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Optimization of Seed Priming Techniques for Seed Enhancement in Sunflower (*Helianthus annus* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Seed invigoration treatments are crucial for enhancing germination and seedling growth, particularly under adverse environmental conditions. Various techniques, such as hydropriming, seed hardening, osmopriming, and pre-sowing dry heat treatments have been proved to improve seed quality. Sunflower is an important oilseed crop, however, its area is now confined to specific niches and therefore any efforts to increase its production and bring back its glory are highly welcome. Sunflower crop is highly susceptible to environmental stresses further limiting its cultivation. Hence identification and standardization of suitable techniques to improve the germination capacity and

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planting value of sunflower especially under stress conditions is the need of the hour. The present study is taken up in aged and fresh seeds of two sunflower hybrids *viz*., DRSH-1 and GK-2002 to standardize hydropriming, thermo priming and pre-chilling treatments so as to the enhance planting value when sown under sub optimal conditions. It was found that hydropriming for 16 hrs, thermopriming for 6hrs at 35^{o}C and pre chilling for 7 days at 10^{o}C showed highest improvement in the seed quality parameters like germination percentage, seedling vigour indices I and II, field emergence and speed of germination. These findings offer promising avenues for improving seed quality and viability in sunflower.

Keywords: Sunflower; priming; enhancement.

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops next to soybean, groundnut, rapeseed and mustard and is the largest source of vegetable oil across the world with 25 to 35 per cent oil of superior quality and low cholesterol content. The crop became popular in India due to its wide adaptability and high yield potential. Sunflower is a highly cross-pollinating crop and therefore requires utmost care during quality seed production. Non-congenial environmental conditions during early stages of crop growth show a major impact on crop yield. Further being an oilseed, it is very sensitive to the harsh environmental conditions and it is hypothesized that oxidation of oil readily occurs under storage leading to its deterioration [1]. Hence it is important to enhance its vigour and germination through appropriate seed invigoration treatments to enable better performance under sub optimal conditions like drought, heat and cold stresses.

Seed invigoration or seed enhancement is a post-harvest treatment given to seed for improving germination and seedling growth or to facilitate the delivery of seeds and other materials required at the time of sowing [2]. To boost seedling establishment under natural and stressful conditions, many seed invigoration treatments like hydropriming, seed hardening, on-farm priming, osmopriming, osmohardening, humidification, matrix priming, priming with plant growth regulators, polyamines, ascorbates, salicylicates, ethanol, osmolytes, coating technologies *etc*. are used in a variety of field crops. Recently pre sowing dry heat treatments are also being used to invigorate seed [3]. Seed priming has the potential to improve not only viability and germination of normal seeds but also has ability to rejuvenate partially aged seeds and boost their germination. Scientifically, priming is a controlled hydration and dehydration process or exposing seed to high or low temperatures to create primary memory. Priming improves uniformity, rate of germination of seed lots and is found beneficial in many field crops. The enhanced performance of primed seed is due to higher protein synthesis, repair of membranes and nucleic acids, higher activity of antioxidant system *etc*. Seed priming is influenced by many factors such as water potentiality of priming agent, duration of soaking, temperature, seed vigour and seed storage conditions [4]. In view of the vast scope of sunflower crop and its sensitivity to moisture and temperature stresses, it is important to standardize appropriate seed invigoration techniques suitable for various growth conditions.

2. MATERIALS AND METHODS

The present study was taken up in two sunflower hybrids DRSH-1 developed from Indian Institute of Oilseeds Research, Hyderabad and GK-2002 from Ganga Kaveri seeds. Due to non availability of aged seed, technique of accelerated ageing was adopted to generate proxy aged seed in both the hybrids. For this, seed was exposed to a temperature of 40°C for 48 hrs at 90% relative humidity. The accelerated aged seeds were subjected to 21 different priming treatments (Table 1) and replicated twice. The primed seed along with control was studied for various seed quality parameters as follows:

2.1 Germination (%)

The germination test was conducted as per ISTA, 2019 [5] through rolled paper towel method. Two replications of fifty seed each from each treatment were uniformly placed and incubated at a constant temperature of 25 ± 0.5°C and 95% relative humidity and percent normal seedlings were recorded on 10th day.

