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Hydropriming and Physiological Enhancement in Sunflower Seeds

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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

Hydropriming is a seed pre-treatment technique that involves controlled imbibition in water, allowing the seed to initiate the germination process without radicle protrusion. This study investigated the effects of hydropriming on the quality and vigour of different sunflower (Helianthus annuus) seed lots. A completely randomised experimental design was employed in a 4 × 2 factorial arrangement, comprising four seed lots and two treatments (with and without hydropriming), with four replications of 50 seeds per treatment. The following assessments were conducted: seed viability, germination, radicle protrusion, first germination

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count, germination speed index, shoot length, and root system length. Hydropriming improved the physiological performance of sunflower seeds in lots 3 and 4 but showed no positive effects in lot 1. The performance of seeds in lot 2 remained high regardless of treatment application. The efficiency of hydropriming depends on the initial seed performance, and it is a promising technique to enhance the quality of seed lots with compromised vigor.

Keywords: Viability; Helianthus annuus; osmotic conditioning; seed vigor; physiological quality.

1. Introduction

Sunflower (*Helianthus annuus* L.) is a crop of great economic and social importance, standing out as one of the main oilseed species cultivated worldwide. In addition to its use in oil production, sunflower is valued for their versatility, being widely used in the food, pharmaceutical, and biofuel industries. Its broad adaptation to different climatic conditions makes it a strategic crop in several regions of the world, contributing significantly to food and energy security (Malunjkar*et al*., 2024).

The germination and vigor of sunflower seeds are determining factors for crop success, as they directly influence stand uniformity and field productivity. Seeds with high physiological quality exhibit faster and more uniform germination, resulting in more vigorous seedlings with greater productive potential. In this context, the adoption of methods that enhance the germination process is essential to maximize seed performance, promoting greater uniformity and faster seedling emergence. Pre-treatment techniques such as hydropriming have proven effective in improving these characteristics, helping seeds overcome adverse conditions and achieve better initial establishment (Corbineau*et al*., 2023; Gris et al., 2010). Hydropriming is a seed pre-treatment technique that involves controlled imbibition in water, allowing the seed to initiate the germination process without radicle protrusion (Marcos Filho, 2015; Vasquez, 1995).

Hydropriming positively influences the pre-germinative metabolism of seeds by activating physiological mechanisms associated with protection against oxidative damage and more efficient early seedling development (Forti *et al*., 2021). This treatment is based on controlled seed hydration, enabling the reactivation of germination metabolism and the reorganization of cellular systems without radicle protrusion (Marcos Filho, 2015).

Evaluating the effects of hydropriming on seeds with different quality levels can provide relevant support for improving more efficient and sustainable agricultural practices. In this context, studies have been conducted to assess the benefits of hydropriming in sunflower seeds (Bouriouг*et al*., 2020; Matias *et al*., 2018; Zoucarli et al., 2011).

In view of these considerations, this study aimed to evaluate the effects of hydropriming on the quality and vigor of different sunflower seed lots.

2. Material and Methods

The experiment was conducted at the Seed Analysis Laboratory of the Department of the State University of Montes Claros (UNIMONTES), Janaúba Campus, MG, Brazil. Sunflower seeds from four different lots, acquired in the municipality of Araguari, MG, were used.

Lot 1 consisted of seeds from the cultivar *Helianthus annuus* ‘Russian Mammoth,’ stored for three months after harvest. Lots 2, 3, and 4 were composed of sunflower seeds of the cultivar ‘GraúdoComum,’ M734, stored for 3, 7, and 11 months, respectively. Seeds from all four lots were stored under laboratory environmental conditions (25 ± 5 °C).

Initially, a seed imbibition curve was established to determine the appropriate duration for hydropriming, corresponding to the moment when seeds completed Phase II of the triphasic imbibition pattern without progressing to Phase III, characterized by radicle protrusion. Hydropriming consists of controlled seed imbibition sufficient to activate the initial stages of germination (Phases I and II) without primary root protrusion (Phase III) (Bewley *etal*., 2013).

