in the seed was initiated, and eventually, protrusion of the radical and subsequent germination (Zare et al., 2011; Rasebeka et al., 2014; Mensah and Ekeke, 2016). However, the results under similar conditions proved that H_2SO_4 was not effective in producing healthy and vigorous quality seedlings. This is evident in the poor seedling length and seed vigour index revealed under laboratory conditions. Poor seed vigour index was associated with seeds unable to germinate under the tunnel conditions.

However, HCL, HW, and HNO3 results were expected because they have been previously reported as ineffective in breaking seed dormancy (Kambizi et al., 2006; Bhardwaj et al., 2016). For H_2SO_4, the findings of the current study are in contradiction with Ansari et al. (2016), who reported that scarification of the seed coat with 95 % H_2SO_4 stimulated the germination of *Malva sylvestris* seeds. As a result, germination of 88 % was recorded under acid scarification, and no germination was recorded under control (Ansari et al., 2016). Mensah and Ekeke (2016) also reported that H_2SO_4 enhanced the germination of *Senna obtusifolia* to 100 % against intact seeds that did not germinate.

On the other hand, KNO_3 is well-known as a compound that promotes the germination of photo-dormant seeds (Rouhi et al., 2013). "KNO_3 increases ambient oxygen levels by reducing the amount of oxygen available for the tricarboxylic acid (TCA) cycle" (Rouhi et al., 2013), which is a critical metabolic pathway for germination as it unifies carbohydrate, fat, and protein metabolism (Zhang et al., 2017). "KNO_3 is related to the activity of the enzyme nitrate reductase in the production of nitrite/nitric oxide (NO)" (Lara et al., 2014). "NO breaks a seed dormancy through the interaction with phytochrome signaling pathways, ethylene biosynthesis and interplays with reactive oxygen species" (Lara et al., 2014). Hence, it was effective in breaking the seed dormancy of cannabis landraces. However, the results of the current study contrast the findings by Mensah and Ekeke (2016), who reported that KNO_3 was ineffective in breaking seed dormancy and promoting seed germination of *Senna obtusifolia* species (Mensah and Ekeke, 2016).

For GA_3, the results of the current study were associated with the fact that "GA_3 acts as a natural plant growth regulator, releasing the seeds from dormancy by promoting protein synthesis, elongation of coleoptiles, and production of ethylene" (Camara et al., 2018; Polaiah et al., 2020). Similar results have been previously reported on plants such as *Papaver rhoeas* L. and *Papaver dubium* L. where GA_3 promoted germination when exogenously applied in these plants (Golmohammadzadeh et al., 2015). GA_3 being effective in breaking seed dormancy may also be associated with that both with abscisic acid (ABA) are key for inducing, maintaining, and releasing seed dormancy (Longo et al., 2020). These hormones are known to act antagonistically; ABA promotes dormancy during seed maturation and inhibits seed germination, whereas GA promotes seed germination (Longo et al., 2020; Yan and Chen, 2020). Thus, the seed dormancy gets released when the ABA/GA ratio is low, and eventually, germination commences (Yang et al., 2020).

Therefore, taking into consideration that tunnel conditions simulate the actual cannabis growing conditions, the summary of findings is that GA_3, KNO_3 and prechilling were the most effective pre-treatments to improve the germination and seedling growth of cannabis genotypes. Also taking into consideration of the pre-treatments that improved the germination, it was found that the seed coat of the cannabis genotypes had no effect on germination as the pre-treatments that were effective are associated with breaking physiological dormancy. Notably, prechilling is known to induce cold environmental conditions that leads to increased synthesis of GA_3 in the embryo and thereby aiding seed germination. Thus, the physiological dormancy in cannabis genotypes was largely associated with ABA/GA ratio.

