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Optimization of Seed Priming Protocols for Enhance Germination and Vigour of Oat Seeds (*Avena sativa* 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

Unfavourable environmental conditions (high temperature, high moisture, and drought) during seed growth and development in the field and seed storage in storehouse can reduce germination, vigour and processing quality of seeds of field crops. Priming stimulates germination by inducing a variety of metabolic changes in the seed leading to improved germination, increased germination rate, and germination in a wider range of conditions. In the present research work, we made an attempt to test 26 different seed treatment viz., hydropriming, thermo-priming, pre-chilling for enhancement of seed quality, germinability of oat seeds. We hypothesized that seed priming lead to enhance seed quality attributes of oat seeds enhancing seed vigour and germination. Seed priming studies revealed that out of 26 different seed treatments, first count was found to be maximum for thermopriming of seeds at 40^{o}C for 36 hrs (94.50%) followed by thermopriming of seed at 35^{o}C for 12 hrs (94.00%), with increment over control in the tune of 3.8% and 3.29%, respectively. Germination percentage observation revealed superior performance of thermopriming of seeds at 35^{o}C for 12 hrs (96.00%), followed by thermopriming of seeds at 40^{o}C for 6 hrs (95.50%), with increment over control in the tune of 3.7% and 3.24%, respectively. Seed vigour studies revealed superior performance for seed vigour index I for thermopriming of seed at 45^{o}C for 36 hrs (3998.44) with increment over control in the tune of 27.84 %. Seed vigour index II found superior for thermopriming of seeds at 35^{o}C for 12 hrs (13.89), with increment over control in the tune of 17.31%. Time for maximum number of radicle emergence studies revealed superior performance of thermopriming of seed at 30^{o}C for 6 hrs (77.88 hrs), with increment over control in the tune of 52.70%. Therefore, thermopriming of oat seeds at 35^{o}C for 12 hrs is recommended for seed treatment to enhance germination and seed vigour of oat seed consequently resulting into better final plant stand establishment and seed yield.

Keywords: Oats; seed priming; Thero-priming; pre-chilling germination; seed vigour.

1. INTRODUCTION

Oat (*Avena sativa* L.) is a popular annual crop in the poaceae family that has a self-pollinated hexaploid nature (2n=6x=42) and is generally referred to as *Avena Sativa*. When it comes to the amount of grain produced each year, oats are considered a minor cereal crop. Considering its various uses as cereal food, feed, green or conserved fodder, *Avena sativa* L. or white oat demonstrated high energy reflection in its hull-free grain to facilitate the observed progression on the crop. It is a good source of protein, vitamin B complex, phosphorus, iron, (Mehra., 1978) and rich inadequate soluble carbohydrates and fibers (Peterson *et al*. 2005). The oat's health-promoting properties increased its utility as a dual-purpose crop (Suttie and Reynolds., 2004). To meet the present need for high standards in the agricultural sector, increasing seed quality has become a priority. 'Priming' is a well-established treatment for enhancing seed quality. Priming allows some of the metabolic processes necessary for germination to occur without germination take place. Priming of seeds to improve the germination rate and uniformity of growth thereby reducing the emergence time of many crops.

Germination and seedling establishment are critical stages in the plant life cycle. The three early phases of germination are: (i) imbibition, (ii) lag phase and (iii) protrusion of the radicle through the testa (Simon, 1984). Priming is a procedure that partially hydrates seed, followed by drying of seed, so that germination processes begin, but radicle emergence does not occur. A method to improve the rate and uniformity of germination is the priming or physiological advancement of the seed lot (Finch-Savage, 2004; Halmer, 2004). Seed priming is the soaking of seeds in a solution of any priming agent followed by drying of seeds that initiates germination related processes without radicle emergence (McDonald, 1999).

