Effects of hydropriming on maize seeds (*Zea mays* L) on growth, development, and yield of crops

Efecto del hidroacondicionamiento de semillas de maíz (*Zea mays* L) en el crecimiento, desarrollo y rendimiento del cultivo

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ABSTRACT

Seed germination is a process that involves several phases, beginning with the uptake of water by dry seeds and ending with emergence. Based on current knowledge, several methodologies have been developed to manipulate this process in order to produce beneficial effects on crops. The hydropriming of maize seeds is one technique that has been used to lower the in-field germination time. The objective of the present study was to measure the effect of different hydropriming times on maize seeds and the subsequent growth, development, and yield of plants. The results demonstrated that hydroprimed seeds for 12 and 18 hours, germinated more rapidly in comparison with the control and 36-hour treatment. Yield was also affected as a function of the imbibition time. The generated data allowed for an optimal soaking time of 22.12 hours to be determined, resulting in an estimated yield of 16.6 t per hectare.

Keywords
hydropriming • Seed corn • germination of seed corn

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RESUMEN

La germinación de las semillas es un proceso que inicia con la toma de agua por la semilla seca y concluye con la emergencia. Con base en los conocimientos actuales se han desarrollado metodologías para manipular este proceso y obtener efectos benéficos en los cultivos. El hidroacondicionamiento de las semillas de maíz ha sido utilizado para obtener menores tiempos de germinación en campo. El objetivo del presente trabajo fue medir el efecto de diferentes tiempos de hidroacondicionamiento en agua de semillas de maíz sobre su crecimiento, desarrollo y rendimiento. Los resultados mostraron que las semillas hidroacondicionadas por 12 y 18 horas germinaron más rápidamente en comparación con el tratamiento testigo y 36 horas. El rendimiento fue afectado en función de los tiempos de imbibición. Los datos permitieron estimar un tiempo óptimo de imbibición que fue de 22,12 horas con lo que se obtiene un rendimiento estimado de 16,6 toneladas por hectárea.

Palabras clave
Hidroacondicionamiento • semillas de maíz • germinación de semillas de maíz

INTRODUCTION

Germination begins with the uptake of water by a dry seed (imbibition) and ends when a portion of the seed (the embryonic axis in dicotyledons or the radicle in monocotyledons or gymnosperms) emerges from the surrounding structure, known as the emergence phase (15). The uptake of water by seeds is triphasic and begins with the rapid initial absorption of water (phase I), followed by a plateau phase (phase II). Finally, a subsequent increase in water absorption (phase III) corresponds with the elongation of the embryonic axis and the aperture of the surrounding sheath (17). During these phases, important physiological changes occur assuring the survival of the seedling. These events have been widely studied by different authors. For example, during phase I, the structures damaged during the previous dehydration phase are repaired, and during phase II, protein synthesis resumes (4). The duration of each phase is variable and depends on seed-specific characteristics, including size, content of hydratable substrates, permeability of seed covering, and available O₂ and CO₂, in addition to other external conditions during imbibition, such as temperature, substrate composition, and moisture content (19).

For sowed crops, large volumes of water are commonly used to provide optimal germination conditions. However, during this period, water losses may also be significant due to a lack of vegetation cover, leading to greater water evaporation. Consequently, recently emerged seedlings experience a greater level of stress. In this context, Mullan and Reynolds (2010) list several genotypes that are capable of rapidly developing their leaf area, increasing the surface of shaded soil and decreasing water evaporation. This results in a more efficient use of water. In this sense, it is preferable that seeds initiate the imbibition-germination process as quickly
as possible (7), as this favours a more efficient usage of water and minimizes the time of exposure to pathogens and other adverse environmental factors present in agricultural systems. In addition, this technique can improve other corn production systems such as corn silage, which represents an important alternative in several countries of America (24).

Based on current knowledge, various methods have been attempted for manipulating imbibition and germination to obtain beneficial effects on crops. One of the most studied techniques for achieving such benefits is hydropriming. In this method, seeds are placed in contact with water or an osmotic solution to initiate the imbibition process, but without arriving at the germination stage (12).

