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RESEARCH ARTICLE

Standardization of *in-vitro* seed germination testing protocol for basella (*Basella alba*) and mustard green (*Brassica juncea* var. *rugosa*)

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Abstract

The demand and value for underutilized vegetables, particularly leafy vegetables like basella *(Basella alba*) and mustard green *(Brassica juncea* var. *rugosa),* have been gaining momentum as a source of nutritional substitutes. Information on seed testing protocols, including their dormancy management, are meagre. The seed testing protocols are neither available in ISTA rules, AOSA rules, nor in the IBPGR compendium. In the absence of prescribed seed testing protocols, practical difficulties are faced in the seed quality evaluation and/or by its end users (farmers) when raising a good crop. The presence of seed dormancy is beneficial in arresting seed germination; however, a long period of seed dormancy poses a problem in immediate sowing and its proper evaluation. In view of the above, the present study was conducted to standardize the seed testing protocol in basella and mustard green at ICAR-Indian Institute of Vegetable Research, Varanasi, India, using 5 genotypes of basella and 4 genotypes of mustard green during 2023 and 2024. The results showed that basella seed germinated best on between paper (BP) at 20 to 25°C. The ninth day and 21st day were found suitable for the first and final count, respectively. As basella seed exhibits physical dormancy, the dormancy management treatments are recommended. Among various dormancy management treatments, presoaking in 1.0% KNO_3 for 12 hrs significantly improved the seed germination. Whereas, in the case of mustard green suitable substratum is the top of the paper (TP) and temperatures of 20 to 30°C, are recommended, with the 5th and 7th day as the first and final count and the seeds of mustard green do not possess any dormancy.

Keywords: *Basella alba*, *Brassica juncea* var. *rugosa*, seed germination testing, dormancy, ISTA rules.

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Introduction

Basella (*Basella alba*) is a nutrient-rich, viny and heat-tolerant leafy green vegetable, and native to India and Southeast Asia, which thrives in tropical and subtropical regions. It belongs to the family Basellaceae and is commonly known as Malabar spinach, Indian spinach, or vine spinach (Singh et al., 2018). In the winter season, basella can be used as a substitute for spinach beet as it has similar nutritional and medicinal value. In Ayurveda, basella is called Upodika, Potaki, Malvaa, Amritvallari, and in Siddha/Tamil as Vaslakkirai (Khare, 2004). In Andhra Pradesh, the curry of *Basella* and Yam is popularly known as Kanda Bachali Koora. Its leaves, stems, and flowers are edible and offer a rich source of protein, fibre, carotenoid, organic acid, vitamin A and C, iron, calcium, and antioxidants (Singh et al., 2018). Additionally, the oil obtained from its seeds can be used as a source of safe vegetable oil. Basella is a valuable crop for small-scale farmers and home gardeners due to its ease of cultivation, rapid growth, and high yields (Pragya et al., 2018). It can be grown in a variety of environments and can tolerate drought, heat, and poor soil conditions, making it

an ideal crop for sustainable agriculture and food security. Additionally, basella has been used in traditional medicine for its anti-inflammatory, antioxidant, and antimicrobial properties.

Brassica juncea var. *rugosa*, commonly known as mustard green or vegetable mustard or leafy mustard or laipatta, belongs to the Cruciferae/ Brassicaceae family and is native to central and Eastern Asia (Singh et al., 2014; Pant et al., 2020). Its lush green foliage ranges in color from light green to deep purple and is widely consumed as a green leafy vegetable globally, with Asian countries like India, China, and Japan being top growers and suppliers (Pant et al., 2020). In India, it is cultivated in small patches in home backyards, cultivated land, and hilly regions of North India (Pant et al., 2020). The peppery, crispy leaves have high moisture content and a thick, tender stem, making them ideal for saag preparation (Rauniyar & Bhattarai, 2017). The green leaves can be eaten raw in salads or cooked and are rich in protein, fiber, vitamins (A, B, C, and E) and minerals (iron, calcium) (Macready et al., 2014). Regular consumption of leafy mustard can help protect against iron deficiency, osteoporosis, and cardiovascular diseases, and may also combat arthritis, asthma, and nervous system disorders (Macready et al., 2014). In addition, it has been used as a diuretic, stimulant, and expectorant to treat various diseases. The seeds of this leafy mustard are very fine in nature and are small, round, and dark brown in color (Pant et al., 2020). The seeds contain high oil content, ranging from 30 to 40%, and are used as a spice and have a pungent flavor, similar to mustard seeds (Rauniyar & Bhattarai, 2017).