Seed priming and other advances in seed technology have increased seed performance. Priming is a simple process that partially hydrates seed in a controlled atmosphere before drying it, allowing germination to occur without radicle emergence. The main goal of seed priming technique is to improve seed performance under specified environmental conditions. Priming stimulates germination by inducing a variety of metabolic changes in the seed, the results of which typically endure after desiccation and become available once the seeds collect moisture after sowing. Furthermore, priming increases seed vitality, resulting in early and uniform emergence as well

as strong stand establishment. Priming has several practical applications, including improved percentage germination, increased germination rate, and germination in a wider range of conditions.

Research on priming has proved that crop seeds primed with water germinated early, root and shoot development started rapidly, grew more vigorously and seedling length was also significantly greater than non- primed seeds. It could also improve the performance of crop by alleviating the effect of salts under saline soil conditions (Mohammadi et al., 2008). Soaking seed in water overnight before sowing can increase the rate of germination and emergence even in soil conditions where moisture content is very low (Clark et al., 2001). Very few studies were conducted on oat seed priming and were confined to studying its effect on germination percentage. Hence the present research was conducted to study the effect of different seed priming treatment viz., thermopriming, pre-chilling and hydro-priming on different seed quality attributes in Oat.

2. MATERIALS AND METHODS

The experiment was conducted at Seed Technology Research Unit, Department of Plant Breeding and Genetics, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh during 2021-2022. The experiment includes 26 priming treatments viz., T_1 Non-priming (Control), T_2 (Vitavax @ 5g/kg of seeds), T_3 (Hydropriming – Soaking in water for 22 hrs (at 20°C for oat), and air-drying at 25°C for 48 hrs, T_4 (Thermopriming- 30°C for 6 hrs), T_5 (Thermopriming- 30°C for 12 hrs), T_6 (Thermopriming- 30°C for 24 hrs), T_7 (Thermopriming- 30°C for 36 hrs), T_8 (Thermopriming- 30°C for 48 hrs), T_9 (Thermopriming- 35°C for 6 hrs), T_{10} (Thermopriming- 35°C for 12 hrs), T_{11} (Thermopriming- 35°C for 24 hrs), T_{12} (Thermopriming- 35°C for 36 hrs), T_{13} (Thermopriming- 35°C for 48 hrs), T_{14} (Thermopriming- 35°C for 6 hrs), T_{15} (Thermopriming- 40°C for 12 hrs), T_{16} (Thermopriming- 40°C for 24 hrs), T_{17} (Thermopriming- 40°C for 36 hrs), T_{18} (Thermopriming- 40°C for 48 hrs), T_{19} (Thermopriming- 45°C for 6 hrs), T_{20} (Thermopriming- 45°C for 6 hrs), T_{21} (Thermopriming- 45°C for 12 hrs), T_{22} (Thermopriming- 45°C for 24 hrs), T_{23} (Thermopriming- 45°C for 36 hrs), T_{24} (Pre-chilling- 5°C), T_{25} (Pre-chilling - 7°C), T_{26} (Pre-chilling - 10°C). The experiment was laid out in completely randomized design with three replications. Data on time for maximum number of radicle emergence, first count percentage, germination percentage, seed vigour index I and seed vigour index II were recorded as follows:

2.1 Time (hrs.) for Maximum Numbers of Radicle Emergence (≥2mm)

Seeds of oats were placed on two filter papers in Petri dishes. Make filter papers moistened with distilled water with three dishes (replicates) per treatment. Radicle emergence was checked daily, and germination was defined as radicle emergence of ≥2 mm

2.2 First Count Percentage

Three replications, each containing 100 seeds taken randomly from each lot. Seeds were kept in between towel paper then the samples were placed in germinator at 25°C. Normal seedling were counted at 5th day after the test.

2.3 Germination Percentage

Germination percentage was recorded by using Paper towel method. From each treatment 100 seeds were placed in three replications on moist towel paper, rolled properly and kept in seed germination at constant temperature (25°C) and relative humidity (90 %) first count of germination was taken on 5th day. Whereas, final germination recorded on 10th day (ISTA, 1999).

2.4 Seed Vigour Index-I

Seed vigour index I was computed by adopting the formula suggested by Abdul-Baki and Anderson (1973) and expressed as an index number.