Several studies have shown this method to be effective in increasing the germination percentage and vigour of seedlings. A study spanning back several decades, as well as a more recent investigation (26) that tested four methods of hydroconditioning, found a resultant improvement in the germination and vigour of okra seedlings (*Abelmoschus esculentus* L. Moench). Previous research highlights that physiological changes may be initiated during imbibition, and these remain even after seeds are dehydrated (3). For this reason, osmoconditioned seeds rapidly re-initiate their metabolism, improving the percent and uniformity of germination (8).

Because water is a limiting factor in agricultural systems, the use of the aforementioned technologies may shorten the imbibition-germination time and improve the initial vigour of seedlings. Thus, the objective of the present study was to evaluate the effect of different periods of soaking in water on maize seeds and to examine their effects on growth, development, and yield of crops.

**Materials and Methods**

**First phase: Determination of the imbibition-germination times**

For this phase, hybrid maize seeds (1503 by Aspros®) were placed in a humidity chamber with 90% relative humidity at a temperature of 27±3°C. The humidity chamber consisted of a plastic container. Paper saturated with sterile water was placed in the bottom of the container. Subsequently, the seeds were placed, the container was sealed and kept at constant temperature in complete darkness. Twenty seeds were placed in 50 humidity chambers (1000 seeds in total). Initial weight along with increases in weight every six hours, were recorded. The number of germinated seeds was recorded over the period of evaluation. A seed was considered as germinated when it showed a radicle measuring at least, 2 mm. The recorded data were tested for normality and homoscedasticity. When assumptions of normality were not met, the data were transformed using the following formula:

\[ Y = \sqrt{X + 10} \]

where:

- \( Y \) = transformed data
- \( X \) = data

The previous procedure facilitated the determination of the germination times and weight gain due to water absorption. Based on the generated data, three periods of hydroconditioning and control were then selected (0, 12, 18, and 36 hours). For each time period, 20 seeds were placed in humidity chambers for the corresponding treatment time. As mentioned, their initial weights, as well as weight gains assessed every 6 h, were recorded.
Temperature and RH were kept constant (27±3 °C and 90% HR). Once the imbibition time was finalised, the seeds were removed from the humidity chamber and replaced on absorbent paper at room temperature until they returned to their original weight prior to imbibition (approximately 120 hours). Afterwards, the seeds were placed in humidity chambers to determine the number of germinated seeds over time; measurement were recorded every six hours. The recorded data were subjected to a regression analysis in Microsoft Excel®.

Second phase: Field experiments

First, 200 g of maize seed were placed in a humidity chamber at 90% humidity and 25±2°C for each one of the previously established time periods (12, 18, and 36 hours and control). After the treatment periods, the seeds were removed from the humidity chamber and placed on absorbent paper at room temperature until they recovered their initial weight (approximately 120 hours). Afterwards, the seeds were sown in an open-field plot located in the experimental field of the Polytechnic University of Francisco I. Madero on Hidalgo estate México (14Q 490716.79 m E y 2236223.96 m N). The study was carried during 2014-2015. The experimental desing was a completely randomised block. For each treatment, a surface area of 50 m² was sown with seeds, with a distance of 0.75 m between furrows and 0.13 m between plants. Each treatment was replicated four time. Drip irrigation system was used, and sowing was carried out at the same time for all treatments under consideration. Weed control was mechanical in all treatments and no fertilizer was applied.

The mean of temperature in the experimental field was 26.2 °C and the HR was 22%.

Variables analysed during the fieldwork

For each treatment (period of hydro-conditioning), the number of emerged seeds was counted six days after sowing (DAS). A plant was considered to have emerged if the coleoptile had a minimum height of 4 cm. The number of plants was also determined at the harvest time.

The variables plant height, foliar temperature, and soil temperature were also recorded. Plant and soil temperatures were measured with a Benetech® infrared thermometer at an approximate distance of 20 cm from the foliage or ground surface. This variables have been previously used for estimation of the water use status on plant (10) in addition to the percentage of ground area covered by foliage every 7-9 days.