For the characterization of the imbibition curve, four replications of 50 seeds per lot were sown between two sheets of Germitest® paper moistened with water equivalent to 2.5 times the dry paper weight and maintained in a germinator at 25 °C. Seeds were weighed on an analytical balance (0.0001 g precision) at thirteen imbibition times (1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 24, and 28 hours) until radicle protrusion was observed in at least one seed per replication.

At the end of each period, seeds were removed from the Germitest® paper, gently dried with paper towels to remove surface moisture, and weighed to obtain fresh weight. The percentage of water absorbed at each time was calculated using the following expression:

$$\%I = [(PF - PI) / PI] \times 100$$

Where:
%I = percentage of imbibition relative to the initial sample weight;
PF = final weight;
PI = initial weight.

Data were subjected to analysis of variance, and when significant, fitted to regression models, with parameter estimates evaluated by the *t* test at 5% probability.

To evaluate the effects of hydropriming on seed physiological quality, a completely randomized design in a 4 × 2 factorial scheme was adopted, consisting of four seed lots and two treatment conditions (with and without hydropriming), with four replications of 50 seeds per treatment.

Seed moisture content was initially determined using the standard oven method at 105 ± 3 °C for 24 hours, with five replications of 50 seeds each, and results expressed as percentage (Brasil, 2025).

For hydropriming, four replications of 50 seeds were placed on Germitest® paper pre-moistened with water equivalent to 2.5 times the dry paper weight and maintained in a germinator at 25 °C for 20 hours. Subsequently, the seeds were removed from the substrate and dried on paper towels at room temperature (approximately 30 °C) for 72 hours (Neves, 2014). The percentage of imbibition was calculated based on the initial seed weight (Brasil, 2025).

After treatment application, seeds were evaluated for viability, germination, radicle protrusion, first germination count, germination speed index (GSI), and initial seedling growth through shoot and root length measurements.

For the tetrazolium test, seeds from different lots were preconditioned by immersion in 200 mL of distilled water for 18 hours in a B.O.D. chamber at 20 °C to facilitate seed coat removal. After removing the pericarp and endosperm membrane, embryos were immersed in a 1.0% solution of 2,3,5-triphenyl tetrazolium chloride and kept in the dark in a B.O.D. chamber at 30 °C for 3 hours for staining (Brasil, 2025). Embryos were then washed in running water and individually evaluated. Viability was determined based on tissue staining, considering the sum of seeds classified in classes 1 to 3, according to Bheringet *et al.* (2005), with results expressed as percentage of viable seeds.

The standard germination test was conducted using four replications of 50 seeds per treatment. Seeds were sown on Germitest® paper moistened with distilled water at a volume equivalent to 2.5 times the dry substrate weight. The rolls were placed in a digital germinator maintained at 25 °C under a constant photoperiod. The first count was performed on the fourth day, and the final evaluation on the ninth day, recording the percentage of normal seedlings—those exhibiting well-developed, proportional, and healthy essential structures (root system and shoot)—with results expressed as a percentage (Brasil, 2025).

Radicle protrusion was assessed 48 hours after test initiation, recording seeds with radicles of at least 2 mm in length, and results were expressed as a percentage (Pereira et al., 2012). The first germination count was also determined on the fourth day, concurrently with the germination test, by evaluating the number of normal seedlings (Brasil, 2025).

The germination speed index (GSI) was calculated by counting the number of normal seedlings daily for nine days, using the formula proposed by Maguire (1962). At the end of the germination test, shoot and root lengths of ten normal seedlings per replication were measured using a millimetre ruler, and the results were expressed in cm per seedling.

Data were subjected to analysis of variance, considering the effects of seed lots, treatment conditions (with and without hydropriming), and their interaction at the 5% significance level. Treatment means were compared using the F test, while seed lot means were compared using Tukey's test, both at 5% probability. Statistical analyses were performed using Sisvar software (Ferreira, 2014).