Seed vigour Index-I = [Root length(cm) + Shoot length(cm)] ×Germination percentage

2.5 Seed Vigour Index-II

Seed vigour index II by weight is determined by the multiplication of seedling dry weight on the final count and germination percentage (Abdul-Baki and Andreson, 1973).

Seed vigour index- II = Germination percentage x seedling dry weight at final count

3. RESULTS AND DISCUSSION

Seed priming is a simple and low-cost hydration technique in which seeds are partially hydrated to a point where pre-germination metabolic activities start without actual germination, and then re-dried until close to the original dry weight (Rehman et al. 2011). Priming significantly increases the quantity of mitochondria and upregulation of proteins for cell division (α- and β-tubulin). Rehydration through seed priming brings major cellular changes in seeds such as de novo synthesis of nucleic acids and proteins; ATP (adenosine tri phosphate) production; activation of sterols and phospholipids; and repairing DNA damaged during threshing (Marthandan 2020). The radicle emergence (RE) test for seed vigour classification is an ingenious protocol that will lead to a fast and reliable automated procedure for verifying seed quality using image analysis (Onwimol *et al.,* 2016). Significant differences were observed for time to maximum radicle emergence, first-count percentage, and germination percentage, whereas seed vigour Indices I and II did not differ significantly across treatments.

Significant difference was observed for the time for maximum number of radicle emergence ($P \geq 0.05$). The range for the parameter is 49.75 hrs - 77.88 hrs. Treatment T_{25} i.e., pre-chilling @ 7^{o}C exhibited minimum time (49.75 hrs) at par with treatment T_5 i.e., heat treatment of seeds at 30^{o}C for 12 hrs (49.94 hrs). In contrast treatment T_4 i.e., heat treatment of seeds at 30^{o}C for 6 hrs exhibited maximum time for radicle emergence (77.88 hrs). The superior treatment T_{25} (Pre-chilling @ 7^{o}C) and T_5 (Thermopriming of seeds at 30^{o}C for 12 hrs) shows superiority over control in the tune of 2.45% and 2.07%, respectively. Khajeh-Hosseini *et al.* (2009) also reported that a single count of Radicle Emergence (RE) taken after six days at 13°C or 66 hours at 20°C predicted Mean Germination Time (MGT), and that both (Radicle Emergence) RE and (Mean Germination Time) MGT identified the same vigour differences as the cold and accelerated ageing tests (Matthews *et al.* 2011).

First count percentage and germination percentage are an important seed quality trait determining the final plant stand population, consequently affecting crop yield. A significant difference was observed for first count percentage and germination percentage among the seed priming treatments. Seed priming treatment studies revealed superior performance of treatment T_{17} (Thermopriming of seeds at 40^{o}C for 36 hrs) at par with treatment T_{10} (Thermopriming of seeds at 35^{o}C for 12 hrs). The range for first count percentage is 81.50%-94.50%. The superior treatment T_{17} (Thermopriming of seeds at 40^{o}C for 36 hrs) shows superiority of 3.84% over untreated seeds. In contrast, minimum first count percentage was observed for treatment T_{24} (Pre-chilling @ 5^{o}C) and treatment T_{26} (Pre-chilling @ 10^{o}C) (81.50%). Germination studies revealed superior performance of treatment T_{10} (Thermopriming of seeds at 35^{o}C for 12 hrs) (96.00%) at par with treatment T_{17} (Thermopriming of seeds at 40^{o}C for 36 hrs) (95.00%). The range for germination percentage is 83.00%-96.00%. The superior treatment T_{10} (Thermopriming of seeds at 35^{o}C for 12 hrs) shows superiority of 3.78% over control. Thermopriming improves seed germination by exposing seeds to controlled high temperatures before sowing, which activates metabolic processes, enhances enzyme activity, accelerates DNA and protein repair, and strengthens antioxidant systems. This pre-treatment reduces seed dormancy, improves membrane integrity, and leads to faster, more uniform germination under both normal and stress conditions (Farooq et al., 2010; Paparella et al., 2015) In contrast, minimum germination percentage was observed for treatment T_{26} (Pre-chilling at 10^{o}C) (83.00%). Seed treatment at different temperatures carried out before sowing is also known as 'thermopriming'. Thermopriming is achieved by pre-sowing seeds at different temperatures thermopriming at high temperatures has been used in some species, resulting in advantages in germination especially for plants adapted to warm climates (Khalil *et al*.1983). Seed priming facilitates the buildup of germination, metabolites and assists with metabolic repair (Farooq *et al.* 2006). Thermopriming combined with other treatments resulted in beneficial effects on germination parameters of white spruce (*Picea glauca* L.), enough to improve nursery practices for commercial seedling production (Liu *et al.* 2013).