Among various seed quality attributes, the germination of seeds is paramount important as it determines the field establishment, including the initial stage of seedling development, followed by subsequent growth, development, and productivity of the crop, which depends on various factors such as temperature, light exposure, water availability, and sowing depth (Nayak et al., 2020). Under the Indian seed legislation system, it is mandatory to label seed bags or containers and specify seed germination and other seed quality attributes (Seed Act, 1966). Labelling of these seed quality parameters provides the quality assurance to the end users (farmers) of the seed. These labelling prescriptions are based on testing protocols developed in a laboratory and subsequently tested for their proficiency. The information on seed testing protocols, including germination requirements, seed dormancy and its management and other related seed quality parameters for these leafy vegetables is very meager (Parihar et al., 2005). The seed testing protocols for these duo leafy vegetables are neither available in the International Seed Testing Association (ISTA) rules, AOSA rules, or with IBPGR compendium. In the absence of prescribed seed testing protocols, practical difficulties are faced in its seed quality evaluation and/or by its end users during raising a good crop. The presence of seed dormancy is more common in wild species and land races than in cultivated species (Gao and Yamata, 1991). However presence of seed dormancy in cultivated species for a short period is beneficial in arresting seed germination within a fruit. Whereas, a long period of seed dormancy poses a problem in cultivating a species, when immediate sowing and/or seed testing is required. Since some seed enhancement treatments are reported to be effective in optimizing germination in seeds of various plant species (Butola and Butola, 2004), the application of seed enhancement treatments (both physical and chemical) was also investigated in both leafy vegetables.

Considering the above facts, the present study was conducted (1) standardization of seed germination testing method for basella and mustard green (2) to evaluate the presence of seed dormancy and its management through seed enhancement treatments. Elucidating the optimal germination conditions and understanding the physiological mechanisms underlying seed germination and dormancy in both leafy vegetables will help in seed quality assurance and inform strategies to enhance crop yields, improve agricultural sustainability, and promote food security.

Materials and Methods

Experimental site and seed material

The seeds of both the leafy vegetables (Basella: VRB-74, VRB-11, VRB-8, VRB-10, VRB-38; and mustard green: VRLP 35-2, VRLP 7, VRLP 5, VRLP 34-1-2) were procured from ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, India and the study was conducted at the seed technology laboratory of ICAR-IIVR, Varanasi. To nullify the environment of seed production, two seed lots of each genotype, consisting of fresh and one-year old seed, were used in the study.

Standardizing the method for seed germination testing

A germination study was conducted on basella and mustard green seeds using three standard germination media/substrata, including Roll towel media or between paper (BP), Top of paper (TP) and sterilized sand (S). For determining the effect of photoperiod, the germination of 400 seeds consisting of 100 seeds each in four replications was kept in both dark and light conditions. Additionally, the temperature requirements were determined by subjecting the seeds to three constant temperature conditions, i.e., 20, 25, and 30°C, as well as one alternating temperature (20°↔25°C) for basella and one alternating temperature (20°↔30°C) for mustard green. 16 h light and 8 h dark for determining the germination substratum, photoperiod and temperature requirements, the germination test was conducted in accordance with the rules outlined by the International Seed Testing Association (ISTA), with minor

modifications. A total of 400 seeds in four replications, each comprising 100 seeds, were placed on moist germination substrate and then incubated under various temperature regimes as specified in the treatment details. The seeds were placed in a controlled germination chamber (single door germinator) under fluorescent lighting with a relative humidity of 90 ± 2%. It was meticulously observed that the specified temperature regimes and photoperiod should be consistently maintained throughout the duration of the experiment. After the designated test period, the number of normal seedlings that contained normal shoots and roots was counted, and the germination percentage was calculated using the following formula.

Germination (%) = (Number of normal seeds germinated/ Total number of seeds sown) × 100

Simultaneously, the number of seeds germinated per day was calculated on a daily basis by counting the germinated seeds. Mean germination time was used to determine the first count (day) and the maximum germination as the final count (day) was recorded. Whereas the mean germination time was calculated using the following formula.

Mean Germination Time (MGT) = $\sum n \sum (n \times t)$

Where n= number of seeds germinated on day t, t = day of germination

Dormancy management treatments

Freshly ungerminated seeds (FUS) during the standard germination test were subjected to the tetrazolium (Tz) test to determine the viability and confirm the presence of seed dormancy. Mustard greens did not show any presence of dormancy, whereas basella seeds possess dormancy. Therefore, FUS of basella were soaked in 1.0% 2,3,5-triphenyl tetrazolium chloride (Tz) solution for 18 hours at 25°C for colour development. Based on the red color developed due to formazan, the viability of the seed was confirmed. Further, the seeds of 5 genotypes containing both fresh and one-year old seed of basella were subjected to the following various physical and chemical dormancy-breaking treatments (Table 1).

For hydro-priming, seeds were primed in double the volume of water. Pre-washing of the seeds was done by placing the seeds in a muslin cloth bag and exposing them to running tap water for 24 hours under laboratory ambient temperature. For chemical treatments, the seeds were soaked in KNO_3, GA_3 and thiourea solutions for durations of 12 hours, followed by drying at room temperature. For heat treatments, seeds were exposed to temperatures of 60 and 70°C for periods ranging from 5 to 10 minutes in a hot air oven. After the application of these seed treatments, a standard germination test was conducted following the guidelines outlined by ISTA. Observations were recorded for germination percentage using the normal seedlings.

