Research Article

Do methods for overcoming dormancy affect the physiological quality of okra seeds?[1]

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ABSTRACT

One of the main obstacles in the okra production is the physical dormancy imposed by the tegument of the seeds. Although several methods for overcoming tegumentary dormancy have been proposed, little is known about their effect on the physiological quality of seeds. Thus, this study aimed to verify the effectiveness of methods for overcoming dormancy (control - no treatment; pre-soaking - seed soaking in water at 30 ºC, for 6 h; thermotherapy - immersion in water at 60 ºC, for 3 min; dry heat - oven at 70 ºC, for 5 min; thermal shock - immersion in water at 30 ºC, for 30 min, followed by 5 ºC, for 24 h; and sand scarification - friction between seeds and sand for 20 min), as well as to analyze their impacts on the physiological quality of okra seed lots. After the treatments were applied, images of the outer seed coat were obtained and the moisture content of the seeds, first germination count, germination, emergence, germination speed index and seedling length were measured. The treatments of pre-soaking, thermotherapy and thermal shock remove cell layers in the chalaza region, allowing a greater water absorption and showing to be efficient in overcoming dormancy, without affecting the physiological quality of the seeds. On the other hand, the dry heat treatment can affect the vigor of okra seeds.

KEYWORDS: *Abelmoschus esculentus* L., hard seeds, integumentary hardness, seed vigor.

RESUMO

Métodos de superação de dormência afetam a qualidade fisiológica de sementes de quiabo?

Um dos principais entraves na produção de quiabo é a dormência física imposta pelo tegumento das sementes. Embora vários métodos de superação de dormência tegumentar tenham sido propostos, pouco se sabe sobre o efeito destes na qualidade fisiológica de sementes. Assim, objetivou-se verificar a eficácia de métodos de superação de dormência (controle - sem tratamento; pré-embebição - embebição das sementes em água a 30 ºC, por 6 h; termoterapia - imersão em água a 60 ºC, por 3 min; calor seco - estufa a 70 ºC, por 5 min; choque térmico - imersão em água a 30 ºC, por 30 min, seguida de 5 ºC, por 24 h; e escarificação em areia - fricção entre as sementes e a areia por 20 min) e analisar seus impactos na qualidade fisiológica de lotes de sementes de quiabo. Após a aplicação dos tratamentos, foram obtidas imagens do tegumento externo das sementes e aferidos o grau de umidade das sementes, primeira contagem de germinação, germinação, emergência, índice de velocidade de germinação e comprimento de plântula. Os tratamentos de pré-embebição, termoterapia e choque térmico removem camadas de células da região da chalaza, permitindo maior absorção de água, mostrando-se eficientes na superação da dormência, sem afetar a qualidade fisiológica das sementes. Por outro lado, o método do calor seco pode afetar o vigor das sementes de quiabo.

PALAVRAS-CHAVE: *Abelmoschus esculentus* L., sementes duras, dureza tegumentar, vigor de sementes.

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is an important vegetable of the Malvaceae family that has a good commercial acceptance (Sun et al. 2023). Besides the use of the pods for human consumption, its leaves can be used to prepare salads, while the seeds are mainly employed in the production of oils and in medicinal treatments, due to their beneficial properties in fighting intestinal diseases, colon cancer, and in reducing the risk of blood clots (Elkhalifa et al. 2021).

Brazil has an advantage in its production, because it is a vegetable that adapts well to tropical and subtropical climates (Dantas et al. 2021). However, one of the main problems that producers face is seed dormancy, which is characterized by a temporary blockage to seed germination, even when exposed to ideal conditions of water, temperature and oxygen (Sohindji et al. 2020). Okra seeds exhibit physical dormancy, in which the tegument acts as a barrier to water absorption, delaying germination (Marcos Filho 2015).