2.2 Seedling Length (cm)

Ten normal seedlings in each replication of a treatment were randomly selected from

germination test for measuring the seedling length on 10th day of germination. The mean was computed and expressed in centimeters.

2.3 Seedling Dry Weight (mg/10 Seedlings)

Ten normal seedlings from each replication selected for seedling length were placed in a butter paper bag and oven dried at 100°C for 24 hours. The dried seedlings were removed, cooled in a desiccator for 30 minutes and the dry weight of ten seedlings was recorded with an electronic balance and expressed in milligrams.

2.4 Seedling Vigour Indices I and II

Seedling vigour I and II were computed as per the procedure suggested by Abdul Baki and Anderson [6].

Seedling vigour index I = Germination (%) x Seedling length (cm)

Seedling vigour index II = Seedling dry weight (mg) x Germination (%)

2.5 Speed of Germination

Speed of germination test was determined in two replicates of 50 seeds placed in sand followed by recording germination counts on day to day basis for ten days. An index of the speed of germination was calculated by adding the quotients of the daily counts divided by the number of days of germination (ISTA,1999) [7].

$$\text{Speed of germination} = \frac{N_1}{T_1} + \frac{N_2}{T_2} + \frac{N_3}{T_3} \ldots \frac{N_x}{T_x}$$

Where 'N' is number of seeds germinated at days 'T'

2.6 Field Emergence (%)

The potential of the seed to emerge in the field was evaluated using the technique outlined by Shenoy et al. [8]. In this method, a random selection of fifty seeds was made from each treatment. These selected seeds were then planted on well prepared field at a depth of 2.0 to 2.5 cm and subsequently covered with soil. After ten days from the sowing date, a count of emerged seedlings in the field was recorded, and the field emergence percentage was computed using the formula provided below.

$$\text{Field emergence (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2.7 Statistical Analysis

The data was analyzed in factorial CRD using INDOSTAT package.

3. RESULTS AND DISCUSSION

3.1 Germination Percentage

Significant differences among the treatments and between the hybrids were observed along with significant interaction for germination. In the hybrid DRSH-1, T4 (Table 1) (84%) exhibited highest germination percentage among hydropriming, thermopriming and pre-chilling treatments. Accelerated aged seeds showed 63% germination rate (T22), while seeds with high vigour displayed notably higher germination rate of 85% (T23). T2, T3 and T4 were found to be on par with high vigour control T23. Similarly, in GK-2002, treatment T10 showed highest germination percentage of 76%. Treatment T10 demonstrated comparable results, placing it on par with T23. Notably, aged seed lot showed a germination rate of 59%, while seeds from fresh seed lot displayed a significantly better germination percentage of 79%. These findings showed the varying germination potential of both hybrids across different seed treatments and vigour levels.

Sunflower seeds were soaked in water for different durations of 4,8,12 and 16hrs and it was found that, the longest soaking time of 16hrs (T4), resulted in the highest seed germination rate in both DRSH-1 (84%) and GK-2002 (73%) while the unprimed seeds (T22) showed lower germination percentages of 63% and 59% respectively. Thermo priming was taken up at 30, 35, 40 ^{o}C temperatures each for different durations of 6, 12, 24, 36 and 48 hrs in both fresh and aged seeds of both hybrids. It was observed that thermo priming at 35^{0}C for 6hrs (T10) showed high germination percentage in DRSH-1 (77%) and GK-2002 (76%) followed by thermo priming at 30 ^{o} C for 6hrs (T5), (72% and 71% respectively). Among the various pre-chilling treatments given it was observed that pre-chilling at 10 ^{o}C for 7 days resulted in highest germination percentage (76% and 71% respectively) (Fig. 1).

Improvement of germination potential in primed seed may be attributed to the enhancement of

physiologically active state of pre-germinated seeds due to priming [9] as the α-amylase activity is activated by water absorption with seed priming and the metabolic potential is preserved in the seed during the dry period after seed priming. Similar results were observed in cumin [10] *Brassica napus* [11], American cotton [12] and rice [13].