Statistical analysis

The data recorded were subjected to a factorial completely randomized design analysis. Significance was determined at $p < 0.05$. The data taken as percent based on count value were transformed to the respective angular (arcsine values) before subjecting them to statistical analysis. The data were analyzed statistically using SPSS software (version 16.0).

Results

Standardization of germination substratum and photoperiod

Irrespective of genotypes of basella and mustard green, significant differences were observed in germination *wrt* different substrate treatments. Basella seed lots registered the maximum germination on the between paper method (BP) among most of the genotypes, with a mean germination of 48.40%, followed by 43.35% germination with the sand (S) method. Least germination (38.75%) was observed in seeds germinated on top of the paper (TP) (Fig 1). Whereas mustard green seed lots registered the maximum germination percentages on top of paper (TP) among most of the genotypes, with a mean germination value of (97.50%), followed by the sand method (75.81%). Least germination (66.68%) was observed with between paper (Fig 1b). Non-significant differences were observed in germination of genotypes under dark and light conditions at 25°C in both basella and mustard green (Fig 1).

Standardization of temperature requirements

The analysis of germination across both crops under different temperature conditions reveals significant differences in germination percentages. For basella genotypes, the highest germination was observed at alternating temperatures (20°↔25°C), i.e., 25°C for 16 hours and 20°C for 8 hours, with a mean germination of 56.3%. Which was followed by 25°C (52.95%) and 20°C (49.9%), and the lowest germination was at 30°C (46.0%) (Fig. 2). This indicates that alternating temperatures promote better germination performance in basella. Besides, examination of speed of germination showed that alternate temperatures (20↔25°C) led to the fastest germination (10.26), followed by 25°C (9.42) and 20°C (8.91), and the slowest at 30°C (8.07) (Fig. 2). The result of mean germination time of basella genotypes align with the speed of germination, showing that germination occurs more quickly at alternate temperatures, i.e., 20 to 25°C (8.54) followed by 25°C (8.89) and slower at 30°C (9.08) (Fig. 2); whereas mustard green genotypes showed the highest germination at 20°C (97.68%). Second-highest germination was observed with alternate temperatures (20°↔30°C), i.e., 20°C for 16 hours and 30°C for 8 hours, with mean germination of 97.56 and 97.50% at 25°C. Lowest germination was observed at 30°C (97.56%) (Fig. 3). However, no significant differences in speed of germination were

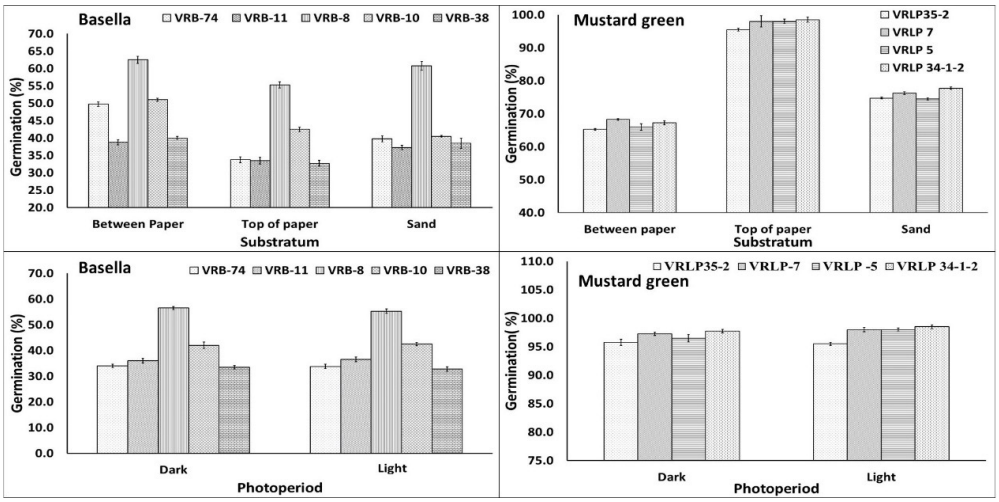

Figure 1: Germination percentage of basella and mustard green genotypes in various substratum and photoperiod