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Seeds with water-impermeable integuments usually show absent or slow and irregular emergence, generating seedling unevenness in the field (Leite et al. 2019). According to Baskin & Baskin (2014), the physical dormancy, as impermeability and tegument hardness, is due to the presence of a waxy layer, lignin deposition and fatty acids in the palisade cell layers, blockage of the hilar cleft in dry seeds and/or low density of pores in the superficial layers. The deposition of various substances, such as suberin, lignin, cutin, tannin, pectin and quinone derivatives, also contributes to tegument impermeability (Nicolás-García et al. 2022). The dormancy imposed by the tegument of okra seeds is caused by the structure of the palisade cells, with the outermost layer of the tegument being rich in hydrophilic substances and the innermost highly lignified, with a transition layer between them (Valentine et al. 1992).

Although the phenomenon of dormancy allows nature to distribute germination over time, promoting and ensuring the survival of species in different situations of adversity (Zhang et al. 2020), for the agricultural production, it is a serious problem. An alternative that can overcome this problem in okra seeds is the adoption of treatments to overcome the integument hardness without compromising the physiological quality of the seeds.

Several methods have been employed and adopted for the overcoming of dormancy in hard seeds, such as pre-soaking, where seeds are soaked in water at 30 ºC, for 6 h (Martins et al. 2011); thermotherapy, which consists of soaking the seeds in water at 60 ºC, for 3 min (Martins et al. 2011); use of dry heat by heating the seeds in an oven at 70 ºC, for 5 min (Erickson et al. 2016); and mechanical scarification in the integument by means of fine sand (Roversi et al. 2002). In addition to these, seed production companies in Brazil, such as Isla Sementes (unpublished data), have recommended the use of heat shock, soaking the seeds for 30 min in water at 30 ºC, subsequently followed by exposure to low temperatures (5 ºC) for 24 h.

However, the application and efficiency of these methods depend on the cause and degree of dormancy, which are quite variable among species. Furthermore, depending on the method applied, even if it is effective in overcoming dormancy, it may damage the vigor and viability of the seeds and may not be recommended as a pre-treatment for planting.

Thus, this study aimed to verify the effectiveness of the main methods for overcoming dormancy and analyze their impact on the physiological quality of okra seed lots.

MATERIAL AND METHODS

The study was carried out at the Universidade Estadual de Maringá, in Umuarama, Paraná state, Brazil (23º47'S and 53º15'W), in 2022.

Seeds of three commercial lots of the Santa Cruz 47 okra cultivar were used. The seeds were obtained under the same growing conditions by the Isla Sementes company. The recently harvested seeds were subjected to six treatments to overcome dormancy: T0: control - seeds without any pre-treatment; T1: pre-soaking by immersing the seeds in water at a temperature of 30 ºC, for 6 h; T2: thermotherapy by soaking the seeds in water at 60 ºC, for 3 min; T3: dry heat, with the conditioning of the seeds in an oven at 70 ºC, for 5 min; T4: heat shock, with the immersion of the seeds in water at 30 ºC, for 30 min, and then conditioned in refrigeration at 5 ºC, for 24 h; T5: scarification of the seeds in the sand, for 20 min. For the scarification, the proportion of one part of seeds to three parts of fine sand was used, obtained by a round wood rim sieve, with 3.35 mm mesh six.

After the application of the pre-treatments, the seeds were subjected to the following tests and conditions:

Moisture content: determined before the beginning of the tests and after the application of the treatments to overcome dormancy. To achieve that, the oven method was employed at 105 ºC, for 24 h (Brasil 2009), with two replications of 50 seeds performed per treatment;

First germination count: performed together with the germination test, computing the average percentage of normal seedlings on the fourth day after the test (Brasil 2009);

Germination test: the seeds were placed to germinate on two sheets of *germitest* paper moistened with distilled water equivalent to 2.5 times the weight of the dry substrate, using plastic boxes (11.0 x 11.0 x 3.5 cm). These were kept in a Biochemical Oxygen Demand (BOD) germination chamber with alternating temperatures of 20-30 ºC and a photoperiod of 8-16 h. The evaluations were performed as established by measuring the percentage of normal seedlings at 21 days (Brasil 2009), and also by analyzing the germination at 8 days after sowing. A normal seedling was considered the one that had all the essential structures of a seedling and showed absence of abnormalities along with the potential for development in the field;