Table 1. Details of different priming treatments for standardization in DRSH-1 and GK-2002

T1	Hydropriming for 4hrs	**Hydro-priming***
T2	Hydropriming for 8hrs	
T3	Hydropriming for 12hrs	
T4	Hydropriming for 16hrs	
T5	Thermo priming at 30° C for 6hrs	**Thermopriming**
T6	Thermo priming at 30° C for 12hrs	
T7	Thermo priming at 30° C for 24hrs	
T8	Thermo priming at 30° C for 36hrs	
T9	Thermo priming at 30° C for 48hrs	
T10	Thermo priming at 35° C for 6hrs	
T11	Thermo priming at 35° C for 12hrs	
T12	Thermo priming at 35° C for 24hrs	
T13	Thermo priming at 35° C for 36hrs	
T14	Thermo priming at 35° C for 48hrs	
T15	Thermo priming at 40° C for 6hrs	
T16	Thermo priming at 40° C for 12hrs	
T17	Thermo priming at 40° C for 24hrs	
T18	Thermo priming at 40° C for 36hrs	
T19	Thermo priming at 40° C for 48hrs	
T20	Pre chilling at 8° C for 7 days	**Pre-chilling**
T21	Pre chilling at 10° C for 7 days	
T22	Untreated accelerated aged seed	
T23	Fresh seeds	

** With seed to water ratio of 1:1*

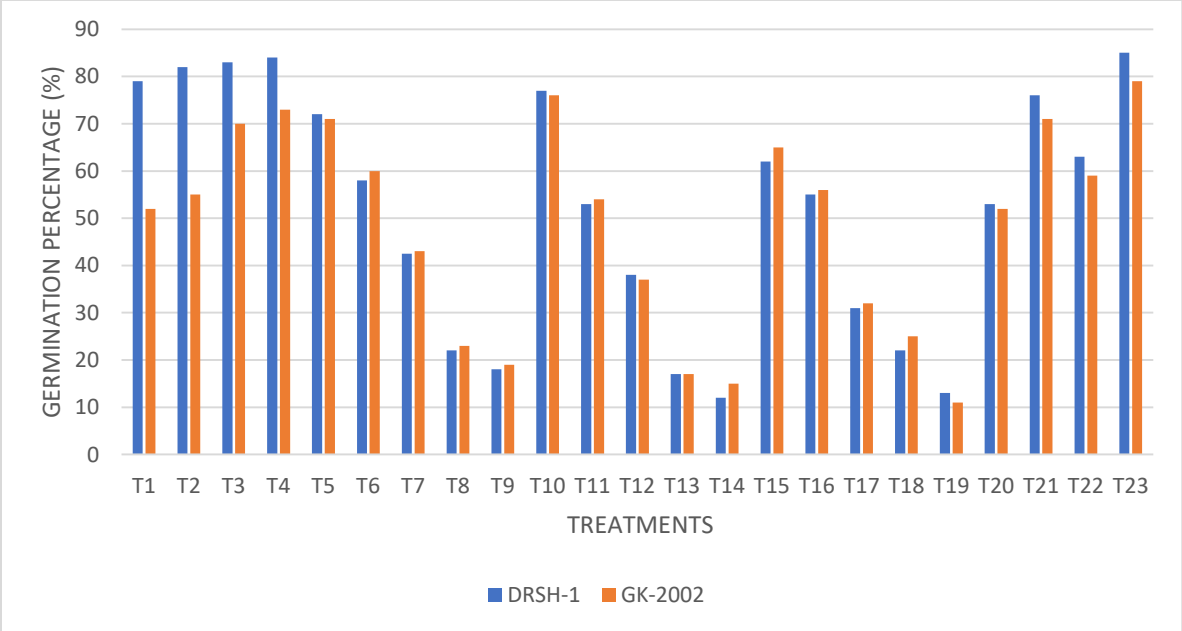

Fig. 1. Effect of priming on germination percentage in two hybrids

3.2 Vigour Indices I and II

There is a significant difference among the treatments, between the hybrids and interaction for seedling vigour index I. For seedling vigour index II, no significant difference was found between two hybrids, however a significant variation was observed among treatment and interaction of hybrids and treatments. Among all the treatments, highest VI-I was observed in T4 in DRSH-1 (2606) and GK-2002 (2707). However high vigour seed lots had better values in both hybrids (2653 and 2635 respectively) while aged seed lot was inferior in both the hybrids (1899 and 1195.25). Treatments T3 and T4 were on par with T23. In DRSH-1, it was observed that treatment T4 exhibited highest seedling vigour index II (29887). Conversely, the low-vigor seed lot demonstrated a notably lower seedling vigour index II score of 19882, while the unaged (fresh seed) high-vigor seed lot showed a substantial higher seedling vigour index II value of 29998. Treatment T21 and T4 displayed comparable results (on par), similar to those of the unaged high-vigor seed (T23). In the case of the GK-2002 hybrid, T4 (28116) treatment showed highest value of seedling vigour index II among all the priming treatments, however none of them matched with control (T23). The accelerated aged seed recorded a seedling vigour index II of 22001, whereas the control seed recorded a value of 31166.

