# PHYSIOLOGICAL CONDITIONING AS A SEED VIGOR IMPROVER: A META-ANALYSIS 

# CONDICIONAMENTO FISIOLÓGICO COMO FERRAMENTA PARA AUMENTAR O VIGOR DE SEMENTES: UMA METAANÁLISE 

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## RESUMO

O setor agrícola vem ganhando destaque tanto no cenário brasileiro quanto no mundial, e o setor de sementes tem sido muito valorizado. Técnicas para otimizar o processo de produção de sementes têm sido cada vez mais buscadas com o objetivo de reduzir a perda de tempo e, consequentemente, aumentar o lucro dos produtores. Nesse sentido, o condicionamento fisiológico de sementes serve como exemplo de uma técnica que acelera e padroniza o processo de germinação. Com o objetivo de reunir resultados de experimentos nessa área, este estudo foi desenvolvido por meio da técnica de meta-análise, que investiga se o condicionamento fisiológico interfere na porcentagem de germinação das sementes com ou sem exposição ao estresse. Uma revisão sistemática foi realizada por meio de pesquisa bibliográfica nas bases de dados Web of Science e Periódicos Capes, utilizando como palavras-chave priming, osmocondicionamento, hidrocondicionamento, estresse ambiental, metabolismo e hidratação. Foram estabelecidos como moderadores as diferentes culturas, condicionamentos e estresses induzidos. Por meio disso, foi possível observar que o condicionamento fisiológico não apresentou resultados significativos, independentemente do moderador utilizado. Embora seja uma técnica promissora, a ausência de resposta das sementes utilizadas se deve ao fato de que as sementes comerciais são geneticamente melhoradas e, consequentemente, já possuem maior vigor, o que não justifica o uso dessa técnica para sementes de alta qualidade.
Palavras-chave: Tamanho do efeito; culturas agrícolas; moléculas; estresse abiótico; germinação.


#### Abstract

The agricultural sector has been gaining ground in both Brazilian and worldwide scenarios, with the seed sector highlighted. Techniques for optimizing seed production processes have been increasingly targeted to reduce time loss and consequently increase producer profit. Thus, seed physiological conditioning serves


as an example of a technique that accelerates and standardizes the germination process. Aiming to reunite the results from experiments regarding this area, this study was developed through a meta-analysis technique, which investigates whether physiological conditioning interferes with the seed germination percentage with or without stress exposure. A systematic review was carried out by a literature search of the Web of Science and Periódicos Capes databases using priming, osmoconditioning, hydroconditioning, environmental stress, metabolism, and hydration as keywords. Different cultures, conditioning, and induced stresses were established as moderators. Through this, it was possible to observe that physiological conditioning has not shown significant results, independent of the moderator used. Although being a promising technique, the absent response from seeds used is because commercialized seeds are genetically improved and, consequently, already have already higher vigor, which does not justify the use of this technique for high-quality seeds.
Keywords: Effect size; crop cultures; molecules; abiotic stress; germination.

## INTRODUCTION

Using seed as a technology is a practice that has been developed since the 1970s. To reach this objective, researchers initially improved the seed germination process with pregermination treatments involving metabolism initiation. Among the developed procedures, physiological conditioning stands out, especially due to its benefits for seed and seedling performance ${ }^{1}$. The term seed physiological conditioning is used by the seed industry and research to comprise techniques that aim to improve or benefit the performance of seed lots or produced seedlings. The main objective of this technique is to ensure seed germination uniformity and seedling establishment ${ }^{(1-3)}$.

Physiological conditioning consists of improving seed quality for those with deterioration, which results in effects on vigor, allowing better use for these seeds ${ }^{(4)}$. This method induces repair of seed cell membranes, improving seed batch vigor ${ }^{(1,2)}$. Once the on-field germination time is reduced and uniformized, this method allows germination under variable conditions of temperature, light, soil, and water availability, also allowing higher development of aerial parts and plant growth ${ }^{(5)}$. Metabolic activity during conditioning includes repair of mRNA, hydrolytic enzymes and other macromolecules that act in reserve mobilization ${ }^{(3)}$.

