PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–09
https://doi.org/10.14719/pst.6048

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Evaluation of seed dormancy-breaking techniques for enhancing germination potential, seedling growth and vigour of *Carissa carandas* L.

Sivakumar V[1]*, V Santhoshini[1], S Selvaganapathy[1], B Adarsh[1], C Kavitha[2], R Balakumbahan[3], M Rajavel[4], K Vanitha[1] & M Kumar[1]

[1]Department of Fruit Science, Horticultural College & Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[2]Turmeric Research Centre, Tamil Nadu Agricultural University, Bhavanisagar 638 451, Tamil Nadu, India

[3]Horticultural Research Station, Tamil Nadu Agricultural University, Thadiyankudisai 624 212, Tamil Nadu, India

[4]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Email: sivakumarv@tnau.ac.in

OPEN ACCESS

ARTICLE HISTORY
Received: 21 October 2024
Accepted: 07 November 2024
Available online
Version 1.0 : 28 December 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Sivakumar V, Santhoshini V, Selvaganapathy S, Adarsh B, Kavitha C, Balakumbahan R, Rajavel M, Vanitha K, Kumar M. Evaluation of seed dormancy-breaking techniques for enhancing germination potential, seedling growth and vigour of *Carissa carandas* L. Plant Science Today.2024;11(sp4):01-09. https://doi.org/10.14719/pst.6048

Abstract

Carissa carandas L. is a versatile evergreen shrub of the Apocynaceae family and its fruit is rich in vitamins and antioxidants. It is commonly propagated by seeds, which are characterized by low germination rates due to the presence of a hard seed coat that causes physical dormancy and limits water absorption and gas exchange. In this study, the effects of different dormancy-breaking treatments *viz.,* chemical treatment with potassium nitrate and thiourea at two concentrations (1% and 2%), hormonal therapy with gibberellic acid (GA_3) at two concentrations (500 and 1000 ppm) and physical treatments (hot water and simple water immersion) were investigated on germination and seedling growth using a Randomized Block Design (RBD) with three replications and the statistical significance of each treatment was tested at the five percent probability level ($p \leq 0.05$). Key germination indices, seedling growth parameters and seedling vigour were observed. Among all treatments, 2% KNO_3 was most effective in breaking seed dormancy that achieved significantly higher (86.33%) germination with accelerated completion of germination in 18 days and the highest (4.80) mean daily germination (MDG). Thiourea 2% attained a germination percentage of 82.91% and MDG of 4.36 while GA_3 treated seeds had a lower germination percentage and MDG than KNO_3 and thiourea treatments. The significantly highest seedling length vigour index (LVI) of 1085.1 and weight vigour index (WVI) of 12.67 were also obtained in 2% KNO_3 treated seeds which was followed by 2% thiourea with LVI of 1003.6 and WVI 10.12 . Both LVI and WVI were significantly lower in GA_3 treatments compared to KNO_3 and thiourea treatments. The significantly lowest germination percentage (61.19%), MDG (2.78), LVI (575.8) and WVI (3.67) were recorded in the control. This study offers valuable insights into the efficient techniques for seed dormancy breaking and optimizing seed propagation techniques for *C. carandas* L.

Keywords

germination capacity; pre-treatments; seed dormancy; seedling growth; seedling vigour

Introduction

Carissa carandas L. grows in arid and semiarid areas of the world and is found in forests, savannas and cultivated gardens (1). It is majorly distributed in South Asia and predominantly found in the Himalayas (India), Pakistan, Sri Lanka, Afghanistan, Malaysia, Nepal and Myanmar (2). It is popularly known as karonda, karwand, Bengal currant, or Christ's thorn. The shrub is primarily valued for its edible miracle fruits loaded with vitamins (particularly vitamin C), dietary fiber, minerals and phytochemicals (3). It is traditionally valued for its great potential health benefits and medicinal applications in treating ailments such as digestive disorders, skin conditions and diabetes (4) and used for the preparation of a range of value-added products like pickles, jam, jellies, sauces, etc., (3, 5, 6). Despite its numerous benefits, one of the key challenges in *C. carandas* L. cultivation is seed dormancy, primarily due to the hard seed coat, which acts as a barrier to water uptake and gas exchange and thus significantly impedes germination rates. Seed dormancy leads to poor germination and emergence. Therefore, it is necessary to identify an effective technique to break seed dormancy and promote seed germination and seedling growth.

Seed pre-sowing treatments with certain chemical agents like nutrients, phytohormones and antioxidants could improve seed germination under varied environmental conditions (7). Various approaches of pre-sowing treatments have been proven to break seed dormancy and enhance the seed germination rate of several plant species (8). Thiourea, with its potential seed priming effects, may improve germination by boosting seed metabolic activities (9). Seed germination process in numerous species was enhanced when pre-treated with thiourea (10). Thiourea serves two main functions such as boosting the growth potential of the embryo and weakening the tissue of the layers that surround the embryo to remove the mechanical restraint imposed by these layers (11). Thiourea and nitrate compounds accelerated seed germination in *Atriplex prostrata* (12). Seed germination enhancement studies of *Prunus avium* indicated that thiourea treatment overcame seed dormancy and increased the germination rate (13). Studies in several alpine medicinal plant species demonstrated that pre-treatment with thiourea enhanced seed germination (14).

Gibberellic acid (GA_3) is a plant hormone known to stimulate seed germination by breaking down dormancy barriers (15, 16). GA_3 influences the physiological and also metabolic activities of seeds that enhance the germination (17). GA_3 improves seed germination by promoting the production of the hydrolytic enzymes and stimulating the synthesis of proteins and other needed metabolites for the embryo (18). Gibberellins regulate the mobilization of starch during the respiration process (19) and also increasing the mineral availability and thus enhancing the germination process. GA_3 has been reported to promote the seed germination of *P. acinose* (20). The previous studies have reported that GA_3 improves seed germination potential in several economically important species (21). The function of GA_3 in breaking physiological dormancy and as a germination enhancer has been widely known in a broader spectrum of plant species (22).

Potassium nitrate acts as an osmotic agent that enhances water uptake by seeds and consequently facilitates the germination process (23, 24). In several plant species, KNO_3 and GA_3 have been identified as functional chemical substances that improve seed germination and enhance enzyme activity (25, 26). Inorganic salts including KNO_3, KH_2PO_4 and $CaCl_2$ increase nitrogen and some other nutrients required for protein synthesis during the seed germination process. KNO_3 regulates the seed hormones and consequently, it reduces the germination inhibitors like abscisic acid (27). KNO_3 acts as a nutrient source and aids in stimulating the growth of plant tissues (19). Seeds treated with KNO_3 had increased germination percentage in *Phytolacca acinosa* and *Silphium integrifolium* (20). Soaking seeds in hot water and plain water are traditional methods employed to break dormancy and encourage germination (28). Water soaking of seeds assists in softening the seed cover, eliminating inhibitors decreases the time needed for germination and escalates the germination percentage (29).