5. Conclusions

The current study revealed the significancy of adequate growing conditions in cannabis genotypes for assessing their germination and seedling growth studies. The current study concluded that using prechilling, GA_3, and KNO_3 pre-treatments enhanced the germination capacity and seedling growth of cannabis landraces, and alleviated seed dormancy. However, the effect on the germination of cannabis varied with landrace, treatment, and seed viability. It was concluded that the physiological state of the embryo caused dormancy in cannabis landraces, ABA/GA_3 levels, and possibly a decreased gas permeability of the seed coat was the major barrier to cannabis seed germination. This is because the pre-treatments prechilling and GA_3 are associated with the increased synthesis of GA_3 which "promotes protein synthesis, elongation of coleoptiles, and production of ethylene". On the other hand, KNO_3 is associated with the increased ambient oxygen levels; oxygen is associated with metabolism reactivation during seed imbibition. Prechilling is known to induce cold environmental conditions that leads to increased synthesis of GA_3 in the embryo and thereby aiding seed germination. The current study, therefore, recommends further investigation of the effect of growth hormones or chemicals at different concentrations and duration of seed exposure on seed reserve mobilization during germination of cannabis seeds of known ABA/GA_3 ratio.

Funding

This research was funded by MOSES KOTANE INSTITUTE (Grant no. 211526424).

Data availability

Research data is available upon request and can be requested by contacting the corresponding author of the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sabeliwe Langa: Conceptualization, Methodology, Investigation, Resources, Formal analysis, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Lembe Samukelo Magwaza:** Conceptualization, Resources, Writing – review & editing, Supervision. **Asanda Mditshwa:** Conceptualization, Writing – review & editing, Supervision. **Samson Zeray Tesfay:** Conceptualization, Writing – review & editing, Supervision.

Acknowledgments

We would like to pass our gratitude to the cannabis growers in Ladysmith, Msinga, Hammersdale and Bergville who assisted us with the cannabis landrace seeds.

References

Aliloo, A.A., Darabinejad, S., 2013. Evaluation of different techniques for breaking seed dormancy of *Heliotropium europaeum* L. (Boraginaceae). J. Biol. Environ. Sci. 7 (20), 87–91.

Amini, V., Zaefarian, F., Rezvani, M., 2015. Effect of prechilling and environmental factors on breaking seed dormancy and germination of three foxtail species. Acta Agric. Slov. 105 (2), 269–278.

Ansari, O., Gherekhloo, J., Kamkar, B., Ghaderi-Far, F., 2016. Breaking seed dormancy and determining cardinal temperatures for *Malva sylvestris* using nonlinear regression. Seed Sci. Technol. 44 (3), 447–460.

Ascrizzi, R., Ceccarini, L., Tavarini, S., Flamini, G., Angelini, L.G., 2019. Valorisation of hemp inflorescence after seed harvest: cultivation site and harvest time influence agronomic characteristics and essential oil yield and composition. Ind. Crops Prod. 139, 111541.

Awasthi, P., Karki, H., Bargali, K., Bargali, S.S., 2016. Germination and seedling growth of pulse crop (*Vigna* spp.) as affected by soil salt stress. Curr. Agric. Res. J. 4 (2), 159–170.

Bano, H., Rather, R.A., Bhat, J.I., Bhat, T.T., Azad, H., Bhat, S.A., Hamid, F., Bhat, M.A., 2021. Effect of pre-sowing treatments using phytohormones and other dormancy breaking chemicals on seed germination of *Dioscorea deltoidea* Wall. Ex Griseb.: an endangered medicinal plant species of north western Himalaya. Eco. Environ. Cons, 27, 253–260.

Bhardwaj, A.K., Kapoor, S., Naryal, A., Bhardwaj, P., Warghat, A.R., Kumar, B., Chaurasia, O.P., 2016. Effect of various dormancy breaking treatments on seed germination, seedling growth and seed vigour of medicinal plants. Trop. Plant Res. 3 (3), 508–516.

Camara, M.C., Vandenberghe, L.P., Rodrigues, C., de Oliveira, J., Faulds, C., Bertrand, E., Soccol, C.R., 2018. Current advances in gibberellic acid (GA_3) production, patented technologies and potential applications. Planta 248, 1049–1062.

Chahtane, H., Kim, W., Lopez-Molina, L., 2017. Primary seed dormancy: a temporally multilayered riddle waiting to be unlocked. J. Exp. Bot. 68 (4), 857–869.