The percentage of ground area covered by foliage was determined by digital imaging, according to the method proposed by Mullan and Barcelo-García (2012). The digital images were then processed with Adobe Photoshop CS5 Extended® software, adjusting the parameters of saturation and luminosity to constant values (+60 and -20, respectively) aiming to contrast and compare the colours corresponding to leaves and ground surface. With this method, the area of the image corresponding to the green colours of leaves was substituted by absolute white (R=255, G=255, and B=255) in an RGB colour system, and the area corresponding to soil and the related colour gamma was substituted by absolute black (R=0, G=0, and B=0) in the same colour system.

The last step involved determining the ratio of white to black pixels in the RGB colour system, using the measuring tools provided by the software. With this information, the percentage of soil cover with foliage was calculated according to the following formula:
\[ \% \text{SCPAT} = \left( \frac{\text{AWP}}{255} \right) \times 100; \]

where:
\( \% \text{SCPAT} \) = percentage of soil covered with photosynthetically active tissue
\( \text{AWP} \) = average number of white pixels

Once a female maize flower appeared, the length and diameter of each cob was measured. During harvest, the average percentage humidity of the seeds for each treatment was determined, and the grains of each cob per treatment were separately weighed in order to calculate yield per hectare.

**Data analysis**

The generated data were submitted to analysis of variance, and a means comparison test was performed if the results were significant (Tukey, \( P=0.05 \)). For the data on plant height and length, diameter of the cob, and percent of ground cover, the area under the curve was calculated following the polygon method. The analysis of the area under the curve is a method used to estimate the total growth through time and the result is a dimensionless value. This method was described by Liengme, 2002. Yield data were analysed by Regression analysis.

**RESULTS**

**Determination of the imbibition-germination times**

Time of imbibition was correlated with seed weight. Before the seeds were placed in humidity chambers, average weight of 20 seeds was 8.3 g. Thirty six hours after the start of imbibition, average weight was 11.8 g (figure 1b, page 77). The greatest number of germinated seeds after reaching an average imbibed weight of 12.4 g, occurred after 70 hours in the humidity chamber. Seed weight had a correlation coefficient of 0.53 with germination percentage. However, time of imbibition was statistically more significant in explaining germination (data not shown). According to the regression model (figure 1a, page 77), 50% germination (\( G_{50} \)) was achieved after 54.8 hours of imbibition.

During the initial hours (0-20 hours), a rapid increase in weight was observed, corresponding to the first phase of imbibition, characterised by a rapid increase in water absorption. Afterwards, increases in weight were stable, followed by another increase in weight corresponding to the appearance of the radicle.

**Germination of hydroprimed seeds**

The seeds showed a distinct behaviour as a function of imbibition time. The most rapid germination time was found for the 36-hour imbibition treatment, resulting in 50% germination of seeds (\( G_{50} \)) after 16.7 hours in the humidity chamber. The 12-hour (T12) and 18-hour (T18) imbibition treatments took more time to germinate (\( G_{50} \)) at 33.4 and 30.27 hours, respectively. The control treatment (unimbibed seeds) had the highest germination time (\( G_{50} \)) at 48.2 hours. Seeds that were treated for 12 hours had an overall rate of germination of 94.1% after 48 hours in a humidity chamber (figure 2, page 78).

**Field experiments**

**Emergence of seeds**

Hydropriming time affected seed emergence emergence in the field. The emergence of seedlings at six DAS, increased in direct proportion with the imbibition time of seeds up to 18 hours, when the number of emerged seeds began to diminish. The 36-hours treatment had the lowest number of emerged seedlings (figure 3, page 78). A similar behaviour has been observed in...
Figure 1. Effect of imbibition time on A) germination percentage of maize seed and B) seed weight.

Figura 1. Efecto del tiempo de imbibición sobre A) porcentaje de germinación de semillas de maíz y B) peso de semillas de maíz.
$G_{50} =$ time (hours) to reach 50% germination; $G_{48h} =$ percentage of germinated seeds at 48 hours. All equations are significant with $\alpha \leq 0.05$.