Given the varied role of different pre-sowing techniques to break seed dormancy by altering the permeability or physiology of seed coat and metabolic activities of seed, thus improving seed germination, it is prudent to test the effectiveness of various pre-sowing treatments including chemical, hormonal and physical treatments on seed dormancy breaking, germination and seedling growth parameters. There is limited or no information available on the promising pre-sowing techniques with an optimum dose of chemicals and hormones to break the seed dormancy effectively and improve seed germination and seedling vigour in *C. carandas* L. The study aimed to standardize the seed dormancy-breaking treatments with optimum concentration of chemicals or hormones for enhancing seed germination potential and augmenting seedling growth and vigour of *C. carandas* L. Therefore, this study aimed to determine the effect of various seed treatments, including potassium nitrate, thiourea and gibberellic acid (GA_3) with different concentrations, hot water and simple water soaking treatments, on germination, seedling growth and vigour and improve the seed germination and seedling growth in *C. carandas* L.

Materials and Methods

Plant material, experimental design and treatments

The present investigation was carried out at the college orchard and laboratory, Department of Fruit Science, Horticultural College and Research Institute, TNAU, Coimbatore in 2024. The seed extraction process adopted in the study is depicted in Fig. 1. *C. carandas* L. seeds were selected based on uniform size and appearance. The cleaned and sorted seeds were used for the experiment. Seeds of *C. carandas* L. are recalcitrant, desiccation-sensitive and lose their viability in a shorter period. Therefore, seeds were subjected to different pre-sowing treatments soon after extracting from fruits and then treated seeds were sown in portrays. The experiment was laid out using a randomized block design (RBD) and each treatment was replicated three times.

Seed pre-treatment with chemicals and reagents

In the hormonal treatments, the seeds were soaked in GA_3 solutions at two concentrations (500 ppm and 1000 ppm) for 1 h. In the chemical treatments, the seeds were soaked in KNO_3 at two concentrations (1% and 2%) and similarly in thiourea at two concentrations (1% and 2%) for 1 h. The seeds were subjected to physical treatment including overnight soaking in simple water and 50˚C hot water soaking for 10 min.

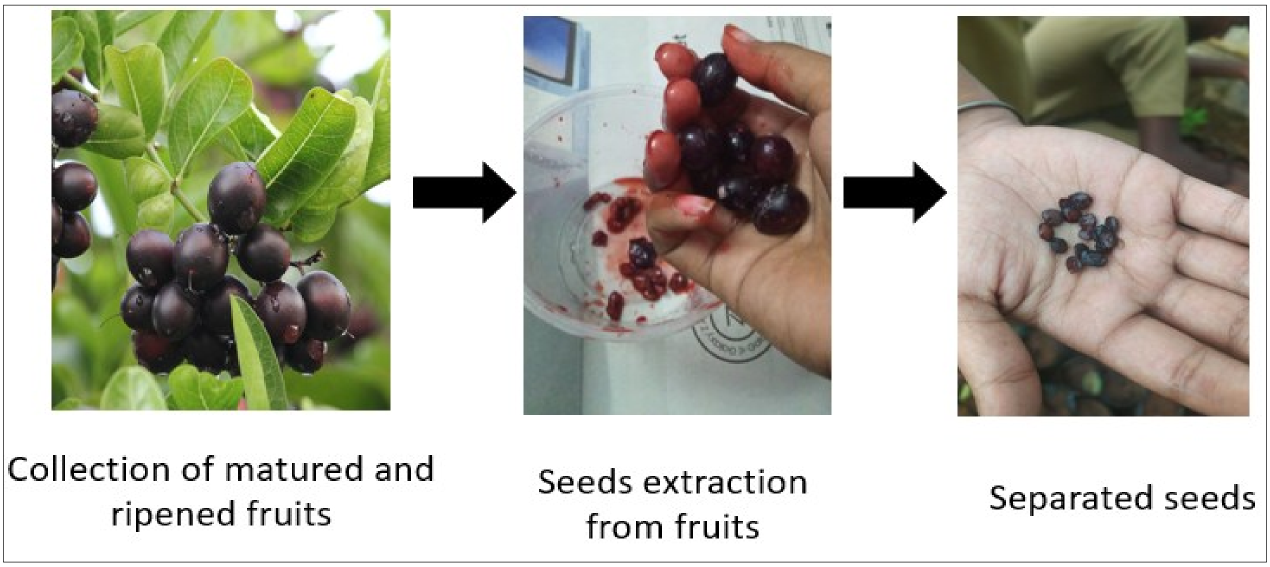

Fig. 1. Seed extraction for sowing.

Statistical analysis

The data collected from the experiment were subjected to statistical scrutiny by employing the SPSS statistical software package version 20. Statistical analysis was carried out using analysis of variance (ANOVA) and wherever the treatment differences were found significant critical differences were worked out at 5% probability level ($p \leq 0.05$).

Germination indices

The days to germination, 50% germination and complete germination were observed. The days to initiation of germination were recorded when the first seeds exhibited visible germination (emergence of the radicle) after sowing. Days to 50% germination was defined as the number of days needed for 50% of the seeds in a treatment to germinate. The days required for complete germination were indicated by the number of days taken until seed germination reaches a constant number and thereafter no germination takes place in a treatment.

Germination percentage was calculated as the percentage of seeds that successfully germinated compared to the total number of seeds sown, measured till the completion of germination. The germination indices were calculated using the following equations (30-33):

Germination percentage (GP) $= Ng/Ns \times 100$

Ng- Total number of seeds germinated, Ns- total number of seeds sown

Mean daily germination (MDG) = FGP/D

FGP - Final germination percentage, D- Number days to completion of germination

During the seed germination phase, the average maximum and minimum temperature recorded were 36.49°C and 24.13°C respectively and the average relative humidity that prevailed was 60.15%.

Growth and biomass measurements

Shoot and root length

Both shoot and root length were measured 20, 30 and 40 days after sowing (DAS) and expressed in centimetres. The height of each seedling was recorded from the base of the seedling to the tip of the shoot using a standard ruler. To measure root length, the longest root of each seedling was carefully extracted from the soil and its length was measured from the base of the seedling to the tip of the root using a ruler.

Seedling fresh and dry weight

The fresh weight of the seedlings was recorded after removing soil that adhered to the root. Dried the seedlings in the oven at 70°C until they reached a constant weight. Once seedlings dried, dry weight was taken using a precision balance. The dry weight of seedlings was recorded 40 DAS and expressed in grams. Both fresh and dry weights of the shoot and root were also recorded separately.