3. Results and Discussion

The seed moisture content of the evaluated lots differed significantly, with higher values observed in lots 3 and 4 (8.9 and 9.2%) compared to lots 1 and 2 (5.6 and 5.1%). Despite the statistical difference, all values were within the recommended range of up to 12% for seed testing, ensuring the reliability of the results, as highlighted by Marcos Filho (2015).

Water imbibition varied among lots; however, seeds from all four lots followed the triphasic pattern described by Bewley *et al.* (2013), as shown in Fig. 1. During the first hours of imbibition (1 to 11 hours), seeds from lots 3 and 4 showed a marked increase in fresh weight.

Similarly, lots 1 and 2 exhibited weight increases between 1 and 12 hours. Phase I of imbibition is characterized by rapid water uptake, a predominantly physical process resulting from differences in water potential between the substrate and the seeds (Bechert et al., 2000). Matias et al. (2018) reported comparable results in sunflower seeds, noting greater water absorption during the first 14 hours.

From 12 hours of imbibition in lots 3 and 4, and 13 hours in lots 1 and 2, water absorption decreased, indicating the onset of Phase II. According to Marcos Filho (2015), this phase is marked by reduced water uptake and intensified metabolic activity. After 16 hours, seeds from all lots resumed rapid water absorption, accompanied by radicle protrusion, characterizing Phase III. At this stage, increased water demand is associated with embryonic axis growth and cell expansion (Carvalho and Nakagawa, 2012).

A significant interaction ($p < 0.01$) was observed between hydropriming treatment and seed lots for all evaluated variables, indicating that the response to hydropriming depends on the physiological characteristics of each lot.

The tetrazolium test (Table 1) showed a positive effect of hydropriming in lots 3 and 4, with viability increasing from 53% to 63% and from 50% to 61%, respectively. This improvement may be related to the reparative effect of hydropriming, which reduces storage-induced damage and preserves cellular integrity and metabolic activity (Shukla *et al*., 2018). Hydropriming stimulates protein synthesis and promotes a favourable metabolic balance, enhancing germination and seedling growth (Trigo and Trigo, 1999).

Table 1. Viability of Sunflower Seeds from Different Lots as a Function of Hydropriming

Lots	**Viability (%)**	
	Hydropriming	
	With	**Without**
1	49 Cb	80 Aa
2	84 Aa	81 Aa
3	63 Ba	53 Bb
4	61 Ba	50 Bb
CV (%)	8.9%	7.2%

Means followed by the same uppercase letter in a column and the same lowercase letter in a row do not differ significantly from each other according to Tukey's test at the 5% probability level.

Lot 2 showed no significant response to hydropriming, maintaining high viability regardless of treatment. In contrast, lot 1 exhibited reduced viability after hydropriming, suggesting that seeds with initially high physiological quality may not benefit from the treatment and may even be negatively affected.