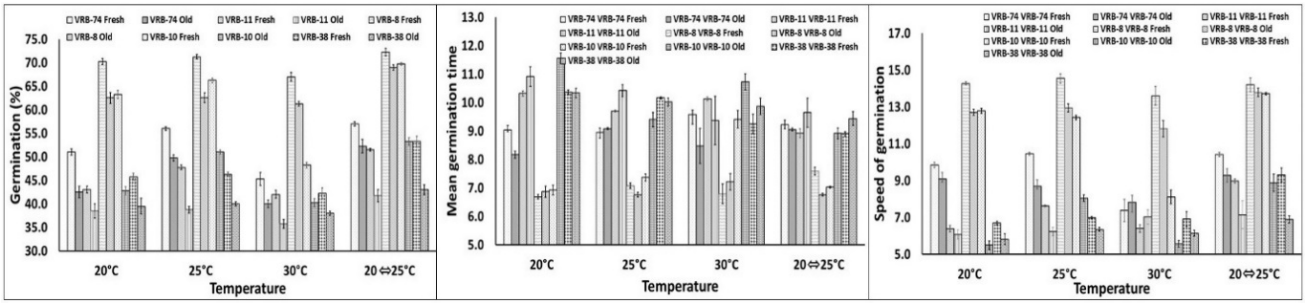

Figure 2: Germination %, mean germination time and speed of germination in basella genotypes under different temperature regimes

observed in the mustard green, where speeds were similar across all temperature's regimes (Fig. 3). For mustard green, the mean germination time across all temperatures indicate that mean germination time occurs more quickly at alternate temperatures 20↔30°C (4.85) followed by 20°C (4.92) and slower at 30°C (5.28), with minimal variation between the temperature conditions (Fig. 3).

Determination of first and final count (days)

Significant differences were observed in the number of days required for the first and final counts in achieving seed germination for both leafy vegetables. In basella, seed germination began on the 3rd day in all five genotypes (10 seed lots), with germination percentages steadily increasing from the 3rd to the 21st day. The maximum seed germination was achieved by the 20th day, which was on par with the 21st day. Based on the mean germination time at various temperatures, the 9th day was most suitable for the first count, and the 21st day was optimal for the final count (Fig 4); whereas, in mustard green, seed germination started earlier, i.e., on the 1st day after planting in all four genotypes. Germination percentages increased from the 6th and were at par with the 7th day, with maximum germination achieved on the 7th day. According to the mean germination time, the 5th day was found ideal for the first count, whereas the 7th day was suitable for the final count (Fig 4).

Identification of suitable dormancy management treatment

In mustard green, no dormancy was observed, as 97.50% mean germination was recorded in mustard green genotypes. Whereas some basella seed lots exhibited seed dormancy, therefore, treatments were applied to release the dormancy. In control with no treatment, 54.2% mean seed germination was observed. Maximum seed germination (68.1%) was registered in the treatment containing 1.0% KNO_3 for 12 hours, which is regarded as the best treatment with the highest mean germination percentage across all genotypes and seed lots (Table 2). On the other hand, the least effective treatment was heat treatment at 70°C for 10 minutes, which resulted in the lowest mean germination percentage (54.3%).

Discussion

Under the Indian seed legislation system, it is mandatory to label seed bags or containers and specify seed germination

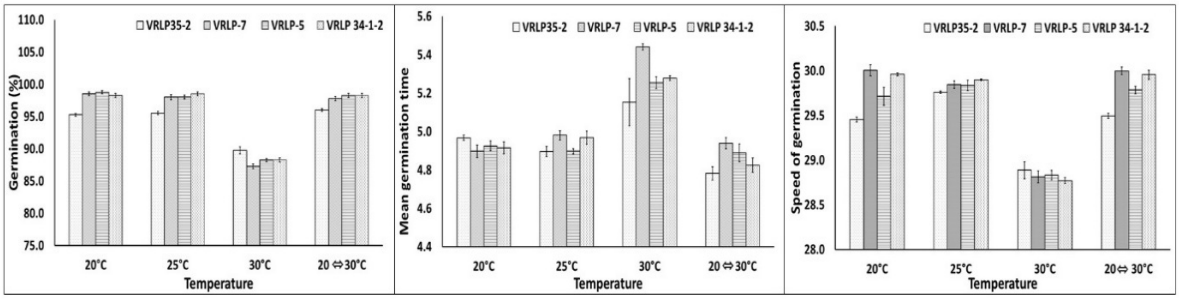

Figure 3: Germination %, mean germination time and speed of germination in mustard green genotypes under different temperature regimes