Seedling vigour Index

The seedling length vigour Index and weight vigour index were calculated using the following equations (33).

Seedling Length Vigour Index (SLVI) = Seedling length × Germination percentage

Seedling Weight Vigour Index (SWVI) = Seedling dry weight × Germination percentage

Relationship between seed germination potential and seedling growth traits

Correlation analysis was performed to determine the relationship between germination indices and seedling growth traits. The correlation coefficient (CC) is used as a statistical measure to study the strength and direction of linear association between two variables. The CC is denoted by r, which ranges from -1 to +1. Higher values indicate a stronger correlation while lower values indicate a weak correlation, -1 signifies an absolute negative correlation, +1 signifies an absolute positive relationship and 0 signifies no relationship between the variables (34). Significance of correlation was tested at 0.01% probability level ($p \leq 0.001$), 1% probability level ($p \leq 0.01$) and 5% probability level ($p \leq 0.05$).

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{(n\sum x^2 - (\sum x)^2)(n\sum y^2 - (\sum y)^2)}}$$

Results

Effect of various dormancy-breaking treatments on seed germination

Results obtained from the investigation on the effect of different dormancy-breaking treatments on seed germination capacity indicate that dormancy-breaking treatments had significantly ($p \leq 0.05$) influenced the *C. carandas* L. seed germination potential. The initiation of germination was most rapid in 2% KNO_3, which occurred 8 days after pre-sowing treatment (Fig. 2). 2% KNO_3 achieved 50% germination in 13 days and reached 100% germination rapidly in 18 days. 2% thiourea also expedited the seed germination process and exhibited better performance next to 2% KNO_3 treatment. In control, germination got delayed and took 9 days for initiation of germination, 15 days for 50% germination and 22 days for completion of germination.

Dormancy-breaking treatments demonstrated a significant ($p \leq 0.05$) effect on seed germination percentage (Table 1). 2% KNO_3 showed the significantly highest germination percentage (86.33%), which was followed (82.91%) by 2% thiourea. GA_3 treated seeds had a lower germination percentage than KNO_3 and thiourea treatments. Hot water soaking treatment recorded a lower germination percentage compared to chemical and hormonal treatments.

Mean daily germination (MDG) under various dormancy-breaking treatments

The results indicate that seed treatment with 2% KNO_3 resulted in a significantly ($p \leq 0.05$) higher MDG (4.80) over other treatments (Fig. 3). Thiourea 2% attained an MDG of 4.36 which was lower than the 2% KNO_3 treatment. 1% KNO_3 had an MDG of 3.82 and 1% thiourea showed 3.66 MDG. It was observed that MDG increased with an increase in KNO_3 and thiourea concentrations. GA_3 had less effect on MDG compared to chemical treatments. MDG slightly varied between GA_3 1000 ppm (3.35) and GA_3 500 ppm (3.30). All chemical and hormonal treatments were found to have higher MDG than overnight night water soaking (3.11) and hot water soaking (3.04) treatments. Control exhibited the least MDG (2.78) compared to all treatments.

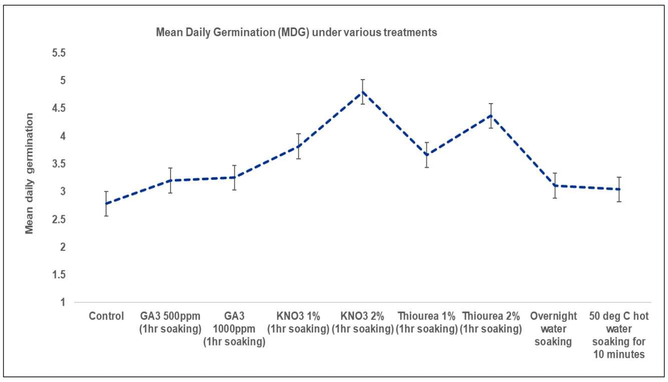

Fig. 3. Effect of dormancy breaking treatments on Mean daily germination (MDG) in *Carissa carandas* L.

Effect of various dormancy-breaking treatments on Length Vigour Index (LVI) and Weight Vigour Index (WVI)

Dormancy-breaking treatments significantly ($p \leq 0.05$) improved the seedling Length Vigour Index (LVI) as shown in Table 1. Among all treatments, 2% KNO_3 was more effective on LVI (1085.1) than the control (575.8). After 2% KNO_3 treatment, 2% thiourea significantly increased the LVI (1003.6) compared to the control. The effect of both KNO_3 and thiourea on LVI was reduced with the 1% concentration compared to 2%. However, KNO_3 and thiourea at 1% obtained higher LVI than GA_3. GA_3 effect on LVI was found to be less than chemical treatments. Water soaking treatments also boosted the LVI but the effect was significantly lower compared to chemical treatments.

Results of the seedling Weight Vigour Index (WVI) revealed significant ($p \leq 0.05$) differences between the treatments (Table 1). KNO_3 and thiourea chemical treatments followed the same trend for WVI with the highest WVI of 12.67 in 2% KNO_3 and 10.12 in 2% thiourea treated seeds. KNO_3 and thiourea at 1% concentration exhibited a lower WVI than at 2%. On the other hand, chemical treatments at both 1% and 2% showed a significant increase in WVI compared to GA_3. WVI was significantly higher with GA_3 1000 ppm than overnight water soaking while GA_3 500 ppm obtained a WVI on par with overnight water soaking. The WVI was lower in the hot water soaking treatment compared to other treatments. The lowest WVI of 3.67 was observed in control.