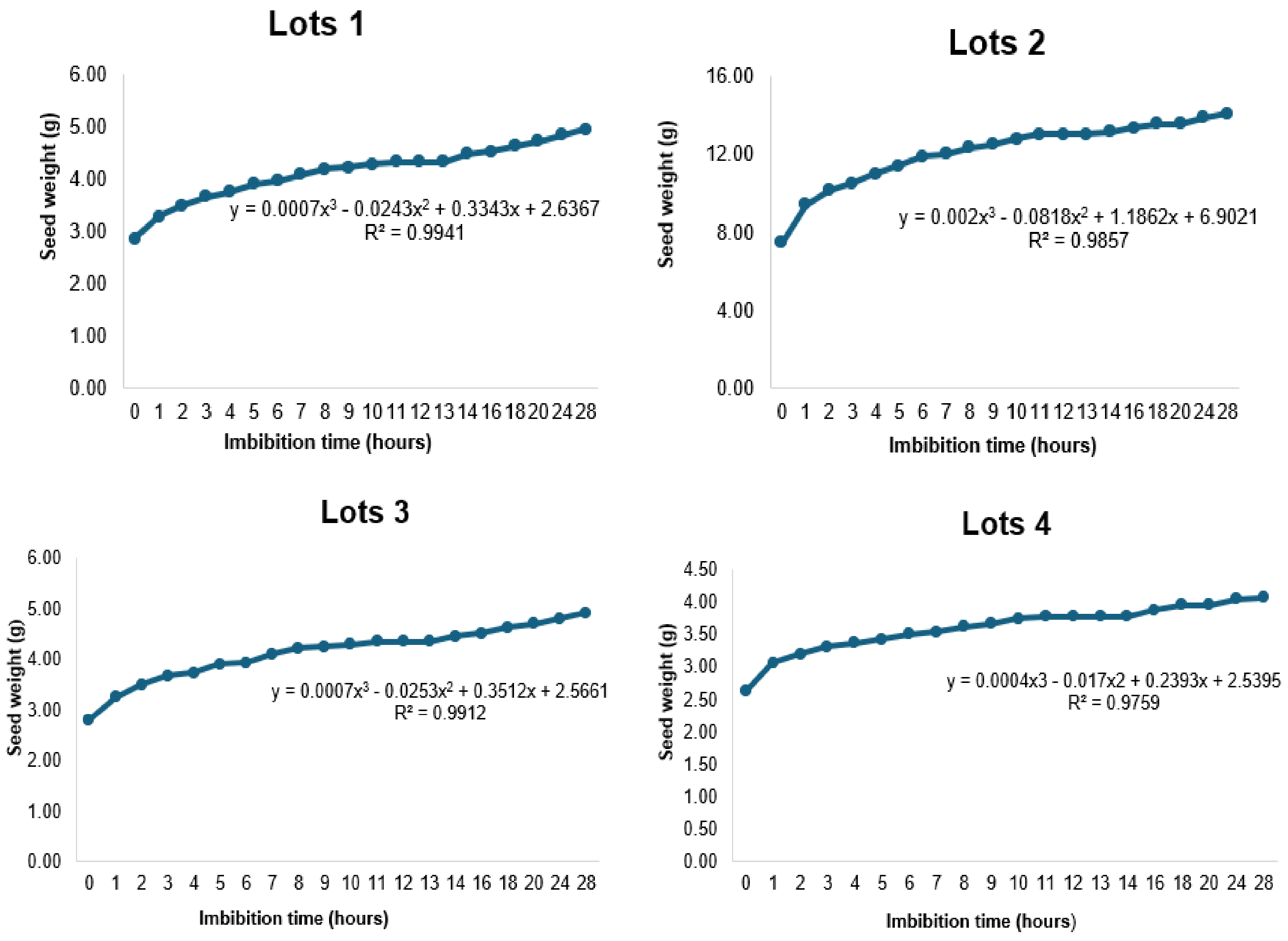

Fig. 1. Imbibition curve of sunflower seeds from different lots

Germination was influenced by hydropriming (Table 2). Lots 3 and 4 showed higher germination percentages under hydropriming, likely due to improved membrane repair and metabolic reactivation (Silva and Villela, 2011; Matias et al., 2018). Conversely, lot 2 was unaffected, and lot 1 showed reduced germination following hydropriming, possibly due to imbibitional damage caused by rapid water uptake, leading to membrane disruption and metabolic impairment (Zoucarliet al., 2008, 2011; Hoekstra et al., 1999).

Table 2. Germination (G), radicle protrusion (RP), first germination count (FGC), and germination speed index (GSI) of sunflower seeds from different lots as a function of hydropriming

Lots	**G (%)**		**RP (%)**	
	Hydropriming		**Hydropriming**	
	With	**Without**	**With**	**Without**
1	25 Db	98 Aa	16 Db	85 Ba
2	99 Aa	99 Aa	95 Aa	93 Aa
3	80 Ba	70 Bb	67 Ba	51 Cb
4	62 Ca	54 Cb	55 Ca	29 Db
CV (%)	6.7		4.3	
Lots	**FGC (%)**		**GSI**	
	Hydropriming		**Hydropriming**	
	With	**Without**	**With**	**Without**
1	15 Db	96 Aa	20 Db	43Aa
2	98 Aa	95 Aa	42 Aa	43 Aa
3	71 Ba	59 Bb	38 Ba	32 Bb
4	57 Ca	29 Cb	30 Ca	25Cb
CV (%)	3.8		8.9	

Means sharing the same uppercase letter in a column and the same lowercase letter in a row are not significantly different from each other according to Tukey's test at the 5% probability level.