Chaohua, C., Gonggu, Z., Lining, Z., Chunsheng, G., Qing, T., Jianhua, C., Xinbo, G., Dingxiang, P., Jianguang, S., 2016. A rapid shoot regeneration protocol from the cotyledons of hemp (*Cannabis sativa* L.). Ind. Crops Prod. 83, 61–65.

Chaves, I.S., Silva, N.C.Q., Ribeiro, D.M., 2017. Effect of the seed coat on dormancy and germination in *Stylosanthes humilis* H. B. K. seeds. J. Seed Sci. 39 (2), 114–122.

Elias, S.G., Wu, Y.C., Stimpson, D.C., 2020. Seed Quality and Dormancy of Hemp (*Cannabis sativa* L.). J. Agric. Hemp Res. 2 (1), 2.

Flores-Sánchez, I.D., Sandoval-Villa, M., Uscanga-Mortera, E., 2022. Breaking Seed Dormancy of *Jaltomata procumbens* (Cav.) JL Gentry Seeds with the Use of KNO3. Crops 2 (2), 99–110.

Flores-Sanchez, I.J., Peč, J., Fei, J., Choi, Y.H., Dušek, J., Verpoorte, R., 2009. Elicitation studies in cell suspension cultures of *Cannabis sativa* L. J. Biotechnol. 143 (2), 157–168.

Forapani, S., Carboni, A., Paoletti, C., Moliterni, V.C., Ranalli, P., Mandolino, G., 2001. Comparison of hemp varieties using random amplified polymorphic DNA markers. Crop Sci. 41 (6), 1682–1689.

Golmohammadzadeh, S., Zaefarian, F., Rezvani, M., 2015. Effects of some chemical factors, prechilling treatments and interactions on the seed dormancy-breaking of two Papaver species. Weed Biol. Manag. 15 (1), 11–19.

Grulichova, M., Mendel, P., Lalge, A.B., Slamova, N., Trojan, V., Vyhnanek, T., Winkler, J., Vaverkova, M.D., Adamcova, D., Dordevic, B., 2017. Effect of different phytohormones on growth and development of micropropagated *Cannabis sativa* L. Mendel Net. 618–623.

Gunes, E., Gubbuk, H., Ayala-Silva, T., Gozlekci, S., Ercisli, S., 2013. Effects of various treatments on seed germination and growth of carob (*Ceratonia siliqua* L.). Pak. J. Bot. 45 (4), 1173–1177.

Islam, M.M., Rengel, Z., Storer, P., Siddique, K.H., Solaiman, Z.M., 2021. Industrial Hemp (*Cannabis sativa* L.) varieties and seed pre-treatments affect seed germination and early growth of seedlings. Agronomy 12 (1), 6.

Jaganathan, G.K., Li, J., Biddick, M., Han, K., Song, D., Yang, Y., Han, Y., Liu, B., 2019. Mechanisms underpinning the onset of seed coat impermeability and dormancy-break in *Astragalus adsurgens*. Sci. Rep. 9 (1), 9695.

Jin, Y.J., Jeong, H.J., Kim, S.Y., Cho, S.H., Lee, J.H., Kim, D.H., 2023. Pelleting of physical dormancy small-seeded species in *Astragalus sikokianus* Nakai. Agronomy 13 (1), 206.

Jovičić, D., Nikolić, Z., Sikora, V., Tamindžić, G., Petrović, G., Ignjatov, M., Milošević, D., 2019. Comparison of methods for germination testing of Cannabis sativa seed. Ratarstvo i povrtarstvo 56 (3), 71–75.

Kambizi, L., Adebola, P.O., Afolayan, A.J., 2006. Effects of temperature, prechilling and light on seed germination of *Withania somnifera*; a high value medicinal plant. South Afr. J. Bot. 72 (1), 11–14.

Khalaki, M.A., Ghorbani, A., Dadjou, F., 2019. Influence of nano-priming on festuca ovina seed germination and early seedling traits under drought stress, in laboratory condition. Ecopersia 7, 133–139.