Seed vigour is a qualitative term encompassing 'the sum total of those properties of the seed which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence (Perry, 1978). A non-significant difference was observed for seed vigour index I and seed vigour index II among the seed priming

Table 1. Effect of seed priming priming treatments on different seed quality parameters

No. of treatment	Treatment details	Time (hrs.) for maximum numbers of radicle emergence (≥2mm)	First count %	Germination %	Vigour index I	Vigour index II
		Mean	Mean	Mean	Mean	Mean
T_1	Untreated	51.00	91.00	92.50	3,127.78	11.843
T_2	State recommended package of practices (Vitavax @ 5gm/kg of seeds)	53.75	89.00	91.25	3124.23	11.76
T_3	Hydropriming @22 hrs	68.69	93.50	94.50	3,463.89	11.76
T_4	Thermopriming of seeds at 30ºC for 6 hrs.	77.88	88.00	90.50	3,600.75	11.623
T_5	Thermopriming of seeds at 30ºC for 12 hrs.	49.94	90.00	92.00	3,423.68	10.475
T_6	Thermopriming of seeds at 30ºC for 24 hrs.	53.50	89.00	93.50	3,666.02	10.68
T_7	Thermopriming of seeds at 30ºC for 36 hrs.	53.13	87.00	88.00	3,498.72	12.103
T_8	Thermopriming of seeds at 30ºC for 48 hrs.	51.94	90.00	91.00	3,575.25	12.543
T_9	Thermopriming of seeds at 35ºC for 6 hrs.	53.31	87.00	90.50	3,446.10	12.33
T_{10}	Thermopriming of seeds at 35ºC for 12 hrs.	55.00	94.00	96.00	3,727.57	13.898
T_{11}	Thermopriming of seeds at 35ºC for 24 hrs.	51.50	89.50	92.00	3,500.55	12.503
T_{12}	Thermopriming of seeds at 35ºC for 36 hrs.	52.63	90.00	93.00	3,611.43	11.938
T_{13}	Thermopriming of seeds at 35ºC for 48 hrs.	54.94	86.50	92.00	3,739.80	12.925
T_{14}	Thermopriming of seeds at 40ºC for 6 hrs.	52.75	93.00	95.50	3,160.44	12.135
T_{15}	Thermopriming of seeds at 40ºC for 12 hrs.	51.94	87.00	90.50	3,002.43	10.87
T_{16}	Thermopriming of seeds at 40ºC for 24 hrs.	56.00	83.00	86.00	2,845.80	11.895
T_{17}	Thermopriming of seeds at 40ºC for 36 hrs.	53.75	94.50	95.00	3,771.48	12.818
T_{18}	Thermopriming of seeds at 40ºC for 48 hrs.	56.31	88.00	89.00	3,158.72	10.53
T_{19}	Thermopriming of seeds at 45ºC for 6 hrs.	53.88	93.50	94.50	3,281.06	11.08
T_{20}	Thermopriming of seeds at 45ºC for 12 hrs.	53.50	93.50	94.50	3,331.56	11.768
T_{21}	Thermopriming of seeds at 45ºC for 24 hrs.	51.00	85.50	90.50	3,124.46	11.635
T_{22}	Thermopriming of seeds at 45ºC for 36 hrs.	52.75	92.00	93.00	3,998.44	12.388
T_{23}	Thermopriming of seeds at 45ºC for 48 hrs.	51.81	86.00	87.50	3,164.92	11.138
T_{24}	Pre-chilling @ 5ºC	52.81	81.50	83.50	3,654.38	11.293
T_{25}	Pre-chilling @ 7ºC	49.75	88.00	89.50	3,744.74	12.375
T_{26}	Pre-chilling @ 10ºC	54.25	81.50	83.00	3,417.95	11.225
	C. D. (P≥0.05)	8.06	5.76	4.90	NS	NS
	SEm (±)	2.86	2.04	1.73	674.18	0.793
	C.V.	10.49	4.60	3.81	37.756	13.405