$G_{50} =$ tiempo (horas) al 50% de germinación; $G_{48h} =$ porcentaje de germinación a las 48 horas. Todas las ecuaciones son significativas con un $\alpha \leq 0.05$.

**Figure 2.** Effect of different hydropriming times on the time to germination of maize seeds.

**Figura 2.** Efecto de diferentes tiempos de hidroacondicionamiento sobre el tiempo de germinación en semillas de maíz.

$y = -0.1702x^2 + 6.3564x$

$R^2 = 0.9149$

$P = 2.3 \times 10^{-10}$

$G_{\text{max}} =$ Maximum number of emerged seeds. / $G_{\text{max}} =$ Máximo número de semillas emergidas.

**Figure 3.** Average number of emerged seeds at different imbibition time, six DAS.

**Figura 3.** Promedio de número de semillas emergidas a diferentes tiempos de imbibición, seis DAS.
other species; for example, Marín Sanchez et al. (2007) found an increase in the percentage of abnormal onion seedlings after 72 hours of hydroconditioning, and the percentage of abnormalities further increased after 96 hours. In another study, Sadeguhi et al. (2011) found an average dry weight of 1.55 g for soy seeds hydroconditioned for 12 hours. However, when the time of osmoconditioning increased to 18 and 24 hours, the average dry weight decreased to 1.3 and 1.2 g, respectively.

**Plant height**

Plant height demonstrated significant differences at 37 and up to 43 DAS. During this period, seeds hydroconditioned for 36 hours showed an average height equal to the control treatment, while the 12- and 18-hour treatments had a greater height. For the rest of the sampled dates, plants did not show significant differences. Overall, and in terms of accumulated growth, seeds imbibed for 12 or 18 hours resulted in plants of greater height in comparison with the control treatment (table 1).

**Plant and soil temperature**

For the 0-, 12-, and 18-hour treatments, plant and soil temperatures significantly differed significantly at 16, 22, and 30 DAS. A lower difference in temperature after 16 days, was found with the 36-hour treatment, while the 12-hour treatment presented the greatest temperature difference. At 22 DAS, an increase in solar energy resulted in a higher soil temperature; causing that plants from imbibed seeds of all treatments showed a difference in relation to non-treated seed. At 30 DAS, only the 12-hour treatment demonstrated a statistically significant difference in comparison with the other treatments. For the rest of the sampling dates, no significant differences were found (table 2, page 80).

**Percent of soil cover**

Although soil cover percentage largely depends on leaf lamina growth, it is also determined by a number of other factors, such as luminosity, nutrient availability in water and soil, leaf insertion angle to the

| Table 1. Plant height expressed in centimeters from seeds with different imbibition times. |
| Tabla 1. Alturas de plantas expresadas en centímetros provenientes de semillas con diferentes tiempos de imbibición. |

<table>
<thead>
<tr>
<th>Imbibition time</th>
<th>Days after sowing</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>0</td>
<td>19.5 a</td>
<td>32.1 a</td>
</tr>
<tr>
<td>12</td>
<td>18.2 a</td>
<td>37.5 a</td>
</tr>
<tr>
<td>18</td>
<td>18.3 a</td>
<td>33.7 a</td>
</tr>
<tr>
<td>36</td>
<td>17.7 a</td>
<td>32.2 a</td>
</tr>
<tr>
<td>P</td>
<td>0.59</td>
<td>0.04</td>
</tr>
<tr>
<td>MSD</td>
<td>3.94</td>
<td>11.13</td>
</tr>
</tbody>
</table>

†ABC = Area under the curve for plant height (Dimensionless number). MSD = Minimum significant difference. Different letters indicate statistically significant differences (Tukey, P=0.05).

†ABC = Área bajo la curva del peso de la planta. MSD = Diferencia mínima significativa. Letras diferentes indican diferencias significativas (Tukey, P=0.05).
main stem, planting density and spatial distribution. In addition, any incidence of plague or disease may also have an effect. In the current study, all treatments showed a similar percentage of soil cover, indicating that the hydropriming treatments did not affect this variable (figure 4, page 81).