Since it was once a theme with extreme importance and easy scientific conduction, physiological conditioning has generated a large number of publications that may be a problem for literature selection and qualified analysis. However, this problem is necessary, beneficial, and essential for knowledge evolution. On the other hand, the large volume of information may impose difficulties for problem contextualization with errors in interpretation and analysis ${ }^{(6)}$. Due to the exposed, a meta-analysis allows researchers to determine factors that have contributed to systematic differences among the studies and to identify neglected areas. In addition, meta-analysis allows us to compare research results not only regarding the substantive aspect but also primarily regarding the methodologic procedures and estimate specific technical measures that are related to a certain pattern of conclusion ${ }^{(7)}$. Given the above exposure, this research was conducted to investigate whether seed physiological conditioning interferes with germination with or without stress exposure through a meta-analysis technique.

## METHODOLOGY

A systematic review of research was carried out in the literature in the Web of Science and Periódicos Capes databases using the terms priming, osmoconditioning, hydroconditioning, environmental stress, metabolism, and hydration. As selection criteria were defined, only studies conducted in Brazil since 1998 and those that reported average and standard deviation values related to germination percentage were used. All reports that did not meet these criteria were excluded. As moderators, we defined cultures, conditioning, and induced stresses.

The effect size was calculated for each studied case as the standardized average difference (d) obtained through Equation 1A. Variance (vd) was also calculated through Equation 1B. We defined that positive values for d represent treatment germinating more than the witness, with the contrary represented by negative values, indicating witness superiority. Effect size was calculated by using random effect models for each response variable. These models were used because
they attribute the effect distribution to real differences among the cases and do not presume that sampling error is the only source of differences. Maximal and minimal values were calculated to determine if the effect size would be different in a normal distribution ${ }^{(8)}$.

$$
\begin{gathered}
d=\frac{(X 1-X 2)}{\text { swithin }} \\
\mathrm{vd}=\frac{(n 1+n 2)}{(n 1 \times n 2)}+\frac{d 2}{2(n 1+n 2)}
\end{gathered}
$$

Equation 1. Equation used to calculate the standardized average difference. Caption: X1 $=$ treatment average, $\mathrm{X} 2=$ average for the witness; Swithin $=$ standard deviation parameter (considering standard deviation, sampling size for both witness and treatment), $\mathrm{n} 1=$ error for the treatment, $\mathrm{n} 2=$ error for the witness.

## RESULTS AND DISCUSSION

Through the defined inclusion criteria, a total of 52 cases (Supplementary Table 1) were found. The effect size aims to observe group characteristics, such as how the population behaves. For the effect size, a value of -0.006 with maximal and minimal limits of -0.17 and 0.16 , respectively, was found, which indicates that conditioner use in a general way does not interfere in seed germination because the study was not significant through the meta-analysis test. We can observe that the effect size was not significant and can be attributed to a few facts, such as the seed quality used in the studies. For most experiments, seeds used already came with some included technology, being genetically improved, with high vigor and sometimes abiotic stress tolerance.

Among the seed improvement techniques used, there is the selection of viable seeds with the best performance during the imbibition phase. For germination to occur, seeds need to reach a hydration level sufficient to allow metabolism reactivation and consequently embryo growth and radicle protrusion ${ }^{(3)}$. Water movement and availability have great importance for seed germination, initial root system growth, and seedling emergence. These factors are affected by
the seed characteristics and the colloidal substrate complex in which the seeds are located. In addition, imbibition phase success depends on hydric potential, i.e., the tension that water exerts over seeds and external media ${ }^{(5)}$.

To observe and study the effects of using conditioners individually, moderators were used, which in this case were the cultures, conditioners, and stresses. For conditioners, we separately analyzed the natural and synthetic conditions, and the established and described synthetic conditions were also analyzed. For the first case (Figure 1), cultures were isolated and established by species, grouping cultivars to observe the characteristics and behavior of that population. The culture moderator had no significant results. This factor may be directly linked to seed vigor because the lots had a high germination percentage. Thus, would be unlikely that with conditioning this value would be improved, and if overcome, would have a significant difference.


Figure 1. Effect size calculated for the culture moderator.