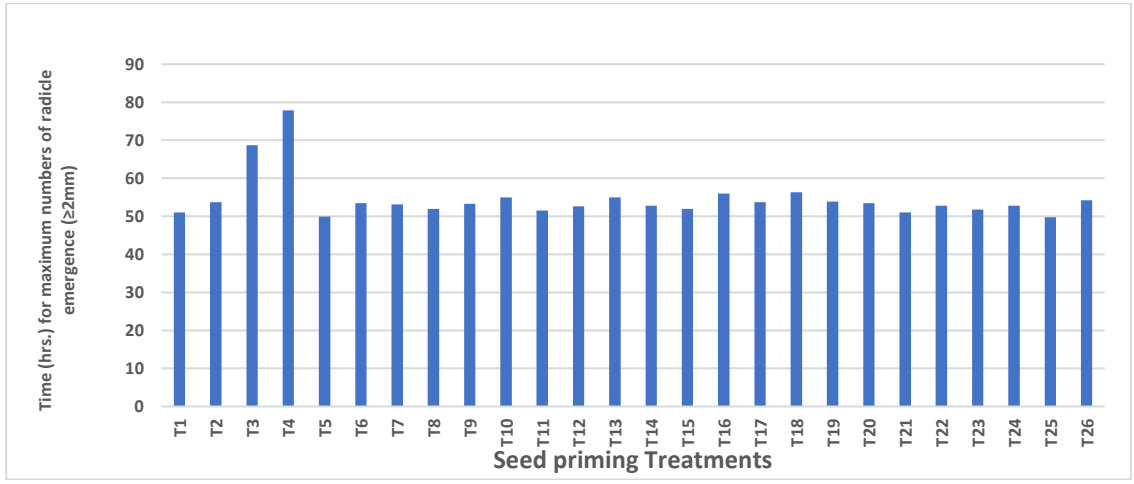

Fig. 1. Depiction of time (hrs.) for maximum numbers of radicle emergence (≥2mm) of oat seeds due to 26 different seed priming treatments