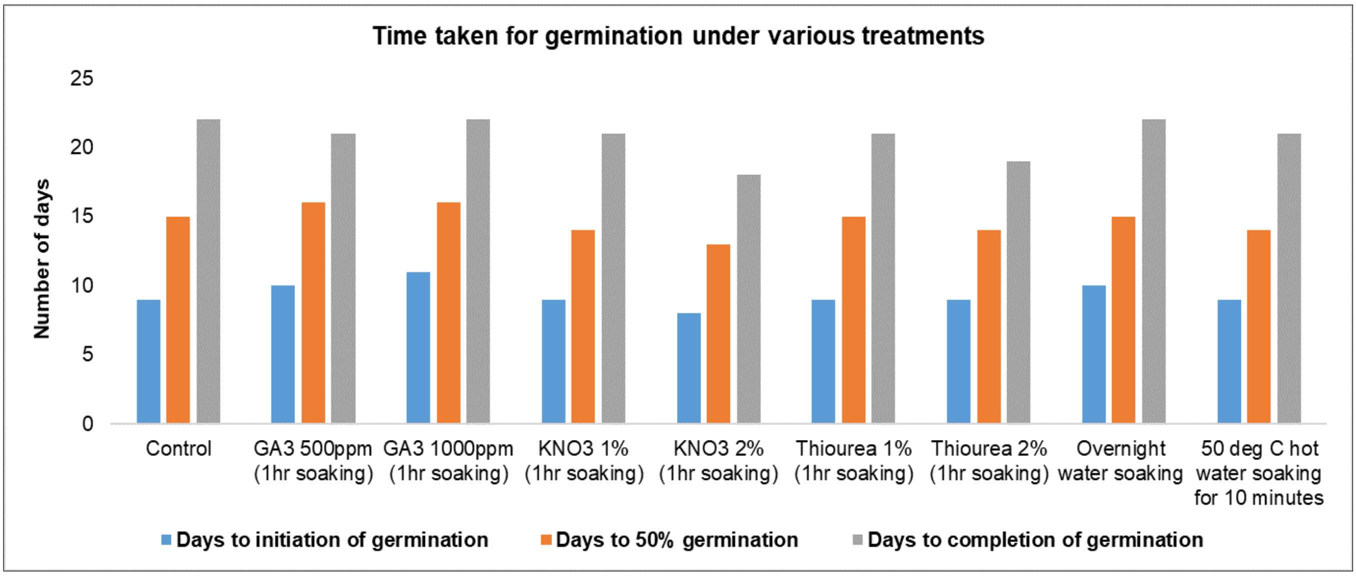

Fig. 2. Response of seed germination to dormancy-breaking treatments in *Carissa carandas* L.

Table 1. Effect of dormancy breaking treatments on germination potential and seedling vigour index of *Carissa carandas* L. seed

Treatments	Germination capacity (%)	Length Vigour Index (LVI)	Weight Vigour Index (WVI)
Control	61.19[h]	575.8[g]	3.67[g]
GA_3 500 ppm -1h soaking	67.15[f]	709.8[e]	6.038[ef]
GA_3 1000 ppm -1h soaking	71.51[e]	770.9[de]	6.54[e]
KNO_3 1% -1h soaking	80.14[c]	924.0[c]	8.76[c]
KNO_3 2% -1h soaking	86.33[a]	1085.1[a]	12.67[a]
Thiourea 1% -1h soaking	76.83[d]	897.4[c]	7.78[d]
Thiourea 2% -1h soaking	82.91[b]	1003.6[b]	10.12[b]
Overnight water soaking	68.35[f]	794.9[d]	5.51[f]
50°C hot water soaking -10 min	63.84[g]	640.3[f]	4.37[g]
SEm ±	1.103	30.05	0.435

Mean data are presented. Mean data within a column with different letters[(a-h)] represent significant differences ($p \leq 0.05$) and the same letters indicate that they aren't significantly different.

Growth performance of seedlings under various dormancy-breaking treatments

Shoot length, root length and seedling length

The longest shoot length was observed in 2% KNO_3 and 2% thiourea which were on par with each other at all times of observation (20, 30 and 40 DAS). The shoot length of seedlings of 2% thiourea treated seeds was 3.86, 4.24 and 5.62 cm and under 2% KNO_3 was 3.71, 4.46 and 5.43 cm at 20, 30 and 40 DAS, respectively. All the pre-sowing treatments had a positive effect on shoot length and increased the shoot length more than the control (Fig. 4). The shortest shoot lengths of 2.13, 3.12 and 3.92 cm were recorded at 20, 30 and 40 DAS, respectively in the control.

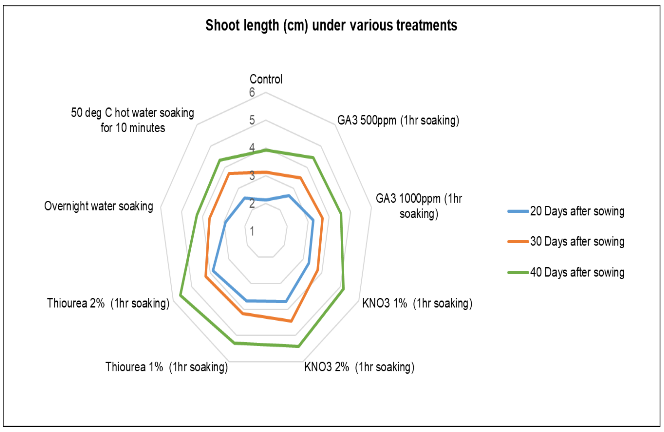

Fig. 4. Effect of dormancy-breaking treatments on shoot length of *Carissa carandas* L.

All the treatments augmented the root length of *C. carandas* L. (Fig. 5). The maximum root length of 5.49 cm at 20 DAS, 6.13 cm at 30 DAS and 7.34 cm at 40 DAS was obtained in 2 % KNO_3. The root length observed in 2% thiourea was also on par with the 2 % KNO_3 at all the times of observations. GA_3 treatment had less effect on shoot and root length than chemical treatments. The control showed the lowest root length of 3.27, 4.31 and 5.13 cm at 20, 30 and 40 DAS, respectively.

The length of the seedlings raised under different dormancy-breaking treatments was measured at 40 DAS and it was found that all the treatments positively influenced the seedling length (Fig. 6). The seedling length was significantly higher (12.57 cm) in 2 % KNO_3 which remained on par with the 2% thiourea (12.1 cm). GA_3 also increased the seedling length but exhibited less effect than KNO_3 and thiourea. The lowest seedling length (9.41cm) was observed in control.

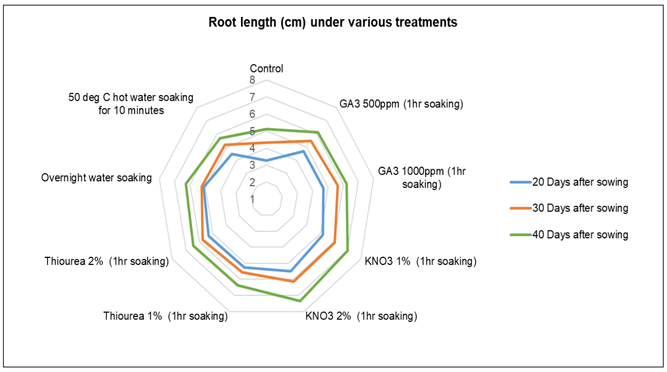

Fig. 5. Effect of dormancy-breaking treatments on root length of *Carissa carandas* L.