Kildisheva, O.A., Dixon, K.W., Silveira, F.A., Chapman, T., Di Sacco, A., Mondoni, A., Turner, S.R., Cross, A.T., 2020. Dormancy and germination: making every seed count in restoration. Restorat. Ecol. 28, S256–S265.

Labbafi, M., Mehrafarin, A., Badi, H., Ghorbani, M., Tavakoli, M., 2018. Improve germination of caper (*Capparis spinosa* L.) seeds by different induction treatments of seed dormancy breaking. Trakia J. Sci. 16 (1), 71–74.

Lara, T.S., Lira, J.M.S., Rodrigues, A.C., Rakocevi, M., Alvarenga, A.A., 2014. Potassium nitrate priming affects the activity of nitrate reductase and antioxidant enzymes in tomato germination. J. Agric. Sci. 6 (2), 72.

Lata, H., Chandra, S., Techen, N., Khan, I.A., ElSohly, M.A., 2016. In vitro mass propagation of *Cannabis sativa* L.: a protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological, biochemical, and genetic fidelity of micropropagated plants. J. Appl. Res. Med. Arom. Plants 3, 18–26.

Longo, C., Holness, S., De Angelis, V., Lepri, A., Occhigrossi, S., Ruta, V., Vittorioso, P., 2020. From the outside to the inside: new insights on the main factors that guide seed dormancy and germination. Genes (Basel) 12 (1), 52.

Maesaroh, S., Demirbağ, N.Ş., 2020. Pretreatment effect on amelioration of seed germination of zollinger's indigo (*Indigofera zollingeriana* Miq.). Yuzuncu Yıl Univ. J. Agric. Sci. 30 (1), 1–8.

Majd, R., Aghaie, P., Monfared, E.K., Alebrahim, M.T., 2013. Evaluating of some treatments on breaking seed dormancy in Mesquite. Int. J. Agron. Plant Prod. 4 (7), 1433–1439.

Mandizvo, T., Odindo, A.O., 2019. Seed coat structural and imbibitional characteristics of dark and light-coloured Bambara groundnut (*Vigna subterranea* L.) landraces. Heliyon 5 (2), e01249. https://doi.org/10.1016/j.heliyon.2019.e01249.

Mensah, S.I., Ekeke, C., 2016. Effects of different pre-treatments and seed coat on dormancy and germination of seeds of *Senna obtusifolia* (L.) HS Irwin & Barneby (Fabaceae). Int. J. Biol. 8 (2), 77–84.

Miraji, K.F., Capuano, E., Fogliano, V., Laswai, H.S., Linnemann, A.R., 2021. Utilization of Pepeta, a locally processed immature rice-based food product, to promote food security in Tanzania. PLoS One 16 (3), e0247870.

Muszyñski, S., Gladyszewska, B., 2008. Representation of He-Ne laser irradiation effect on radish seeds with selected germination indices. Int. Agrophys. 22 (1), 151–157.

Önol, B., Yildirim, M.U., 2021. Breaking seed dormancy and regeneration in *Cannabis sativa* L. Int. J. Agric. Environ. Food Sci. 5 (4), 709–719. https://doi.org/10.31015/jaefs.2021.4.32.

Paiva, E.P.D., Torres, S.B., Almeida, J.P.N.D., Sá, F.V.D.S., Oliveira, R.R.T., 2017. Tetrazolium test for the viability of gherkin seeds. Rev. Ciência Agron. 48, 118–124.

Polaiah, A.C., Parthvee, R.D., Manjesh, G.N., Thondaiman, V., Shivakumara, K.T., 2020. Effect of presowing seed treatments on seed germination and seedling growth of sandalwood (*Santalum album* L.). Int. J. Chem. Stud. 8 (4), 1541–1545.

Quintana, Y.G., Crespo, Y.A., De Decker, M., Castelo, M.D.C., 2023. Seed viability, germination and seedling quality patterns of three forest species for restoration in Amazonian conditions. Enfoque UTE 14 (1), 18–32.