Highest vigour index (I and II) among differently hydro primed seeds was obtained for 16hrs soaking time (T4) in both the hybrids. In thermopriming, the maximum vigour index (I and II) was obtained when thermoprimed at 35^{o}C for 6hrs (T10) in both hybrids. Similarly, pre chilling at 10 ^{o} C (T21) resulted in highest vigour indices in both the hybrids (Figs. 2 and 3).

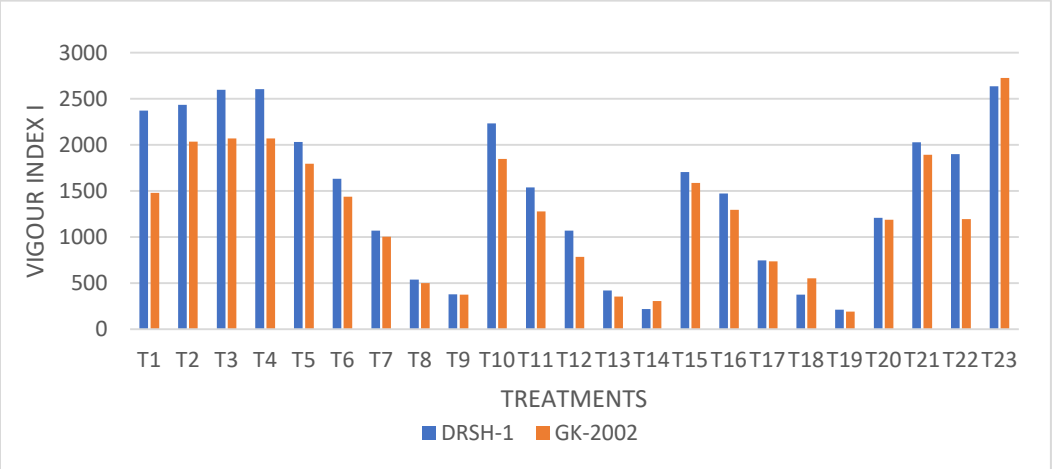

Fig. 2. Effect of priming on vigour index I in two sunflower hybrids

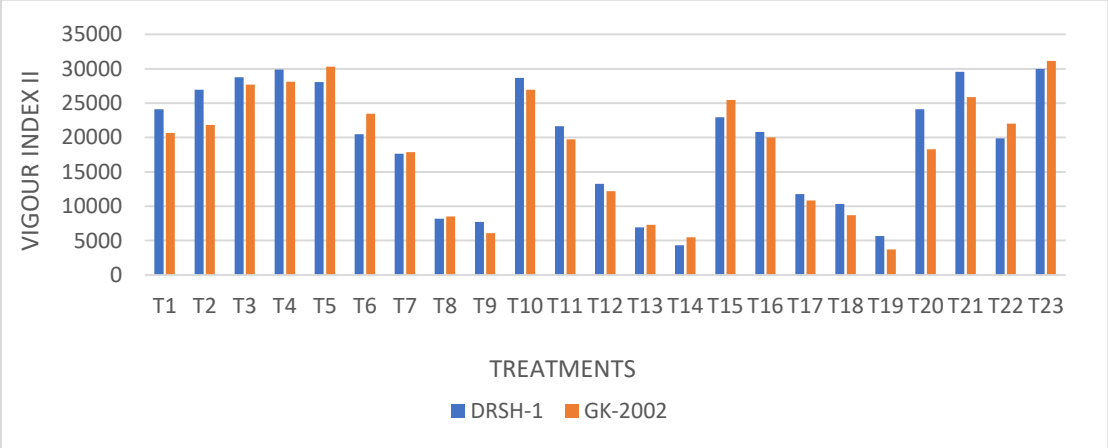

Fig. 3. Effect of priming on vigour index II in two hybrids

The increase in seedling vigour index is mainly due to reduction of lag time in priming treatments [14] and the preparedness of seed to directly enter phase III of germination in case of hydropriming. Priming also causes physiological and biochemical changes in seed leading to enhanced activities of hydrolyzing enzymes and early breakdown of seed reserves thereby resulting in enhanced early growth. These results were similar to those of in rice [13], maize [15,16], alfalfa [17,18], blackgram [19], *Chenopodium quinoa* and *Amaranthus caudatus* [20].