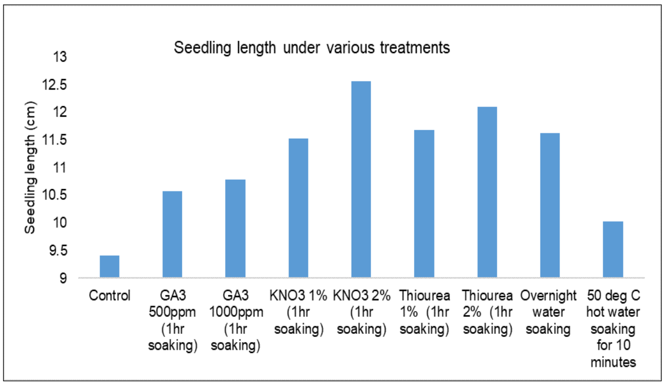

Fig. 6. Effect of dormancy-breaking treatments on seedling length of *Carissa carandas* L.

Effect of various dormancy-breaking treatments on biomass production

The seedlings of the 2% KNO_3 treated seeds had the significantly ($p \leq 0.05$) highest shoot and root fresh weight (0.587 g and 0.1967 g) as compared to control (0.215 g and 0.0726 g). 2% thiourea produced the seedlings with the fresh shoot weight of 0.452 g and root weight of 0.1527 g (Table 2). Similar to the fresh weight of shoot and root, 2 % KNO_3 had the highest dry shoot (0.108 g) and root (0.039 g) weight which was followed by 2% thiourea (0.092 g and 0.0306 g). Hormonal treatments had a moderate effect on shoot and root weight and stood next to the chemical treatments. Among the treatments, hot water soaking showed a little improvement in both fresh and dry root and shoot weight. Control recorded the lowest fresh as well as dry shoot and root weight.

The results of the fresh and dry weight of seedlings indicate that 2% KNO_3 had a significant ($p \leq 0.05$) effect on seedlings fresh (0.784 g) and dry weight (0.147 g) and after this treatment, 2% thiourea was the effective treatment (0.605 g and

Table 2. Effect of various dormancy-breaking treatments on biomass production of *Carissa carandas* L. (40 Days after sowing)

Treatments	Fresh weight (g)		Dry weight (g)		Fresh weight (g)	Dry weight (g)
	Shoot	Root	Shoot	Root	Seedling	Seedling
Control	0.215g	0.0726g	0.041h	0.0187f	0.2876g	0.0600h
GA_3 500ppm -1h soaking	0.319e	0.1043e	0.070e	0.0204d	0.4233e	0.0899e
GA_3 1000ppm -1h soaking	0.327e	0.1060e	0.070e	0.0214d	0.4330e	0.0915e
KNO_3 1% -1h soaking	0.418c	0.1341c	0.083c	0.0268c	0.5521c	0.1094c
KNO_3 2% -1h soaking	0.587a	0.1967a	0.108a	0.0393a	0.7837a	0.1468a
Thiourea 1% -1h soaking	0.374d	0.1287d	0.076d	0.0257c	0.5027d	0.1013d
Thiourea 2% -1h soaking	0.452b	0.1527b	0.092b	0.0306b	0.6047b	0.1221b
Overnight water soaking	0.298ef	0.0953f	0.060f	0.0203d	0.3933e	0.0806f
50˚C hot water soaking -10 min	0.280f	0.0943f	0.050g	0.0189f	0.3743f	0.0684g
SEm ±	0.016	0.0010	0.002	0.0012	0.0198	0.0039

Mean data are presented. Mean data within a column with different letters represent significant differences ($p \leq 0.05$) and the same letters indicate that they aren't significantly different

0.122 g). GA_3 effect on the biomass production of seedlings was less than the chemical treatments. The hot water treatment showed a minimal effect on seedling biomass production compared to other treatments.

Relationship between seed germination potential and seedling growth traits

Correlation analysis between germination indices and seedling growth traits indicates that germination indices viz., seed germination capacity and MDG rate were positively correlated with the seedling growth parameters such as shoot and root length, seedling fresh and dry weight, seedling LVI and seedling WVI. All the parameters exhibited a strong positive correlation with an r-value of more than 0.8 (Fig. 7). Germination capacity demonstrated a significantly positive linear relationship with seedling growth traits by having r values of 0.91, 0.93, 0.97, 0.97, 0.98 and 0.99 with seedling length, root dry weight, shoot dry weight, seedling dry weight, WVI and LVI respectively. Similarly, MDG also proved to have a significantly positive relationship (>0.8) with the seedling growth traits.

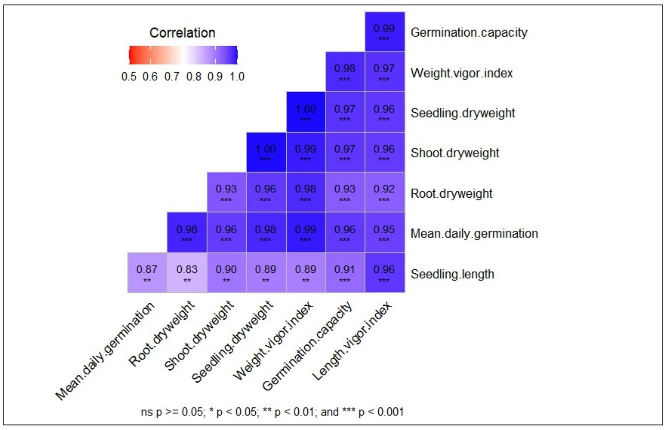

Fig. 7. Association between seed germination potential and seedling growth.

Discussion

Among the treatments, KNO_3 had a maximum germination percentage of 86.33% and showed considerable improvement in seed germination capacity by 25.14% compared to untreated control seeds. The significant positive effect of KNO_3 on germination potential observed in the present study (Fig. 7) is in agreement with the findings of previous studies which reported that KNO_3 enhances cell wall permeability and increases water absorption, which augments the enzyme activity and cellular metabolism that promote germination (25, 26). The higher germination percentage might be because KNO_3 helps stimulate metabolic processes in the seeds (35) and modulates the ABA metabolism or signaling in seed development (36, 37).

Seed treatments with chemicals such as KNO_3 and thiourea showed better results on seed germination and seedling growth traits at a dose of 2% compared to 1%. These results indicate that 2% is the optimum dose of KNO_3 and thiourea for seed treatment to markedly improve the seed germination and seedling growth of *C. carandas* L.

GA_3 treatment also showed a favorable role in germination but its effect in improving seed germination was found to be lower compared to KNO_3 and thiourea. GA_3can break the seed dormancy and improve the germination (38, 39). GA_3 at a higher concentration (1000 ppm) had a higher seed germination percentage compared to a lower concentration (500 ppm). The previous studies indicated that physiological dormancy in seeds hampers radicle protuberance, delaying seed emergence until changes occur in GA_3 and ABA levels (40-42). Changes in chemicals are taking place due to hormone imbalance of growth regulators such as GA_3 and growth inhibitors like ABA (43, 44). Dormant seeds contain high amounts of ABA while GA_3 is present at low levels. Thus, treating the seeds with a high dose of GA_3promotes seed emergence (44). It has been reported that GA_3 might overcome the seed dormancy and result in an improvement in seed emergence (45). The previous research highlighted that GA_3 induces metabolic processes in the embryo, which are needed to instigate the emergence process (35).