In genetic improvement programs, there is a demand for knowledge for the quantitative and qualitative characterization of seeds from different cultures, as this knowledge is responsible for introducing new cultivars into the market. Traditional varieties are replaced by newly improved materials and by technological packages aimed at higher productivity ${ }^{(9)}$ because, as observed through this research's results, all cultures already have higher germination rates.

The second moderator was the different conditioners used (Figure 2), which both natural and synthetic have no significant results. Natural molecules are part of the seed chemical constitution and are already present even before conditioning. The exogenous application is indifferent, justifying the lack of significance for this result. The synthetic molecules applied did not have significant results either, regardless of the fact that the seeds used already had high vigor.


Figure 2. Effect size calculated for the moderator conditioner. Synthetic conditioner is an average for the values calculated for $\mathrm{KNO}_{3}$, PEG 6000, and Stimulate.

Methods employed in physiological conditioning may differ among them. Variations consist of water supply forms, drying, rehydration, and germination stage reached during conditioning. Additionally, osmotic active chemical substances are used to control water uptake. The use of different conditioning molecules does not necessarily increase the final germination percentage, as seen in this research, and is not responsible for increasing germination speed and seedling hypocotyl development ${ }^{(4)}$.

Regarding the third moderator (Figure 3), independent of the stress to which the seed was subjected, the analysis result was not significant. Witness (without stress) had a higher value, which may justify this result, as it was difficult to overcome by any conditioning and/or stress, justified by the high vigor for the used species.


Figure 3. Effect size calculated for the moderator stresses.
Stress tolerance present in most arable seed species is based on the organisms' capacity to recover normal biological functions when they are inserted in an inappropriate environment to activate these functions and metabolic responses. This consists of an important adaptative strategy, maintaining seed viability for long periods in favorable conditions ${ }^{(10)}$.

Physiological conditioning is a technique that involves seed hydration control that is sufficient to activate metabolic processes essential to germination while avoiding radicle protrusion ${ }^{(3)}$. This technique aims mainly to homogenize the germination and, if possible, the number of germinated seeds. Thus, there was no significant effect through meta-analysis for germination in the found studies, once those are conducted in controlled conditions, which are ideal for seeds to germinate ${ }^{(11)}$.

Additionally, we can highlight that all studies reported that the water content was at acceptable values; thus, no interference in seed physiological quality occurred, reinforcing that, in addition to the environmental (external) conditions being ideal, internal ones were also ideal, allowing natural germination, which resulted in the conditioning being not significant enough to change germination results.

Germination variations in seeds submitted or not to conditioning are not expected, as according to Marcos Filho ${ }^{(12)}$, no difference in germination percentage under favorable environmental conditions between samples with or without osmotic conditioning is common because this treatment cannot recover seed germinative capacity or invigorate them, as these effects are expected in parameters regarding germination speed, seedling emergence, and normality.

In addition to the benefits of uniformizing lot performance, physiological conditioning also benefits the germination or seedling emergence speed at plant establishment and reduces seedling exposure to less favorable environmental conditions ${ }^{(11)}$.

One of the processes most used in an attempt to uniformize the germinative period is physiological conditioning, which comprehends seed imbibition at phases I and II without reaching phase III and is characterized by radicle protrusion ${ }^{(4,5)}$. The main conditioning techniques are osmoconditioning, hydroconditioning, and matriconditioning, which consist of the use of chemical products (such as polyethylene glycol 6000), water and solid matrix, respectively, to control seed imbibition ${ }^{(13)}$.

Thus, physiological conditioning is a promising technique that allows seeds to experience the necessary conditions for uniform germination. This mechanism reduces the time that seeds are exposed to the field until germination, reducing chances of injuries and allowing producers to gain time in the process. In addition, this technique standardizes the germination process, increasing vigorous seedlings to be obtained in less time ${ }^{(4)}$.

## CONCLUSIONS

The meta-analysis carried out here allowed us to sort studies conducted in Brazil for many crop cultures.

Physiological conditioning has no significant results, independent of the conditioning, stress, or culture studied.

Studies have shown that physiological conditioning has a direct influence on seed vigor.

The lack of significant influence of conditioning in the observed studies may be the result of the genetic improvement applied in the seeds used.