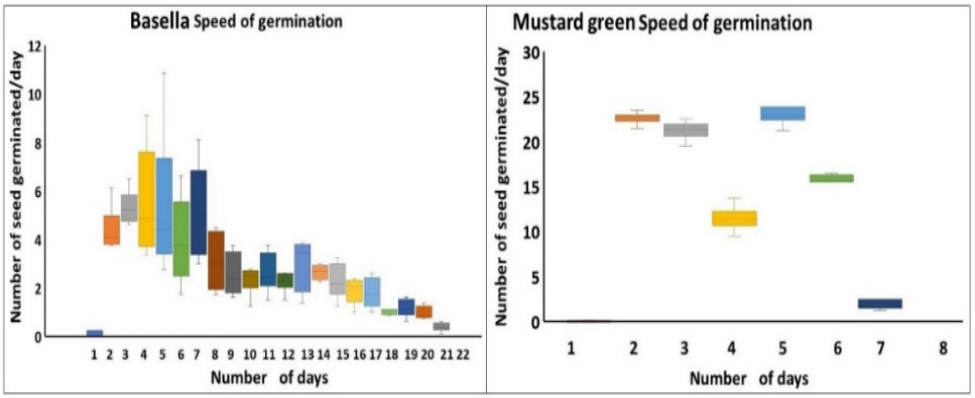

Figure 4: Number of seeds germinated per day in basella and mustard green

and other seed quality attributes (Seed Act 1966). These prescriptions are based on testing protocols developed in a nationally reputed laboratory and subsequently tested for their proficiency. Information on seed germination testing for basella and mustard green is either fragmented or not available in the Indian Minimum Seed Certification Standards (IMSCS) or the International Seed Testing Association (ISTA). These seed germination testing protocols are very useful and are used to determine the maximum germination potential of a given seed lot, which will be used to compare the quality of different seed lots and to estimate the field planting value (Anon 2008). Unlabeled seed does not come under the purview of the seed certification system and is considered spurious in nature, which leads to poor crop stand. The present study determines the standard seed testing methodology for *in-vitro* testing of seeds of basella and mustard green. Simultaneously, also provides recommendations to release the seed dormancy through seed treatments, which are required to break the dormancy prior to germination testing. Seeds of mustard green genotypes do not possess any seed dormancy, whereas basella genotypes exhibit some extent of seed dormancy. Normally, factors to be considered during development of a seed germination protocol are substratum, temperature, light, and methods for management the seed dormancy.

A suitable substrate for seed germination is very crucial and the selection of substrate depends on seed size, light requirements, etc. The ideal substratum for seed germination should be sufficiently firm and dense, retentive of moisture and sufficiently porous. In the present study, between paper method, where the seed is rolled in germination paper and placed vertically in the germinator, was found superior for basella genotypes over other treatments, whereas for mustard green top of the paper was superior, possibly due to the smaller round size of seeds, where seeds were placed on the germination paper in a petri-plate. Higher germination on BP was in conformity with Gupta et al. (2019), who reported that the BP was the best medium for the germination test for *moringa olifera*. Whereas, higher germination on TP was in conformity with Cesevensyes and Baleanu (1987), who reported that the best medium for germination test for *Atropa belladonna, Hyperium pciforatum, Solanum lacinatum, S. sclerea* and *Majorana hortensis* was the TP method. In both the leafy vegetables, photoperiod and the presence of light do not show any significant difference. These results imply that the seeds of both the vegetables are not dependent on light for germination, hence non-photoblastic in nature.

Temperature is one of the most necessary factors for all stages of seed germination, as it affects the cell energy status, protein synthesis, and activity of certain enzymes such as lipase, alanine aminotransferase, aspartate aminotransferase, and especially ribonuclease (Prerna et al., 2021). The optimum temperature results in germination

Table 1: List of various pre-sowing seed treatments used to break seed dormancy in basella.

S. N.	*Treatment*
1.	Control
2.	Hydro-priming (in double volume of water) for 12 hrs
3.	Pre-washing of seeds in running tap water for 12 hrs
4.	Heat treatment @ 60°C for 5 min
5.	Heat treatment @ 60°C for 10 min
6.	Heat treatment @ 70°C for 5 min
7.	Heat treatment @ 70°C for 10 min
8.	KNO_3 @ 0.5% for 12 hrs
9.	KNO_3 @ 1 % for 12 hrs
10.	KNO_3 @ 1.5% for 12 hrs
11.	GA_3 @ 250 ppm for 12 hrs
12.	GA_3 @ 500 ppm for 12 hrs
13.	Thiourea @ 1.0% for 12 hrs
14.	Thiourea @ 0.5% for 12 hrs

in the shortest time possible by enhancing the speed of germination (Ostadian Bidgoly et al., 2018). Temperature stress increases the transcription, translation, and activity of ROS-scavenging enzymes in maize and results in the buildup of H_2O_2 (Gong et al., 2001). In some crop plants, fluctuating temperature on a daily or seasonal basis is required for seed germination. Better germination at alternating temperatures may be attributed to natural diurnal temperature cycles that characterize the season, climate, and microclimate. Alternating temperatures have been reported to increase the rate of germination in spinach (Leskovar et al., 1999).