Germination speed index: performed together with the germination test, with daily evaluations of the number of germinated seeds. Germinated seeds were considered to be those with root protrusion greater than 2 mm. The data from the daily counts were used to calculate the germination speed index, according to Maguire (1962);

Seedling length: performed together with the germination test, taking the average length of 10 normal seedlings per replication. To avoid a tendency to select seedlings, among the 50 seeds that were planted, it was stipulated that the 10 seedlings from the central row were measured for length with a graduated ruler at 8 days after sowing. They were measured from the root base to the shoot apex and the result was expressed in cm seedling^{-1};

Emergence: using five replications of 20 seeds, sowing one seed per cell in trays, 1 cm deep, in a substrate suitable for the production of vegetable seedlings (Plantmax). The trays were kept in a germinator at 25 ºC, for 8 days. After this period, the percentage of emerged seedlings was evaluated;

Tegument images: using four replications of 10 seeds per treatment for each lot. After applying the treatments, images of the tegument regions were obtained. By visual evaluation, differences among the treatments were observed in the chalaza region and in the external color of the seeds. Thus, photographs of the chalaza region and the region opposite to the chalaza were taken. The images were captured using a digital microscope, model CIF 26445, with 1000x optical zoom;

Statistical analyses: The experiment was performed in a 6 x 3 factorial scheme (six treatments and three lots), in an entirely randomized design, with four replications. The data were submitted to analysis of variance, using the F test, and the means compared by the Scott-Knott test at 5 % of probability, using the R software (R Core Team 2023).

RESULTS AND DISCUSSION

All the treatments affected the seed dormancy overcoming; however, there was a significant difference between treatments and seed lots (Figure 1).

There was an interaction effect between treatments and lots for the variables germination, germination at 8 days and germination speed index, while the interaction was not significant for the first germination count, emergence and seedling length.

It was evident that there was a difference in the germination and vigor of the seeds among the seed lots before the application of the treatments. By analyzing the tests of first count, length and seedling emergence, it was possible to notice that the lot 3 had a higher physiological quality than the lots 1 and 2 (Figure 1). All the overcoming dormancy treatments increased the percentage of normal seedlings on the date of the first count. However, T1 (soaking in water at 30 ºC/6 h) presented the best performance to seedling length, if compared to the other treatments. On the other hand, T3 (dry heat in oven at 70 ºC/ 5 min) was the only one that did not increase the seedling emergence, being statistically equal to the emergence of T0 (control).

In the control treatment, that is, without the application of treatments to overcome dormancy, the seeds from the lot 1 presented a germination of 83 %, not differing from that of the lot 3 (89 %), being superior to the germination of the lot 2 (58 %). For germination at 8 days, a similar result was observed, with germination of 80 % to lot 1, 54 % to lot 2 and 89 % to lot 3. So, it was observed that, after 8 days of sowing, the seedlings already had their essential structures, as the roots and shoots developed, not requiring 21 days, as recommended by Brasil (2009). Furthermore, the germination speed index was higher for the lot 3, when compared to the lots 1 and 2 (Figure 1).

It was also observed that the lots presented different degrees of dormancy, being evident that the seeds from the lot 2 presented a deeper dormancy than those from the lots 1 and 3 (Figure 1). When the dormancy breaking treatments were applied, the germination of the lot 1 increased from 83 to approximately 95 %, regardless of the pre-treatment applied. For the seeds of the lot 2, the germination increased from 58 to 96 % after T1 (soaking in water at 30 ºC/6 h) to approximately 80 % after the treatments T2 (water heat treatment at 60 ºC/3 min), T3 (dry heat in oven at 70 ºC/5 min) and T4 (heat shock at 30 ºC/30 min, followed by 5 ºC/24 h); and to 67 % after T5 (sand scarification/20 min). Concerning