Although from 16 to 30 DAS the incidence of solar radiation caused a difference in temperature between soil and plants (table 2), all of the treatments maintained a similar foliage area. Even so, the 12- and 18-hour treatments maintained a greater difference in temperature, which implies that even though none of the plants were affected in terms of growth, only the 12-

Table 2. Soil and maize plant temperatures on different days after planting for a given time of imbibition.

<table>
<thead>
<tr>
<th>T1 (Hours)</th>
<th>Days after planting</th>
<th>Days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>TS</td>
<td>TP</td>
</tr>
<tr>
<td>0</td>
<td>33.7</td>
<td>29.7</td>
</tr>
<tr>
<td>12</td>
<td>39.2</td>
<td>31.1</td>
</tr>
<tr>
<td>18</td>
<td>34.6</td>
<td>29.4</td>
</tr>
<tr>
<td>36</td>
<td>35.3</td>
<td>33.2</td>
</tr>
<tr>
<td>DMS</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

DMS = Minimum significant temperature (Tukey, P = 0.05). The value of temperatures and difference are expressed in °C.

Yield

The imbibition treatments showed an effect on yield. The generated data were used as inputs for a mathematical model to estimate maximum yield, resulting in 16.6 t per hectare and corresponding with 22.12 hours of imbibition. Upon surpassing this timeframe, plant yield decreased to 15.6 t per hectare (figure 5, page 82).
Discussion

The germination value is greater than that reported, who found a G50 value of 46 hours. After 36 hours of imbibition, an 11% increase in seed germination was observed. In the present study, this time period was considered to be the maximum imbibition period before the seeds began to germinate. The germination time behaviour is typical of the majority of seeds (19). For hybrid corn, other authors have reported similar results. For example, by Yu-quin and Song-quan (2008) tested different imbibition times and evaluated the water content of seeds under different treatments. Phase 1 was observed between 0 and 12 hours, followed by a decrease in water content from 12 to 36 hours and, finally, a subsequent increase in water uptake. The hydropirming of seed resulted in earlier germination for maize seeds. However, this also affected the final number of germinated seeds. This behaviour has also been reported for other species. For example, in soybean...
Max yield = maximum yield estimated by the following model: $y = ax^2 + bx + c$, where $y =$ output in kg per hectare and $x =$ time of imbibition.

Max yield = rendimiento máximo estimado por el modelo $y = ax^2 + bx + c$, donde $y =$ rendimiento en kg por hectárea y $x =$ tiempo de imbibición.

**Figure 5.** Effect of imbibition time of maize seeds on yield per hectare.

**Figura 5.** Efecto del tiempo de imbibición de semillas de maíz sobre el rendimiento.

crops (25) 50% germination of seeds was found to increase at imbibition periods of 18 and 24 hours, even though the final germination percentage decreased in comparison with seeds hydroprimed for 12 hours. Hydropriming of seeds induces a series of biochemical changes (11) that are necessary for the germination process to occur; Heydecker and Coolbear (1977) stimulating, for example, the activation of enzymes or the metabolism of germination inhibitors (1). However, during the hydration process, seeds may also suffer temporary changes in the permeability of their cellular membranes, loosing solutes and metabolites of low molecular weight to the surrounding environment (19). These compounds are necessary for development during the first stages of germination (4), and their excessive loss could represent a disadvantage for the seed (5). This described phenomenon may explain what occured in this study, when seeds were hydroconditioned for 36 hours and germinated more rapidly even when the final number of germinated seeds resulted lower. This finding is possibly due to irreparable damage from excessive loss of nutritional compounds during imbibition phase II. In the field experiments, results demonstrated that hydroconditioning treatments are only beneficial, when practiced for an adequate period of time. When the optimal hydroconditioning time is surpassed, physiological damage may occur leading to a lower percentage of emergence. The results of plant height were similar to those reported by Sharma et al. (2014), who found an average height for okra plants of 27.1 cm when the seeds