Since germination involves several stages, each with its own cardinal temperature scale, the temperature response can vary during the germination period due to its complexity (Prerna et al., 2021). The temperature response of a seed depends on variety, seed quality, the length of time after harvest, and other factors (Díaz-Granados et al., 2020). During germination, changes in temperature can significantly impact respiration and sugar metabolism, and any disruption in the homeostasis of reactive oxygen species (ROS) needed for seed germination can lead to abnormal respiration (Bailly, 2019). Temperature thus plays a critical role in determining the duration of seed germination, or the time from hydration to the onset of germination (Ostadian Bidgoly et al., 2018). In the present study, the speed of germination in basella significantly differs, indicating that moderate fluctuations in temperature are more conducive to rapid germination. Both crops exhibited reduced germination at higher temperatures, suggesting a sensitivity to heat, which may affect seed viability. Mustard green was exhibiting similar substratum, temperature and germination period as *Brassica juncea* (ISTA, 2022). Basella seeds exhibited slight dormancy, and potassium nitrate (KNO_3) treatment @ 1.0% for 12 hours was found to be an effective means of overcoming dormancy with improved germination rate (68.1%). These results are consistent with Tapfumaneyi et al. (2023), where KNO_3 helped in `the management of dormancy in various crop species. Possibly, KNO_3 acts as an oxidative substrate and activates the key metabolic pathways, such as the pentose phosphate pathway, which

Table 2: Effect of various dormancy release treatments on seed germination in basella. Values in the table are mean of two seed lot (fresh and one-year old) using three replications ± standard error (SE).

Treatment	*Germination%*					
	VRB-74	*VRB-11*	*VRB-8*	*VRB-10*	*VRB-38*	*MEAN*
Control	52.7 ± 0.88	46.3 ± 1.86	64.7 ± 1.20	59.3 ± 0.88	48.1 ± 1.53	54.2 ± 1.27
Hydro-priming for 12 hrs	57.7 ± 0.88	52.7 ± 0.88	72.0 ± 0.58	70.0 ± 0.58	55.0 ± 0.58	61.5 ± 0.70
Pre-washing for 12 hrs	58.0 ± 1.16	52.3 ± 1.86	72.3 ± 1.20	68.3 ± 0.88	54.7 ± 0.67	61.1 ± 1.15
Heat treatment @ 60°C for 5 min	61.0 ± 1.16	56.7 ± 1.20	72.3 ± 0.88	71.0 ± 1.53	57.7 ± 0.88	63.7 ± 1.13
Heat treatment @ 60°C for 10 min	58.3 ± 0.33	54.0 ± 1.16	71.3 ± 0.88	71.0 ± 0.58	54.3 ± 0.88	61.8 ± 0.77
Heat treatment @ 70°C for 5 min	56.0 ± 0.58	50.3 ± 2.85	70.0 ± 1.16	66.3 ± 0.33	46.0 ± 0.58	57.7 ± 1.10
Heat treatment @ 70°C for 10 min	51.3 ± 1.33	43.3 ± 0.33	69.0 ± 2.08	63.7 ± 0.88	44.3 ± 0.33	54.3 ± 0.99
KNO3 @ 0.5% for 12 hrs	60.7 ± 0.33	58.0 ± 0.58	73.0 ± 1.16	70.0 ± 1.16	58.0 ± 0.58	63.9 ± 0.76
KNO3 @ 1% for 12 hrs	64.0 ± 1.00	62.7 ± 1.20	74.3 ± 0.88	73.3 ± 0.33	66.3 ± 1.20	68.1 ± 0.92
KNO3 @ 1.5% for 12 hrs	57.0 ± 0.58	51.3 ± 0.33	71.7 ± 0.67	69.7 ± 0.33	54.3 ± 0.67	60.8 ± 0.52
GA3 @ 250 ppm for 12 hrs	57.3 ± 0.33	53.0 ± 1.16	71.3 ± 0.88	70.3 ± 1.20	52.7 ± 0.88	60.9 ± 0.89
GA3 @ 500 ppm for 12 hrs	56.3 ± 0.33	51.0 ± 2.52	69.7 ± 0.88	66.0 ± 0.58	47.3 ± 1.45	58.1 ± 1.15
Thiourea @ 1% for 12 hrs	60.3 ± 0.33	58.3 ± 0.33	73.7 ± 0.88	70.7 ± 0.88	60.0 ± 0.58	64.6 ± 0.60
Thiourea @ 0.5% for 12 hrs	59.7 ± 1.45	54.0 ± 0.58	71.0 ± 0.58	69.7 ± 0.88	55.7 ± 0.88	62.0 ± 0.87

facilitates energy production required for germination (Tapfumaneyi et al. 2023). Furthermore, KNO_3 may promote seed germination by modulating the abscisic acid (ABA) signaling pathway, a major regulator of seed dormancy (Chahtane et al., 2017). The results establish a comprehensive protocol for seed germination testing in basella and mustard green, including the selection of appropriate substrates, temperature management, and dormancy-breaking treatments. The successful application of KNO_3 to overcome seed dormancy in basella seeds is a valuable contribution to seed certification standards, ensuring higher germination rates and better seed quality. These findings can be used to improve seed testing protocols, leading to better quality planting material and, ultimately, more successful crop production.