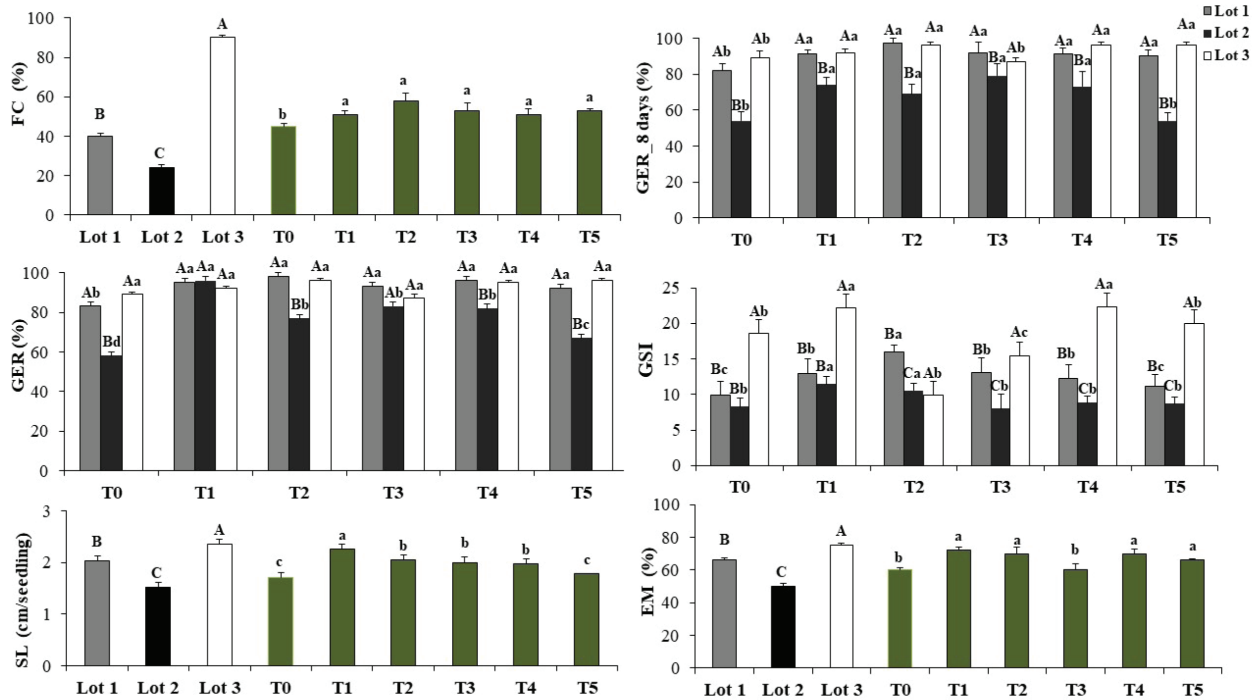

Figure 1. First germination count (FC), germination at 8 days (GER_8 days), germination at 21 days (GER), germination speed index (GSI), seedling length (SL) and emergence (EM), when applied the pre-treatments T0 (untreated/control), T1 (soaking in water at 30 ºC/6 h), T2 (thermotherapy at 60 ºC/3 min), T3 (dry heat in oven at 70 ºC/5 min), T4 (heat shock at 30 ºC/30 min, followed by 5 ºC/24 h) and T5 (sand scarification/20 min) to seeds of three lots of *Abelmoschus esculentus*. Averages followed by the same capital letter, comparing lots, and lowercase letter, comparing treatments, do not differ from each other by the Scott-Knott test at 5 % of probability.

the lot 3, although the treatments did not have a significant effect on the increase in germination (89 %), there was an increase in the germination speed index after the application of the treatments T1 and T4, suggesting that this lot presented a light level of dormancy. For this lot, the T3 treatment was harmful, as it reduced the seed germination speed.

Therefore, the lot that presented a greater dormancy (lot 2) also presented a greater germination after the application of T1 and a higher germination speed after applied the T1 and T2 treatments. The lot that showed light dormancy (lot 3) had no increase in the germination of the seeds with the application of the pre-treatments. However, the germination speed index increased after applied the treatments T1 and T4 and impaired after the T3 treatment. On the other hand, the lot 1, which presented intermediate dormancy, had germination increases by all the treatments applied, but presented the highest germination speed index, after the T2 treatment.