Pre-sowing treatments had a favorable effect on both shoot and root length. Among all treatments, 2% KNO_3 and 2% thiourea had the maximum shoot, root length and seedling vigour index. Accelerated seed germination under KNO_3 and thiourea might have increased the rate of cell division and elongation and consequently increased seedling length. KNO_3 has an advantageous effect on seed germination and seedling growth (39, 46). They pointed out that the enhanced seed germination and seedling growth might be due to faster seed germination and increased enzyme activity with KNO_3 application. KNO_3 priming has been shown to increase the shoot length in tomatoes (47).

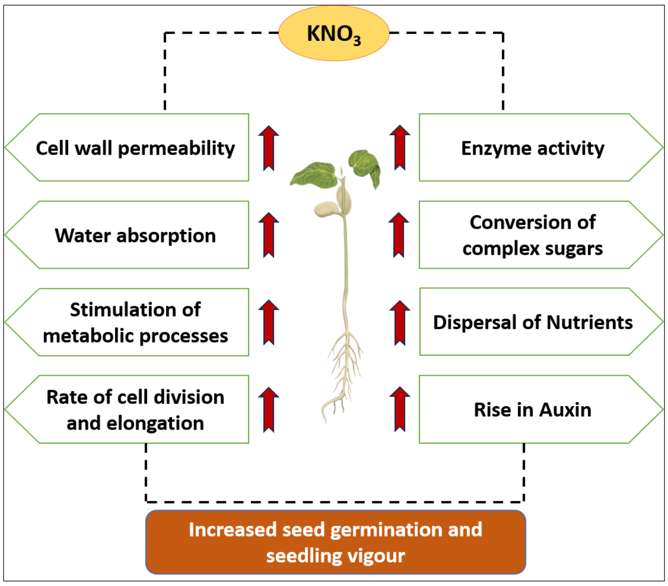

Fig. 8. Effect of KNO_3 in seed germination and seedling vigour

Thiourea application has been reported to enhance plant growth (48). Thiourea actively regulates a series of physiological, developmental and biochemical processes in plants (49). Followed by KNO_3 and thiourea, GA_3 increased the shoot and root length. The increase in shoot length could be attributed to the inherent ability of GA_3 to enhance cell division and elongation (50). GA_3 signaling might increase calcium and protein content and result in increased root growth (51).

It is evident from the results that 2% KNO_3 has substantially increased the fresh and dry biomass of seedlings compared to the control. Seeds treated with 2% thiourea also showed a significant improvement in seedling weight. KNO_3 and thiourea can enhance the metabolic activities of cells and subsequently increase biomass accumulation in the seedlings (46). Increased seedling dry weight might be due to an increase in shoot and root length (52). The results of seedling dry weight corroborate the findings of a previous study (53). Exogenous application of GA_3 raised the auxin amount in the roots which promoted root cell elongation as well as nutrient uptake. This led to an increase in root length and secondary fibrous roots and thereby enhanced the seedlings fresh and dry weight (54). KNO_3 treated seeds have the highest vigour index because KNO_3 participates in catalytic activity and conversion of complex sugar to simple glucose (52). The high vigour index of KNO_3 treated seeds could be attributed to high germination percentage, shoot and root length. KNO_3 might have facilitated the assimilation and efficient dispersal of nutrients in the seedling, thus resulting in a high seedling vigour index compared to other pre-sowing treatments.

The relationship between seed germination potential and seedling growth was found to be significantly positive. This indicates that the pre-sowing treatment with higher germination potential could enhance seedling growth and emphasise the vital role of appropriate pre-sowing technique in improving the seedling growth and vigour.

Conclusion

The study investigated the effect of different dormancy breaking treatments viz., chemical treatment with KNO_3 and thiourea at two concentrations (1% and 2%), hormonal treatment with GA_3 at two concentrations (500 and 1000 ppm) and physical (hot water and simple water soaking) treatments to identify the effective treatment for breaking seed dormancy, improve germination potential and seedling growth in *C. carandas* L. The results obtained from the study indicate that 2% KNO_3 is the best treatment for seed dormancy-breaking over all other pre-sowing techniques. The seeds treated with 2% KNO_3 had shortened germination time, increased seed germination percentage and improved seedling growth, vigour index and biomass accumulation, compared to other treatments. The GA_3 application showed less effect on seed dormancy-breaking, seed germination, seedling growth and vigour compared to chemical treatments. The effect of hot water treatments on the examined traits was comparatively lower than both chemical and hormonal treatments. The findings of the study can be applied for effective seed propagation and enhancement of seedling growth in *C. carandas* L. Seed germination is pivotal for the formulation of conservation strategies and sustainable use of plant species. Further research on evaluating the efficacy of various dormancy-breaking methods in various genotypes of *C. carandas* under diversified environmental conditions will pave the way for enhancing seed germination potential, sustainable utilization and large-scale cultivation of various *C. carandas* genotypes.

Acknowledgements

The authors are thankful to the Tamil Nadu Agricultural University for supporting their research work.

Authors' contributions

SV contributed to the conceptualisation, framing the methodology, obtaining resources, carrying out the investigation, analysis and writing the original draft. VS helped in the statistical analysis, conducting the investigation, formulating the methodology, employing software and writing the original draft. SS has assisted with the analysis, investigation, methodological framework, software application and writing. AB participated in writing, review and editing the manuscript. CK took part in framing the methodology, utilization of software, writing, review and editing. BR involved in writing, review and editing. RM, VK and KM contributed to writing, review and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Jadhav SD, Nangare D, Kakade VS, Baramati P. Karonda: Under-exploited fruit crop for dry land condition. Just Agriculture: Multidisciplinary E-Newsletter. 2022;2.
2. Bilala A, Ayuba MA, Mushtaqa A, Merzaiab AB. A brief study of phytochemical profile and pharmacological applications of *Carissa carandas* (L.). Int J Chem Biochem. 2015;8:92-96.