Conclusion

The seed testing protocols are generally used to determine the maximum germination potential of a given seed lot, which will be used to compare the quality of different seed lots and to estimate the field planting value. In the present study, basella seed germination was found to be temperature dependent. Seed germinated best on between paper (BP) at 20°↔25°Cand 9th day and 21st day were found best suitable for first and final count, respectively. It could be inferred that seeds of basella possess dormancy, due to certain inhibitory chemical substances in the seed coat which act as inhibitors for seed germination. Among the various physical and/or chemical treatments applied, presoaking of seed in KNO_3 1% for 12 hrs was best in releasing seed dormancy; whereas, mustard green seed germination was found statistically similar at 20°C and at 20°↔30°C alternate temperature. The fifth day and the seventh day were found best suitable for the first count and the final count, respectively. Mustard green seeds do not possess any level of dormancy.

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References

Abdul-Baki, A. A., & Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria. Crop Science, 13(6), 630-633.

Baskin, C. C., & Baskin, J. M. (1998). *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. Academic Press.

Butola, J. S., & Badola, H. K. (2004). Seed germination improvement using chemicals in *Heracleum candicans* Wall, a threatened medicinal herb of Himalaya. Indian Forester, 130(5), 565-572.

Cesevensyes, Z., & Baleanu, M. (1987). Evaluation methods for germinating coriander (*Coriandrum sativum* L.) and horned poppy (*Glaucicum flavum* Grantz). Seed Abstracts, 10(1), 34-99.

Chahtane, H., Kim, W., & Lopez-Molina, L. (2017). Primary seed dormancy: A temporally multilayered riddle waiting to be unlocked. Journal of Experimental Botany, 68(4), 857-869.

Deng, B., Yang, K., Zhang, Y., & Li, Z. (2016). Can heavy metal pollution defend seed germination against heat stress? Effect of heavy metals (Cu^{2+}, Cd^{2+} and Hg^{2+}) on maize seed germination under high temperature. Environmental Pollution, 216, 46-52.

Díaz-Granados, V. H., López-López, J. M., Flores-Sánchez, J., Olguin-Alor, R., Bedoya-López, A., Dinkova, T. D., Salazar-Díaz, K., Vázquez-Santana, S., Vázquez-Ramos, J. M., & Lara-Núñez, A. (2020). Glucose modulates proliferation in root apical meristems via TOR in maize during germination. Plant Physiology and Biochemistry, 155, 126-135.

Ellis, R. H., & Roberts, E. H. (1981). The quantification of ageing and survival in orthodox seeds. Seed Science & Technology, 9(2), 373-409.

Farooq, M., Hussain, M., Wakeel, A., & Siddique, K. H. M. (2015). Salt stress in maize: Effects, resistance mechanisms, and management. A review. Agronomy for Sustainable Development, 35(2), 461-481.

Gao, W. H., & Yamata, H. (1991). Germinability of eggplant seeds with different dormancy breaking methods. Plant Physiology, 27, 190-192.

International Seed Testing Association. (2022). *International rules for seed testing*. ISTA.

Khalid, N., Tarnawa, Á., Kende, Z., Kassai, K. M., & Jolánkai, M. (2021). Viability of maize (*Zea mays* L.) seeds influenced by water, temperature, and salinity stress. Acta Hydrologica Slovaca, 22(2), 113-117.

Khare, C. P. (2004). *Encyclopedia of Indian medicinal plants*. Springer Verlag.

Leskovar, D. I., Esensee, V., & Belefant-Miller, H. (1999). Pericarp, leachate, and carbohydrate involvement in thermo-inhibition of germinating spinach seeds. Journal of the American Society for Horticultural Science, 124(3), 301-306.

Macready, A. L., George, T. W., Chong, M. F., Alimbetov, D. S., Jin, Y., Vidal, A., Spencer, J. P., Kennedy, O. B., Tuohy, K. M., Minihane, A. M., Gordon, M. H., Lovegrove, J. A., & FLAVURS Study Group. (2014). Flavonoid-rich fruit and vegetables improve microvascular reactivity and inflammatory status in men at risk of cardiovascular disease—FLAVURS: A randomized controlled trial. The American Journal of Clinical Nutrition, 99(3), 479-489.

Nakul Gupta, Manimuragan, C., Singh, P. M., & Rajesh Kumar. (2019). Standardization of seed germination testing protocol in *Moringa oleifera* Lam. Vegetable Science, 46(1-2), 148-151.

Nayak, P., Panda, S., & Das, S. (2020). Effect of temperature, light and water on seed germination of *Basella alba*. Journal of Applied and Natural Science, 12(2), 434-438.

Ostadian Bidgoly, R., Balouchi, H., Soltani, E., & Moradi, A. (2018). Effect of temperature and water potential on *Carthamus tinctorius* L. seed germination: Quantification of the cardinal temperatures and modeling using hydrothermal time. Industrial Crops and Products, 113, 121-127.

Pant, U., Bhajan, R., Singh, A., Kulshesthra, K., Singh, A. K., & Punetha, H. (2020). Green leafy mustard: A healthy alternative. Electronic Journal of Plant Breeding, 11(1), 267-270.