Thus, it is clear that soaking the seeds in water at 30 ºC for 6 h (T1), conditioning the seeds in hot water at 60 ºC for 3 min (T2) and promoting the heat shock at 30 ºC for 30 min, followed by 5 ºC for 24 h (T4), were efficient treatments to overcome the dormancy of the lots of seeds, without affecting seed vigor (Figure 1), while the dry heat in oven at 70 ºC for 5 min (T3) caused a reduction in the vigor of the seeds of the lot 3.

According to Muimba-Kankolongo (2018), soaking hard seeds in warm or hot water may reduce the impermeability of the tegument and stimulate the germination process. The period that the seeds need to be soaked in water depends on the hardness of the seed tegument (Velempini et al. 2003). Meantime, high temperatures can cause the rupture of membranes and extravasation of primary metabolites that would be used in the germination and seedling development (Machado 2000). It also induces cell dehydration and affects enzyme activation, leading to a decline in physiological quality, as observed for dormant millet seeds (Baskin et al. 2000) and okra seeds after the dry heat treatment (T3) (Figure 1).

In order to check the effectiveness of the treatments, it is essential to verify the water content

Table 1. Moisture content of each lot of *Abelmoschus esculentus* seeds, before and after the application of treatments to overcome dormancy.

Treatments	Moisture content (%)					
	Lot 1		Lot 2		Lot 3	
	Before	After	Before	After	Before	After
T0		11.8		10.3		12.8
T1		15.6		18.2		22.2
T2	11.7	13.3	10.4	12.5	12.9	15.0
T3		11.0		9.8		12.0
T4		12.6		13.3		15.0
T5		11.5		10.4		13.0

T0: control without treatment; T1: soaking in water at 30 ºC/6 h; T2: thermotherapy at 60 ºC/3 min; T3: dry heat in the oven (70 ºC/5 min); T4: heat shock (30 ºC/30 min, followed by 5 ºC/24 h); T5: sand scarification for 20 min.

of the seeds. The moisture content of the seeds was different between lots and treatments (Table 1).

It was observed that the lot with the lowest initial moisture content presented more dormancy, and the one with the highest moisture content had less dormancy (Table 1). Thus, it is suggested that the greater impermeability of the tegument in the lot 2 may have reflected in the lower moisture gain of the seed and, consequently, in a greater difficulty in water absorption, reflecting in a lower percentage of germination in the control treatment (58 %) (Figure 1). According to Lamont & Pausas (2023), the impermeability to water presented by the tegument of the seeds is genetically governed and few genes seem to be related to this inheritance. On the other hand, these same authors consider that the environment and the conditions under which the seeds were produced can influence the permeability of the tegument, what may justify the differences in the dormancy degrees observed in the seed lots.

It was also found that the treatments T1, T2 and T4, which were effective to overcome dormancy (Figure 1), were the ones that promoted a greater moisture gain by the seeds (Table 1). However, T5, which was not the most effective to overcome the seed dormancy of the lot 2, did not change the moisture content of the seeds, while T3 provided a slight reduction in the water content of the seeds and caused damage to the seed quality of the lot 3 (Table 1; Figure 1).

Moreover, by observing the images obtained by the digital microscope, it was possible to notice visual differences among the seeds after the application of the treatments. There were differences in the chalaza region and in the integument color, demonstrating visible differences among the treatments (Figure 2).