![Graph showing imbibition time vs. yield](image-url)
were imbibed for 12 hours. By contrast, with the control treatment, an average height of 14.8 cm was found, but when the imbibition time increased to 18 hours, the seedlings displayed a height of 22 cm. Plant surface temperature is related to transpiration rate; increased transpiration lowers leaf temperature by dissipation of sun heat (20). This has demonstrated that plant temperature is the result of several physiological processes, which, in addition to transpiration, involve stomatal conductance, hydric status of the plant, water use efficiency, leaf area index, and yield (23). In the present study, plants from hydroconditioned seeds showed a greater difference in plant and soil temperatures, compared with non-imbibed seeds. It may be inferred that imbibition causes a physiological effect that enables a greater capacity for transpiration in conditions of high irradiance, which may lead to an elevated rate of photosynthesis as stomata remain open for longer periods of time. Some studies have revealed that the hydroconditioning of seeds results in significant effects during plant development. In the case of wheat, it has been shown that hydroconditioning leads to a decrease in the concentration of sodium ions in plants growing in saline soils, favouring plant growth (22).

Further research has highlighted how hydroconditioned plants behave in comparison with control treatment plants as a function of environmental conditions. For example, Chen and Arora (2011) found that spinach seedlings from hydroconditioned seeds showed an increase in the expression of the CAP85 gene when plants were submitted to stress by desiccation. However, no similar expression of the gene occurred under optimal conditions. This gene encodes for LEA (late embryogenesis abundant) proteins that play an important role in water stress resistance (22). In the specific case of maize (6), an increase in the expression of LEA genes and their respective proteins was found for osmoconditioned seeds compared with normal seeds. Although the function of these proteins is not precisely known, evidence suggests that these proteins protect cellular structures from water stress or cold (27). In the present study, it is likely that 12- and 18-hour treatments would have affected the expression of the genes that encode LEA proteins, enabling these plants to exhibit a greater resistance to stress between 22 and 30 DAS, which corresponds to the period of high irradiance (table 2, page 80).

Dry matter and grain production depend on the ability of crops to capture resources, and radiation is an important factor for yield (14). However, a high solar radiation causes an increase in leaf and soil temperatures. When sun incidence was not sufficient to elevate soil temperature above 50°C, plants did not show significant differences in leaf temperature. This finding suggests that during the hydroconditioning of maize seeds, gene expression may be affected, leading to an improved stress response.

Other studies have demonstrated that seed imbibition may lead to an agronomic advantage. For example, Ghiyasi et al. (2008) found an increase in maize plant yield when seeds were hydroconditioned with water at a potential of -0.5 MPa for 24 hours. A similar behaviour has been observed for other crop species. For example, Arif et al. (2014) found that soy plants from seeds hydroconditioned with polyethylene glycol (PEG) for six hours at -1.1 MPa, had a greater yield, compared with plants from non-treated seeds. Plants from treated seeds also flowered and presented mature seeds before
the control. In line with these findings, recent evidence has shown that hydroconditioning treatments enable moderate resistance to drought because the physiological mechanisms behind this tolerance are activated as a consequence of these treatments (9).

Intermembrane proteins called aquaporins, transport water from cell to cell and therefore, play an important role during germination. Another key process might involve guard cells and their changes in volume with the addition of water. Finally, phloem loading and unloading, as well as stomatal movements (17), may partly explain drought resistance as previously reported. In the current study, plants underwent high temperatures from 16 to 30 DAS (table 2, page 80).

Under these conditions, certain treatments (12 and 18 hours) showed a greater difference between soil and foliage temperature, indicating that these plants had greater resistance. This resistance also led to greater growth at 37 to 43 DAS (table 1, page 79), which may be an indicator of the capacity of the 12- and 18-hour treatments to continue development in conditions under which other treatments (control and 36 hours) were interrupted. As a consequence of these effects, seeds that are hydroconditioned for 12 and 18 hours develop into plants with greater stress tolerance, representing a significant advantage in comparison to non-treated plants.

**Conclusions**

Hydroconditioned maize seeds for 12 and 18 hours presents an agronomic advantage for adult plants. Several of these advantages include more uniform germination, earlier germination, greater growth during periods of thermal stress, and greater grain yields.

**References**


Hydropriming on maize seeds


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