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Supplementary Table 1. List of scientific papers reporting studies on osmoconditioning, hydroconditioning, environmental stress, metabolism, and hydration according to the criteria established for the present research.

| Case | Species | Priming | Origin | Concentration | Purpose of the study | Stress | Germination (\%) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  | Witness | Treatment |
| Balbinot \& Lopes ${ }^{14}$ | Carrot | PEG 6000 | Synthetic | -0.4 MPa | Evaluated the effect of osmotic conditioning and drying of carrot seeds cv. Brasília on germination and vigor. | Drying | 86 | 86 |
|  |  |  |  | $-0.8 \mathrm{MPa}$ |  | Drying | 86 | 86 |
|  |  |  |  | $-0.4 \mathrm{MPa}$ |  | Drying | 84 | 78 |
|  |  |  |  | $-0.8 \mathrm{MPa}$ |  | Drying | 84 | 82 |
|  |  |  |  | $-0.4 \mathrm{MPa}$ |  | Drying | 91 | 83 |
|  |  |  |  | $-0.8 \mathrm{MPa}$ |  | Drying | 91 | 87 |
|  |  |  |  | $-0.4 \mathrm{MPa}$ |  | Drying | 87 | 84 |
|  |  |  |  | $-0.8 \mathrm{MPa}$ |  | Drying | 87 | 89 |
| Gurgel Junior et $\mathrm{al}^{15}$ | Cucumber | Distiled Water | Natural | - | Evaluated the physiological quality | No-stress | 93 | 98 |
|  |  |  |  | - | of cucumber seeds, cv. Aodai, aiming to improve the speed and uniformity of germination, using hydroconditioning. | Drying | 93 | 96 |
| Kikut et al ${ }^{16}$ | Bell pepper | PEG 6000 | Synthetic | -1.10 MPa | Evaluate the efficiency of physiological conditioning on the performance of sweet pepper seeds. | No-stress | 75 | 71 |
| Marcos Filho \& Kikuti ${ }^{11}$ | Cauliflower | Distilled water | Natural | - | Evaluate the effects of physiological | No-stress | 98 | 99 |
|  |  |  |  | - | seed conditioning as well as the | Drying | $98$ | 98 |
|  |  |  |  | - | development and final production of | No-stress | 96 | 96 |
|  |  |  |  | - | plants from conditioned seeds. | Drying | 96 | 96 |
| Costa et al ${ }^{17}$ | Coriander | Distilled water | Natural | - | Evaluate the efficiency of different methods of physiological conditioning in coriander seeds. | No-stress | 84 | 92 |
|  |  | PEG 6000 | Synthetic | -0.4 MPa |  | No-stress | 84 | 78 |
|  |  | PEG 6000 | Synthetic | $-0.8 \mathrm{MPA}$ |  | No-stress | 84 | 83 |

To be continued ...