Okra seeds are rounded, albuminous, with the presence of chalaza traces. The seed coat of the Santa Cruz cultivar is slightly rough and formed by two layers of sclerenchyma cells arranged in a palisade (Martinelli et al. 2022). In figure 2A, it is possible to visualize differences among the treatments in the chalaza region. It was noted that the treatments 1, 2 and 4 removed totally (1) or partially (2 and 3) the layers of cells in this region, and also that the seeds submitted to the treatments 1 and 2 were

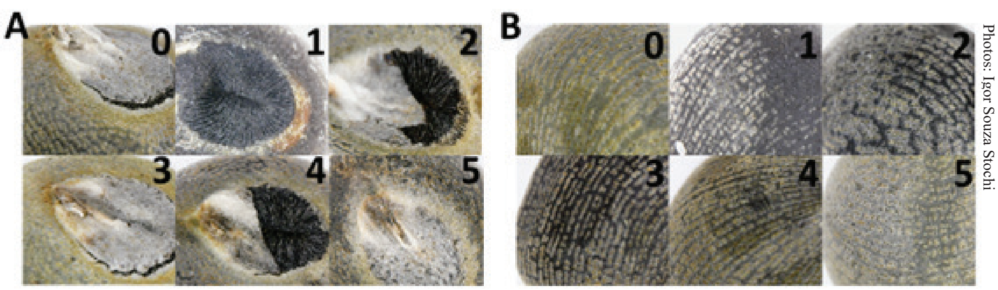

Figure 2. Chalaza region of the integument (A) and external region of the tegument, opposite side to the chalaza (B). T0 (0): control, no treatment; T1 (1): soaking in water at 30 ºC/6 h; T2 (2): thermotherapy at 60 ºC/3 min; T3 (3): dry heat in oven at 70 ºC/5 min; T4 (4): heat shock at 30 ºC/30 min, followed by 5 ºC/24 h; T5 (5): sand scarification for 20 min.

darker (Figure 2B), due to a greater water absorption (Table 1).

Serrato-Valenti et al. (1992) stated that water permeability occurs through the hilar region of the okra tegument, and that the region that controls the onset of water entry is the chalaza area. However, Villavicensio et al. (2007) reported that the mesophyll cell layer in okra is highly lignified and may contribute to integument hardness, and also emphasized that, in okra, the different degrees of permeability and eventual dormancy may be due to the number of substances such as lignins in the integument, especially in the hilar region. Lignins were found abundantly in the mesophyll layer of the hilar region and other sections of the integument of all okra species (Villavicensio et al. 2007). This presents a hydrophobic nature, playing an essential role in the water transport and mechanical resistance of plants. It also acts directly on the permeability and protection of tissues against environmental fluctuations, being responsible for possible changes in the water absorption speed (Zhao & Dixon 2011). Thus, dormancy in okra seeds is related to the deposition of various substances such as suberin, lignin, cutin, tannin, pectin and quinone derivatives, both in the testa and pericarp and nucellar membrane during seed formation, and these compounds, according to Nicolás-García et al. (2022), contribute to tegument impermeability.

The tegument impermeability, particularly the internal tangential walls of the endothelial cells, hinders water penetration in hard seeds (Lamont & Pausas 2023). According to Paula et al. (2012), water absorption is performed by the chalaza cap, which is covered by a cap made of permeable tissue, which projects downward into the chalaza cap. The authors also mention that the exposure of hard seeds to high temperatures promotes the overcoming of seed physical dormancy, allowing the entry of water and, consequently, their germination.

According to Mahajan et al. (2023), water has a strong influence on the germination process, and it has been observed that, in seeds in which the pre-germination treatment is used by pre-soaking in water, germination occurs quickly and uniformly. In fact, the beneficial effect of pre-soaking on germination speed is directly related to water uptake during the first stage of the germination process (Martins et al. 2011).

As observed in the present study, soaking the seeds in warm water (30 ºC/6 h), thermotherapy (60 ºC/3 min) and heat shock did not compromise their viability and vigor, while the application of dry heat impaired their physiological quality (Figure 1).

CONCLUSIONS

1. The treatments of pre-soaking (30 ºC for 6 h), thermotherapy (60 ºC for 3 min) and thermal shock (soaking in water at 30 ºC for 30 min, followed by 5 ºC for 24 h) remove cell layers in the chalaza region, allowing a greater water absorption by the seeds. These treatments are efficient in overcoming dormancy, without affecting the seed physiological quality;
2. The dry heat treatment (oven at 70 ºC for 5 min) can affect the vigor of okra seeds.

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