3.3 Field Emergence

There is a significant difference among the treatments, between the hybrids and interaction of hybrids and treatments for field emergence. DRSH-1, it was observed that T4 exhibited highest emergence rate of 77% among all the primed seeds. This performance was compared to an unaged high-vigour seed lot (T23), which showed an emergence rate of 83%, however, lowest field emergence of 58% was recorded in aged low vigour seed lot (T22). In GK-2002, T4 recorded the highest field emergence of 66% among the primed seed, while aged low-vigour seed lot exhibited a lower emergence rate of 53% (T22) and unaged high-vigour seed lot displayed a robust emergence rate of 77% (T23).

The field emergence among the hydroprimed seeds of both hybrids was observed to be highest in T4 *i.e*., hydropriming for 16hrs recorded a mean value of 77% and 66% in DRSH-1 and GK-2002 respectively. The field emergence among the thermo primed seeds of both hybrids was observed to be highest in T10 with a mean value of 70% and 65% in DRSH-1 and GK-2002 respectively. The field emergence in pre-chilled seeds of both hybrids was observed to be highest in T21with a mean value of 67% and 63% in DRSH-1 and GK-2002 respectively (Fig. 4).

Priming has a positive impact on seedling development as it accelerates hypocotyl elongation thereby promoting early seedling emergence [21]. Primed seeds exhibit improved germination rates, reduced germination time, and enhanced uniformity [22], potentially due to better plasma membrane structure. These results were similar to those in okra [22], *Brassica napus* [23], wheat [24,25] and watermelon [26].

3.4 Speed of Germination

There is a significant difference among the treatments, between the hybrids and interaction of hybrids and treatments for speed of germination. In both DRSH-1 and GK-2002, the seeds in treatment T4 exhibited fastest germination rates, with respective values of 13.095 and 14.460 compared to different priming treatments. The germination speed of accelerated aged seed of DRSH-1 seeds decreased to 9.01 (T22), while the high-vigour seeds maintained a strong germination speed of 14.78 (T23). Similarly, for GK-2002, accelerated aging reduced the germination speed to 9.41, whereas the high vigour seeds displayed a robust germination speed of 15.12. T4 exhibited on par speed of germination with control (T23).