3. Tomer V, Kumar A. Karonda (*Carissa carandas* L.): a miracle fruit with multifaceted potential. JAgric Food Res. 2024;101417. https://doi.org/10.1016/j.jafr.2024.101417

4. Tesfaye T, Ravichadran Y. Traditional uses, pharmacological action and phytochemical analysis of *Carissa carandas* Linn. A review. Nat Prod Chem Res. 2018;6(5):1-20. https://doi.org/10.4172/2329-6836.1000334

5. Wani R, Prasad V, Hakeem S, Sheema S, Angchuk S, Dixit A. Shelf life of Karonda jams (*Carissa carandas* L.) under ambient temperature. Afr J Agric Res. 2013;8(21):2447-49. https://doi.org/10.5897/AJAR2013.6854

6. Rafique N, Mamoona T, Ashraf N, Hussain S, Ahmed F, Ali Shah T, Salamatullah AM, Mekonnen AB, Bourhia M. Exploring the nutritional and sensory potential of karonda fruit: Physicochemical properties, jam production and quality evaluation. Food Sci Nutr. 2023;11(11):6931-44. https://doi.org/10.1002/fsn3.3619

7. Kamran M, Wang D, Xie K, Lu Y, Shi C, Sabagh AE, Gu W, Xu P. Pre-sowing seed treatment with kinetin and calcium mitigates salt induced inhibition of seed germination and seedling growth of choysum (*Brassica rapa* var. *parachinensis*). Ecotoxicol Environ Safety. 2021;227:112921. https://doi.org/10.1016/j.ecoenv.2021.112921

8. Yeddula N, Topno S, Srivastava V, Bahadur V, Prasad V, Singh S. Effect of chemical priming on seed germination and seedling growth in papaya (*Carica papaya* L.). The Pharma Innov J. 2022;11(5):2542-46

9. Hore J, Sen S. Role of presowing seed treatment on germination, seedling growth and longevity of ber (*Zizyphus mauritiana* Lam.) seeds. Indian J Agric Res. 1994;28:285-89.

10. Mahadi S, Nulit R, Mohtar M, Ibrahim M, Ab Ghani N. Synergistic effect of KCl, thiourea, GA_3 and SA on the germination and early seedling growth enhancement of drought-stressed Malaysian indica rice cv. MR220. Biocatal Agric Biotechnol. 2020;29:101779. https://doi.org/10.1016/j.bcab.2020.101779

11. Finch�Savage WE, Leubner�Metzger G. Seed dormancy and the control of germination. New Phytol. 2006;171(3):501-23. https://doi.org/10.1111/j.1469-8137.2006.01787.x

12. Getlawi A, Shahba M, Hughes H. *Glaucium* species seed germination at different salinity levels as influenced by growth regulators. Hortic Int J. 2019;3:77-85. https://doi.org/10.15406/hij.2019.03.00115

13. Çetinbaş M, Koyuncu F. Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. Hortic Sci. 2006;33(3):119-23. https://doi.org/10.17221/3750-HORTSCI

14. Dey AN, Bhowmick N, Chakraborty A, Dey K, Ghosh A. Influence of different pre-sowing treatments on germination potential of Bakul (*Mimusops elengi* L.). Pharma Innov J. 2021;10:236-38. https://doi.org/10.22271/tpi.2021.v10.i4d.5930

15. Palepad K, Bharad S, Bansode G. Effect of seed treatments on germination, seedling vigour and growth rate of custard apple (*Annona squamosa*). J Pharmacogn Phytochem. 2017;6(6):20-23.

16. Nimbalkar S, Jadhav Y, Adat S, Savvashe A. Effect of different seed treatments on germination and growth of karonda (*Carissa congesta* W.). Green Farming. 2012;3(3):340-42.

17. Datt G, Chauhan JS, Ballabha R. Influence of pre-sowing treatments on seed germination of various accessions of Timroo (*Zanthoxylum armatum* DC.) in the Garhwal Himalaya. J Appl Res Med Aromat Plants. 2017;7:89-94. https://doi.org/10.1016/j.jarmap.2017.06.004

18. Amri B, Khamassi K, Ali MB, da Silva JAT, Kaab LBB. Effects of gibberellic acid on the process of organic reserve mobilization in barley grains germinated in the presence of cadmium and molybdenum. S Afr J Bot. 2016;106:35-40. https://doi.org/10.1016/j.sajb.2016.05.007

19. Waman AA, Bohra P, Norman A. Chemical pre-treatments improve seed germination and seedling growth in *Semecarpus kurzii*: an ethnomedicinally important plant. J For Res. 2018;29:1283-89. https://doi.org/10.1007/s11676-017-0562-9

20. Krishan R, Sharma RK, Sharma SS. Assessment of seed biology of the Himalayan medicinal herb *Phytolacca acinosa* Roxb., the Indian pokeweed, from the perspective of longevity, conservation and propagation. The Nucleus. 2022;65(3):331-39. https://doi.org/10.1007/s11676-017-0562-9

21. Rashid S, Rashid K, Ganie AH, Nawchoo IA, Khuroo AA. Seed ecology enlightens restoration of endemic species: A case study of *Actaea kashmiriana* from the Himalaya. Ecol Eng. 2023;187:106880. https://doi.org/10.1016/j.ecoleng.2022.106880

22. Singh S, Bhuker A, Kumar S, Kumar A, Dhaka AK. Effects of dormancy-breaking treatments on seed quality parameters in medicinal herb tulsi (*Ocimum tenuiflorum* L.). Seed Res. 2023;51(1):43-49.

23. Kadam A, Singh D, Kade R. Effect of plant growth regulators and potassium nitrate on growth of seedling of Kagzi lime. Asian J Hortic. 2010;5:431-34.

24. Lay P, Basvaraju G, Pashte V, Gowri M. Studies on effect of giberellic Acid (GA_3) and potassium nitrate (KNO_3) on breaking of seed dormancy of papaya (*Carica papaya* L.) cv. Surya The Ecoscan. 2015;9(1 and 2):111-15.

25. Mousavi L, Ishak WRW, Mousavi M. Evaluation of physicochemical methods for dormancy breakage and germination of *Datura stramonium* seeds. J Chem Health Risks. 2019;9(3).https://doi.org/10.22034/jchr.2019.668186

26. Sohindji FS, Sogbohossou DE, Zohoungbogbo HP, Houdegbe CA, Achigan-Dako EG. Understanding molecular mechanisms of seed dormancy for improved germination in traditional leafy vegetables: An overview. Agronomy. 2020;10(1):57. https://doi.org/10.3390/agronomy10010057

27. Gashi B, Abdullai K, Mata V, Kongjika E. Effect of gibberellic acid and potassium nitrate on seed germination of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae*. Afr J Biotechnol. 2012;11(20):4537-42.

28. Patel R, Ahlawat T, Singh A, Momin S, Gavri C. Effect of pre-sowing treatments on stone germination and shoot growth of mango (*Mangifera indica* L.) seedlings. Int J Agric Sci. 2016;8(52):2437-40.

29. Bhavya N, Naik N, Kantharaju V, Nataraj K. Studies on effect of different pre-sowing treatments on germination of karonda (*Carissa carandas* L.) seeds. J Pharmacogn Phytochem. 2017;6(6):352-54.