Rasebeka, L., Mathowa, T., Mojeremane, W., 2014. Effect of seed pre-sowing treatment on germination of three Acacia species indigenous to Botswana. Int. J. Plant Soil Sci. 3 (1), 62–70.

Rouhi, H.R., Aboutalebian, M.A., Saman, M., Karimi, F., Champiri, R.M., 2013. Seed germination and dormancy breaking methods for Pheasant's eye (*Adonis vernalis* l.). Int. J. Agric. 3 (1), 172.

Salentijn, E.M.J., Zhang, Q., Amaducci, S., Yang, M., Trindade, L.M., 2015. New developments in fiber hemp (*Cannabis sativa* L.) breeding. Ind. Crops Prod. 68, 32–41.

Schultz, C.J., Lim, W.L., Khor, S.F., Neumann, K.A., Schulz, J.M., Ansari, O., Skewes, M.A., Burton, R.A., 2020. Consumer and health-related traits of seed from selected commercial and breeding lines of industrial hemp, *Cannabis sativa* L. J. Agric. Food Res. 2, 100025.

Small, E., 2015. Evolution and classification of *Cannabis sativa* (Marijuana, Hemp) in relation to human utilization. Bot. Rev. 81 (3), 189–294.

Smith, V.M., Powell, M., Mungeam, D., Emmons, R.G., 2019. Stakeholder perceptions of the impact of cannabis production on the southern Oregon food system. J. Agric. Food Syst. Commun. Dev. 8 (4), 125–135.

Soltani, E., Baskin, C.C., Baskin, J.M., Heshmati, S., Mirfazeli, M.S., 2018. A meta-analysis of the effects of frugivory (endozoochory) on seed germination: role of seed size and kind of dormancy. Plant Ecol. 219 (11), 1283–1294.

Tapfumaneyi, L., Dube, P., Mavengahama, S., Ngezimana, W., 2023. Effect of gibberellic acid and potassium nitrate seed treatments on the emergence and seedling vigor of Amaranth and Cleome gynandra. Agrosyst., Geosci. Environ. 6 (1), e20359.

Thomas, M., 2012. Cannabis Cultivation: A Complete Grower's Guide. Green Candy Press.

Tiwari, R.K.S., Chandra, K.K., Dubey, S., 2018. Techniques for breaking seed dormancy and its efficacy on seed germination of six important medicinal plant species. Int. J. Agric., Environ. Biotechnol. 11 (2), 293–301.

Utami, E.P., Santika, E., Hidayat, C., 2021, March. The mechanical and chemical scarification to break dormancy and increasing vigor of Sunan candlenut seed. IOP Conference Series: Earth and Environmental Science. 694, IOP Publishing, 012028.

Virgens, P.B.S., Conceição, T.A., Barbosa, R.M., 2019. Tetrazolium test to evaluate viability and vigour in Genipa americana seeds. Seed Sci. Technol. 47 (3), 307–318. https://doi.org/10.15258/sst.2019.47.3.06.

Yan, A., Chen, Z., 2020. The control of seed dormancy and germination by temperature, light and nitrate. Bot. Rev. 86 (1), 39–75.

Yang, L., Liu, S., Lin, R., 2020. The role of light in regulating seed dormancy and germination. J. Integr. Plant Biol. 62 (9), 1310–1326.

Zare, S., Tavili, A., Darini, M.J., 2011. Effects of different treatments on seed germination and breaking seed dormancy of *Prosopis koelziana* and *Prosopis juliflora*. J. For. Res. 22, 35–38.

Zhang, N., Zhang, H.J., Sun, Q.Q., Cao, Y.Y., Li, X., Zhao, B., Wu, P., Guo, Y.D., 2017. Proteomic analysis reveals a role of melatonin in promoting cucumber seed germination under high salinity by regulating energy production. Sci. Rep. 7 (1), 1–15.

Zuk-Golaszewska, K., Golaszewski, J., 2018. *Cannabis sativa* L.–cultivation and quality of raw material. J. Elementol. 23 (3), 971–984.