Parihar, S. S., Kumar, R., & Jain, S. K. (2005). A need for the formulation of seed testing protocols in medicinal and aromatic plants. National Seminar on Medicinal and Aromatic Plants—Biodiversity, Conservation, Cultivation and

Processing, IGAU, Raipur, February 26-27, Abstracts, 180-181.
Pragya, Singh, B., Singh, P. M., Ranjan, J. K., Sagar, V., Bhardwaj, R., & Pandey, C. D. (2018). Flower and fruit colour variability in Indian spinach (*Basella alba*). Vegetable Science, 45(1), 116-117.
Prerna, D. I., Govindaraju, K., Tamilselvan, S., Kannan, M., Vasantharaja, R., Chaturvedi, S., & Shkolnik, D. (2021). Influence of nanoscale micro-nutrient Fe_2O_3 on seed germination, seedling growth, translocation, physiological effects and yield of rice (*Oryza sativa*) and maize (*Zea mays*). Plant Physiology and Biochemistry, 162, 564-580.
Rauniyar, R., & Bhattarai, S. (2017). Nutritional and medicinal properties of leafy mustard (*Brassica juncea* var. *rugosa*). Journal of Food Science and Technology, 54(4), 1056-1063.
Riley, G. J. P. (1981). Effects of high temperature on the germination of maize (*Zea mays* L.). Planta, 151(1), 68-74.
Seed Act. (1966). Retrieved from https://seednet.gov.in/PDFFILES/Seed_Act_1966.pdf
Singh, A., Dubey, P. K., & Chaurasiya, R. (2018). Indian spinach: An underutilized perennial leafy vegetable for nutritional security in developing world. Energy, Ecology and Environment, 3(4), 195-205.
Singh, B.K., Verma, V.K. & Ramakrishna, Y. (2014). Leaf vegetables of Mizoram, pp 383-404. In: Peter, K.V. (ed.) Climate Resilient Crops for the Future. NIPA, New Delhi, ISBN 9789383305599.
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*. Agrosystems, Geosciences & Environment, 6(1), e20359.

सारांश

उपेक्षित पत्तेदार सब्जियाँ जैसे पोई (बेसेलाएल्बा) और सरसों का साग (ब्रासिका जुनसिया वर. रुगोज़ा) पोषण के अच्छे स्रोत के रूप में तेजी से पहचान प्राप्त कर रही हैं। हालांकि, इनकी बीज परीक्षण प्रक्रियाओं और बीज की प्रसुप्तावस्था (डॉर्मेंसी) के प्रबंधन पर जानकारी बहुत सीमित है। अंतर्राष्ट्रीय बीज परीक्षण संघ (ISTA), अधिकारीकृत बीज विश्लेषक संघ (AOSA) और अंतर्राष्ट्रीय पादप आनुवंशिक संसाधन संस्थान (IBPGR) के नियमों में इन बीजों के परीक्षण के लिए कोई निर्धारित प्रोटोकॉल उपलब्ध नहीं है। इस कारण से बीज की गुणवत्ता का सही मूल्यांकन और किसानों द्वारा बेहतर फसल उत्पादन में कठिनाइयाँ उत्पन्न हो रही हैं। बीज की प्रसुप्तावस्था कुछ हद तक अंकुरण को नियंत्रित करने में सहायक होती है, लेकिन अधिक समय तक निष्क्रियता बुवाई में देरी और बीज के मूल्यांकन में बाधा उत्पन्न करती है। इस समस्या को हल करने के लिए, वर्ष 2023 और 2024 में भाकृअनुप - भारतीय सब्जी अनुसंधान संस्थान, वाराणसी में पोई के 5 जीनोटाइप्स और सरसों के साग के 4 जीनोटाइप्स पर बीज परीक्षण प्रोटोकॉल को मानकीकृत करने के लिए अध्ययन किया गया। अध्ययन में पाया गया कि पोई के बीजों के लिए 20°↔25°C तापमान पर "Between Paper" विधि सबसे उपयुक्त रही, जिसमें 9वें दिन फर्स्ट काउंट और 21वें दिन फाइनल काउंट लिया गया। चूंकि इस शोध में पोई के बीजों में शुषुप्त अवस्था पाई गई, इसके सुधार हेतु किए गए विभिन्न बीज उपचारों में से 1.0% पोटैशियम नाइट्रेट (KNO_3) में 12 घंटे तक भिगोना सबसे उपयुक्त पाया गया, जिससे अंकुरण में सुधार हुआ। वहीं, सरसों के साग के लिए उपयुक्त विधि Top of the Paper पाई गई, जिसमें 20°C, 20°↔30°C,तापमानों पर अंकुरण प्रभावी रहा। पाँचवें दिन फर्स्ट काउंट और सातवें दिन फाइनल काउंट को उपयुक्त पाया गया। सरसों के साग के बीजों में किसी प्रकार की शुषुप्त अवस्था नहीं पाई गई।