Continuation:
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| Case | Species | Priming | Origin | Concentration | Purpose of the study | Stress | Germination (\%) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  | Witness | Treatment |
| Araújo et al ${ }^{18}$ | Gherkin | Distilled water | Natural | - | Evaluate the effects of conditioning on the germination and vigor of gherkin seeds. | No-stress | 80 | 82 |
|  |  | Distilled water | Natural | - |  | No-stress | 80 | 87 |
|  |  | Distilled water | Natural | - |  | No-stress | 64 | 78 |
|  |  | Distilled water | Natural | - |  | No-stress | 64 | 61 |
| Silva \& Nascimento ${ }^{19}$ | Pumpkin | PEG 6000 | Synthetic | -0.3 MPa | To evaluate the influence of two | Temperature | 87 | 90 |
|  |  | KNO3 | Synthetic | -0.3 MPa | osmotic solutions in 'Brasileirinha' pumpkin seeds on germination at ideal and adverse temperatures. | Temperature | 87 | 99 |
| Armondes et $\mathrm{al}^{20}$ | Cabbage | PEG 6000 | Synthetic | -1.0 MPa | Evaluate the performance of cabbage seeds, with different quality levels, after osmotic conditioning with PEG solution, for different periods, followed or not by drying. | No-stress | 85 | 90 |
|  |  | PEG 6000 | Synthetic | $-1.0 \mathrm{MPa}$ |  | Drying | 85 | 88 |
|  |  | PEG 6000 | Synthetic | $-1.0 \mathrm{MPa}$ |  | No-stress | 98 | 98 |
|  |  | PEG 6000 | Synthetic | -1.0 MPa |  | Aging 42 h | 58 | 73 |
| Costa Zonetti et $\mathrm{al}^{21}$ | Cotton | PEG 6000 | Synthetic | -0.60 MPa | Evaluate the osmotic conditioning on the physiological quality of colored fiber cotton seeds of different varieties. | No-stress | 58 | 66 |
|  |  | Distilled water | Natural | - |  | No-stress | 58 | 63 |
|  |  | PEG 6000 | Synthetic | $-0.60 \mathrm{MPa}$ |  | No-stress | 61 | 53 |
|  |  | Distilled water | Natural | - |  | No-stress | 61 | 45 |
|  |  | PEG 6000 | Synthetic | -0.60 MPa |  | No-stress | 80 | 62 |
|  |  | Distilled water | Natural | - |  | No-stress | 80 | 73 |
|  |  | PEG 6000 | Synthetic | $-0.60 \mathrm{MPa}$ |  | No-stress | 71 | 49 |
|  |  | Distilled water | Natural | - |  | No-stress | 71 | 51 |

To be continued ...

Continuation:
Supplementary Table 1. List of scientific papers reporting studies on osmoconditioning, hydroconditioning, environmental stress, metabolism, and hydration according to the criteria established for the present research

| Case | Species | Priming | Origin | Concentration | Purpose of the study | Stress | Germination (\%) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  | Witness | Treatment |
| Rodrigues et $\mathrm{al}^{22}$ | Lettuce | Distilled water | Natural | - | Evaluate imbibition, the effects of hydroconditioning on paper, by immersion in distilled water and osmoconditioning in the improvement of lettuce seed vigor and its associated use of sodium hypochlorite solution. | No-stress | 33 | 42 |
|  |  | Distilled water | Natural | - |  | No-stress | 33 | 35 |
|  |  | PEG 6000 | Synthetic | $-1.50 \mathrm{MPa}$ |  | No-stress | 33 | 66 |
|  |  | Distilled water | Natural | - |  | No-stress | 64 | 0 |
|  |  | Distilled water | Natural | - |  | No-stress | 64 | 57 |
|  |  | PEG 6000 | Synthetic | $-1.50 \mathrm{MPa}$ |  | No-stress | 64 | 64 |
| Pereira et al ${ }^{23}$ | Tomato | PEG 6000 | Synthetic | $-0.8 \mathrm{MPa}$ | To evaluate the effects of osmotic | Aging 48 h | 90 | 93 |
|  |  | PEG 6000 | Synthetic | -0.8 MPa | conditioning on germination and vigor of artificially aged tomato seeds. | Aging 96 h | 90 | 90 |
| Oliveira et al ${ }^{24}$ | Cotton | PEG 6000 | Synthetic | -0.4 MPa | To study the effects of drying after | Drying | 31 | 47 |
|  |  | PEG 6000 | Synthetic | -0.4 MPa | conditioning on the physiological | Drying | 67 | 85 |
|  |  | PEG 6000 | Synthetic | -0.4 MPa | quality of seeds of three cotton cultivars. | Drying | 84 | 94 |
| Menezes et al ${ }^{25}$ | Oat | Salicylic acid | Natural | $100 \mathrm{mg} / \mathrm{L}$ | To evaluate the effect of oat seed conditioning with salicylic acid on | Without stress | 93 | 83 |
|  |  | Salicylic acid | Natural | $100 \mathrm{mg} / \mathrm{L}$ | germination and seedling development. | Without stress | 89 | 89 |
| Ramos et al ${ }^{26}$ | Bean | Distilled water | Natural | - | Evaluate the initial growth of seedlings and the physiological | Drying | 89 | 94 |
|  |  | Stimulate | Synthetic | 0.1\% | quality of bean seeds submitted to physiological conditioning and seed treatment with biostimulant. | Drying | 89 | 90 |