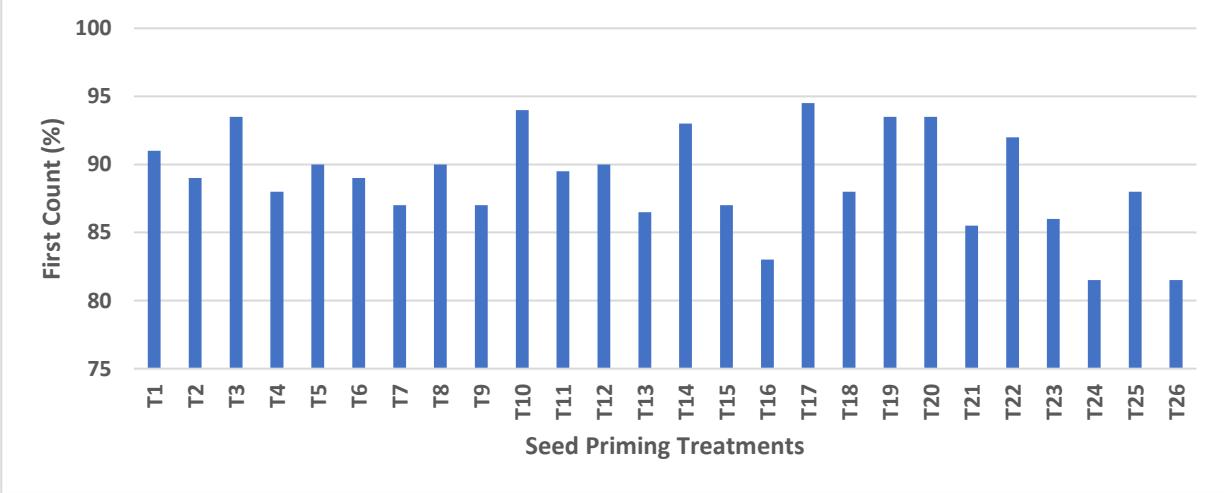

Fig. 2. Depiction first count of oat seeds due to 26 different seed priming treatments

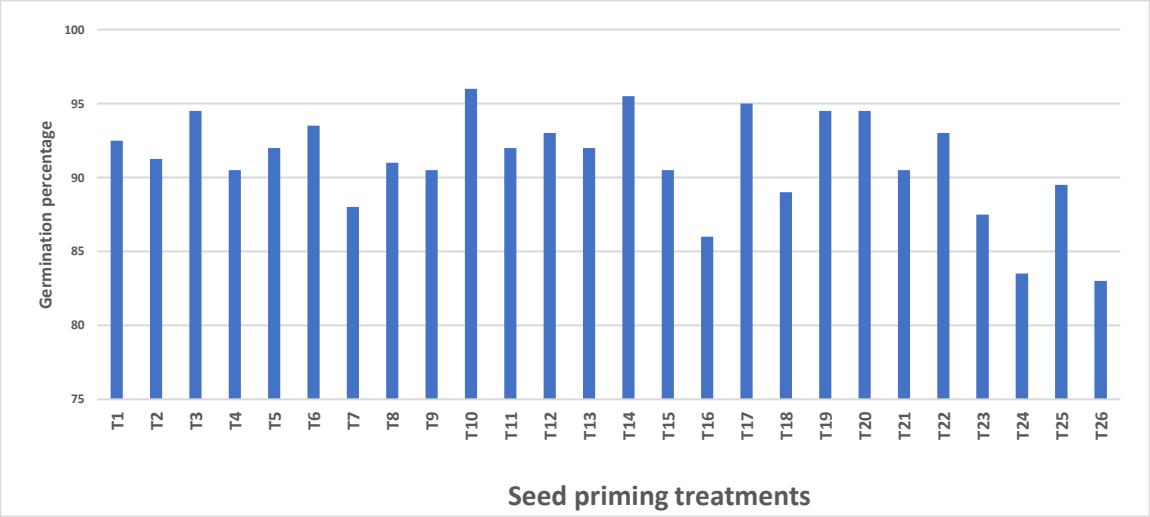

Fig. 3. Depiction of Germination percentage of oat seeds due to 26 different seed priming treatments

treatments. Among all the treatments, T_{22} (Thermopriming of seeds at 45°C for 36 hrs) (3998.440) showed high seed vigour index I and T_{10} (Thermopriming of seeds at 35°C for 12 hrs) showed high seed vigour index II (13.898). When low vigor seeds were primed showed improved germination performance (Bray, 1995). The increased dry root and shoot weight demonstrated the biomass accumulation due to priming. Ramamoorthy *et al*. (2001) reported that priming enhanced seedling vigour, seedling length and dry weight of high and low vigour seed lots in rice.

4. CONCLUSION

Thermopriming is a widely applied method known to enhance seed germination and vigour in various crops by improving physiological and biochemical processes. In this study, thermopriming oat seeds at 35 °C for 12 hours significantly improved both germination percentage (by 3.7%) and Seed Vigour Index II (by 17.31%) compared to untreated controls. These enhancements indicate that thermopriming at the specified temperature and duration can serve as an effective pre-sowing treatment to improve the performance and uniformity of oat seed lots. The practical recommendation is adopting thermopriming at 35°C for 12 hours in seed handling protocols could elevate germination success and seed vigour—ultimately contributing to more robust crop establishment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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