30. Scott SJ, Jones R, Williams W. Review of data analysis methods for seed germination 1. Crop Sci. 1984;24(6):1192-99. https://doi.org/10.2135/cropsci1984.0011183X002400060043x

31. Burnett SE, Pennisi SV, Thomas PA, van Iersel MW. Controlled drought affects morphology and anatomy of *Salvia splendens*. J Am Soc Hortic Sci. 2005;130(5):775-81. https://doi.org/10.21273/JASHS.130.5.775

32. MacGregor DR, Kendall SL, Florance H, Fedi F, Moore K, Paszkiewicz K, Smirnoff N, Penfield S. Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. New Phytol. 2015;205(2):642-52. https://doi.org/10.1111/nph.13090

33. Priyanka P, Jafar M, Ram M, Nitu G, Suaib L, Puja K, Birendra K.. Effect of potassium chloride-induced stress on germination potential of *Artemisia annua* L. varieties. J Appl Res Med Aromat Plants. 2018;9:110-16. https://doi.org/10.1016/j.jarmap.2018.03.005

34. Ratner B. The correlation coefficient: Its values range between+ 1/−1, or do they?. Journal of Targeting, Measurement and Analysis for Marketing. 2009;17(2):139-42. https://doi.org/10.1057/jt.2009.5

35. Nasri F, Khosheh Saba M, Ghaderi A, Mozafari AA, Javadi T. Improving germination and dormancy breaking in *Alstromeria ligtu* hybrid seeds. Trakia J Sci. 2014;12(1):38-46.

36. Matakiadis T, Alboresi A, Jikumaru Y, Tatematsu K, Pichon O, Renou JP, Kamiya Y, Nambara E, Truong HN. The *Arabidopsis* abscisic acid catabolic gene CYP707A2 plays a key role in nitrate control of seed dormancy. Plant Physiol. 2009;149(2):949-60. https://doi.org/10.1104/pp.108.126938

37. Chahtane H, Kim W, Lopez-Molina L. Primary seed dormancy: a temporally multilayered riddle waiting to be unlocked. J Exp Bot. 2017;68(4):857-69. https://doi.org/10.1093/jxb/erw377

38. Forghani A, Almodares A, Ehsanpour A. The role of gibberellic acid and paclobutrazol on oxidative stress responses induced by *in vitro* salt stress in sweet sorghum. Russ J Plant Physiol. 2020;67:555-63. https://doi.org/10.1134/S1021443720030073

39. Kwon HJ, Shin SL, Kim Y-R, Kim S-Y. Effects of temperature, gibberellic acid and KNO_3 treatments on seed germination of the wild plant *Maesa japonica*. Seed Sci Technol. 2020;48(1):65-72. https://doi.org/10.15258/sst.2020.48.1.09

40. Nur M, Baskin CC, Lu JJ, Tan DY, Baskin JM. A new type of non-deep physiological dormancy: evidence from three annual Asteraceae species in the cold deserts of Central Asia. Seed Sci Res. 2014;24 (4):301-14. https://doi.org/10.1017/S0960258514000300

41. Soltani E, Baskin C, Baskin J. A graphical method for identifying the six types of non‐deep physiological dormancy in seeds. Plant Biol. 2017;19(5):673-82. https://doi.org/10.1111/plb.12590

42. Hu D, Baskin JM, Baskin CC, Yang X, Huang Z. Ecological role of physical dormancy in seeds of *Oxytropis racemosa* in a semiarid sandland with unpredictable rainfall. J Plant Ecol. 2018;11(4):542-52. https://doi.org/10.1093/jpe/rtx063

43. Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L. The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. Plant Cell. 2008;20(10):2729-45. https://doi.org/10.1105/tpc.108.061515

44. Si Q, Ma Y, Zang D. The causes of dormancy and the changes of endogenous hormone content in *Cephalotaxus sinensis* seeds. Agric Sci. 2016;7(12):834. https://doi.org/10.4236/as.2016.712076

45. Gurung N, Swamy G, Sarkar S, Ubale N. Effect of chemicals and growth regulators on germination, vigour and growth of passion fruit (*Passiflora edulis* Sims.). The Bioscan. 2014;9(1):155-58.

46. Lara TS, Lira JMS, Rodrigues AC, Rakocevi M, Alvarenga AA. Potassium nitrate priming affects the activity of nitrate reductase and antioxidant enzymes in tomato germination. J Agric Sci. 2014;6 (2):72.https://doi.org/10.5539/jas.v6n2p72

47. Mirabi E, Hasanabadi M. Effect of seed priming on some characteristic of seedling and seed vigour of tomato (*Lycopersicon esculentum*). J Adv Lab Res Biol. 2012;3(3):237-40.

48. Haider FU, Virk AL, Rehmani MIA, Skalicky M, Ata-ul-Karim ST, Ahmad N, Soufan W, Brestic M, Sabagh AE, Liqun C. Integrated application of thiourea and biochar improves maize growth, antioxidant activity and reduces cadmium bioavailability in cadmium-contaminated soil. Front Plant Sci. 2022;12:809322. https://doi.org/10.3389/fpls.2021.809322

49. Perveen A, Wahid A, Mahmood S, Hussain I, Rasheed R. Possible mechanism of medium-supplemented thiourea in improving growth, gas exchange and photosynthetic pigments in cadmium-stressed maize (*Zea mays*). Rev Bras Bot. 2015;38:71-79. https://doi.org/10.1007/s40415-014-0124-8

50. Liopa-Tsakalidi A, Zakynthinos G, Varzakas T, Xynias IN. Effect of NaCl and GA_3 on seed germination and seedling growth of eleven medicinal and aromatic crops. J Med Plants Res. 2011;5(17):4065-73.

51. Zhu XF, Jiang T, Wang ZW, Lei GJ, Shi YZ, Li GX, Zheng SJ. Gibberellic acid alleviates cadmium toxicity by reducing nitric oxide accumulation and expression of IRT1 in *Arabidopsis thaliana*. J Hazard Mater. 2012;239:302-07. https://doi.org/10.1016/j.jhazmat.2012.08.077

52. Lay P, Basvaraju G, Sarika G, Amrutha N. Effect of seed treatments to enhance seed quality of papaya (*Carica papaya* L.) cv. surya. Greener J Biomed Health Sci. 2013;2(3):221-25.

53. Patil M, Desai N, Pawar U, Gaikwad D. Effect of plant growth regulators on seed germination and seedling growth of *Colubrina asiatica* L. Stud Rosenthaliana J Study Res. 2021;12(8):41-46. https://www.researchgate.net/publication/349589019

54. Dilip W, Singh D, Moharana D, Rout S, Patra S. Effect of gibberellic acid (GA) different concentrations at different time intervals on seed germination and seedling growth of Rangpur Lime. J Agroeco Nat Resour Manag. 2017;4:157-65. https://www.researchgate.net/publication/316968909