Efficient DNA repair allows embryonic cells to resume replication, whereas failure in repair mechanisms may result in oxidative damage and cell death (Waterworth et al., 2015), which may explain the sensitivity of lot 1 seeds.

A significant interaction was also observed for the Germination Speed Index (GSI). Hydropriming increased GSI in lots 3 and 4, while lot 2 remained unchanged and lot 1 showed reduced values. Improvements in GSI for seeds stored for 7 and 11 months are associated with enhanced enzymatic activation, reserve mobilization, and physiological synchronization (Adhikari *et al*., 2021; Lutts *et al.,* 2016; Matsunami *et al*., 2022). Lower GSI in non-primed lots 3 and 4 may reflect seed aging effects, including ROS accumulation and membrane deterioration (Haj Sghaier *et al.,* 2023).

Radicle protrusion, an important indicator of germinative potential (De Castro *et al*., 2000), was significantly higher in hydroprimed seeds from lots 3 and 4. Lot 2 showed no significant response, while lot 1 was negatively affected. Hydropriming promotes early enzymatic activation and metabolic readiness, favoring rapid radicle emergence and improved tolerance to environmental stress (Paul *et al*., 2022).

In the first germination count, lots 2, 3, and 4 exhibited higher percentages of normal seedlings under hydropriming, whereas lot 1 showed reduced vigor. This test, considered an indicator of seed vigor, confirmed that hydropriming benefits lots with intermediate physiological quality.

Seedling growth was also influenced by treatment and lot (Table 3). In lots 3 and 4, hydroprimed seeds produced longer shoots and roots, suggesting improved vigor due to enhanced water uptake and metabolic activation (Karki *et al*., 2021). No significant effect was observed in lot 3, while lot 1 showed reduced growth after hydropriming.

Table 3. Shoot length (SL) and root length (RL) of sunflower seedlings originating from seeds of different lots as a function of hydropriming

Lots	SL (cm)		RL (cm)	
	Hydropriming		Hydropriming	
	With	Without	With	Without
1	8.2 Cb	12.6 Ba	10.5 Cb	16.4 Aa
2	17.8 Aa	17.9 Aa	17.7 Aa	17.2 Aa
3	11.1 Ba	9.7 Cb	13.8 Ba	11.9 Bb
4	8.6 Ca	4.1 Db	6.1 Da	3.3 Cb
CV (%)	16.71		8.21	

Means followed by the same uppercase letter in a column and the same lowercase letter in a row do not differ significantly from each other according to Tukey's test at the 5% probability level.

Negative effects may be related to genetic differences, physiological conditions, storage history, water absorption rate, and treatment duration, highlighting the complexity of hydropriming responses.

Overall, hydropriming had variable effects depending on the initial physiological status of the seed lots. Lots 3 and 4 showed significant improvements in germination, radicle protrusion, first count, and GSI, indicating enhanced vigor. Lot 2 maintained high performance regardless of treatment, whereas lot 1 was negatively affected. These findings demonstrate that hydropriming efficiency depends on the initial physiological condition of seeds and is more effective in lots with intermediate or reduced vigor.

4. Conclusion

Hydropriming improved the physiological performance of sunflower seeds in lots 3 and 4 but showed no positive effects in lot 1. The performance of seeds from lot 2 was superior, regardless of treatment application. The efficiency of hydropriming depends on the initial physiological quality of the seeds, and it is a promising technique to enhance the quality of seed lots with compromised vigor.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare 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.

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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