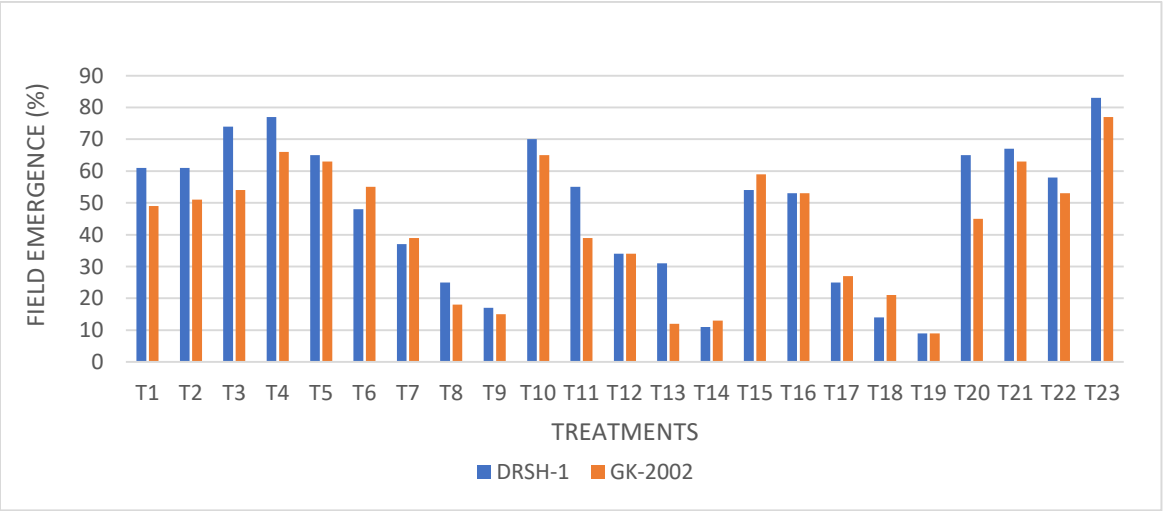

Fig. 4. Effect of priming on field emergence in two hybrids

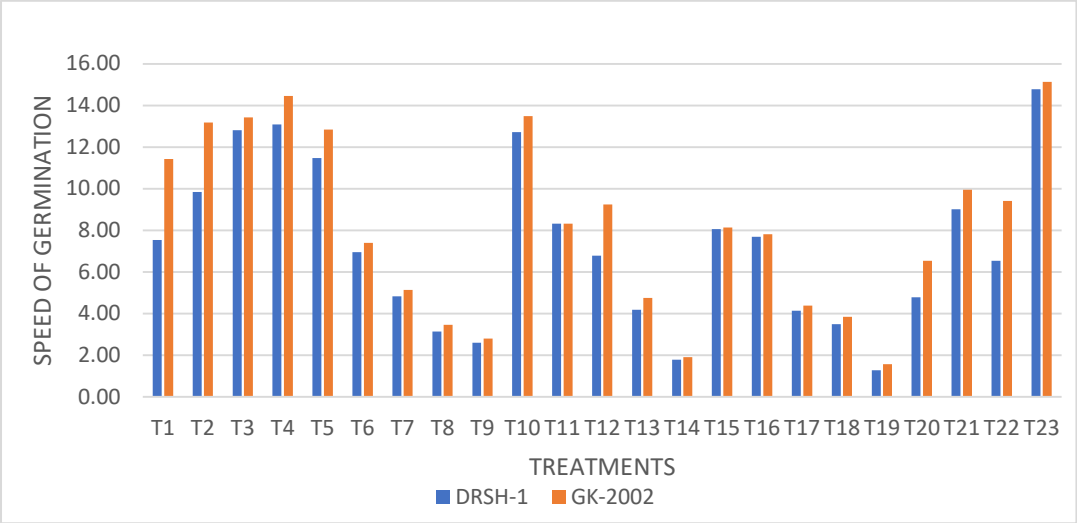

Fig. 5. Effect of priming on speed of germination in two hybrids

Among different durations of hydropriming, T4 showed the highest speed of germination with 13.095 and 14.460 in DRSH-1 and GK-2002 respectively. Among thermo primed seeds significantly highest speed of germination (12.72 and 13.49) was reported in the treatment T10, followed by treatment T5 (11.47 and 12.83). Significantly highest speed of germination (9.01 and 9.95 in DRSH-1 and GK-2002 respectively) was reported in the treatment T21 in both the hybrids for pre chilling treatment (Fig. 5).

Priming improves speed, germination percentage under sub optimal temperatures [27]. The enhanced speed of germination is due to higher and early α-amylase activity leading to early germination in primed seed when compared to aged seed. Similar results were obtained in *Aeluropus macrostachys* [28], rice [29], spinach [30], garden cress and basil [18], chilli [31] and rice [32].

4. CONCLUSION

The comprehensive assessment of various quality parameters revealed that fresh seed lot, characterized by its high vigor without any prior priming, consistently exhibited the highest values across all tested parameters. However, on comparison of the effects of different priming techniques on accelerated aged seed, including hydropriming with varying durations, thermo priming with different durations and temperatures, and pre-chilling at different temperatures, suggested that some specific combinations of priming duration and temperature appeared to significantly enhance the quality and vigour of seeds to the extent that they were comparable to those of fresh lot. Priming conditions for aged seed lot were found to significantly enhance seed quality, which with 16 hours of hydropriming, 6 hours at 35°C in thermo priming, and 7 days at 10°C in pre-chilling demonstrating notable efficacy. These findings underscore the potential for optimizing priming conditions to substantially improve the performance of aged seeds, offering exciting prospects for enhancing seed quality and viability.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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