

Can chemical agents associated with priming attenuate stress in corn seeds during the initial establishment?

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ABSTRACT

Chemical substances for physiological conditioning are employed to reduce the stresses caused by the adversities affecting corn seeds during the seedling emergence and establishment. This study aimed to evaluate different chemical agents used in the physiological conditioning of corn seeds to overcome abiotic stresses at the time of germination. The experimental design was completely randomized arranged in a 2 x 5 factorial scheme consisting of two seed lots, four chemical agents used in physiological conditioning, and the control treatment. The seeds were subjected to different abiotic stresses: low-temperature, hypoxia, salt, and water. The physiologic seed conditioning was carried out through direct immersion, for 18 hours at 25°C, under low-temperature stress condition. All physiologic conditioning methods proved to effectively minimize this type of abiotic stress during the germination process. Using nitrates via physiological conditioning is an alternative to promote beneficial effects in stressful situations.

Keywords: Calcium Nitrate, Potassium Nitrate, Priming, *Zea mays*.

Agentes químicos associados ao priming podem atenuar estresses em sementes de milho durante o estabelecimento inicial?

RESUMO

As substâncias químicas utilizadas no condicionamento fisiológico pode ser uma das técnicas utilizadas para reduzir os estresses causados pelas adversidades que acometem as sementes de milho durante a emergência e o estabelecimento da cultura. Objetivou-se avaliar diferentes substâncias utilizadas no condicionamento fisiológico de sementes de milho para a superação de estresses abióticos no momento da germinação e emergência. O delineamento experimental foi inteiramente casualizado em esquema fatorial 2 x 5, sendo constituído de dois lotes de sementes, e quatro agentes químicos utilizados no condicionamento fisiológico, mais a testemunha. As sementes foram submetidas a diferentes estresse abióticos: baixa temperatura, hipóxia, estresse salino e restrição hídrica. O condicionamento fisiológico de sementes através da imersão direta, no período de 18 horas e com ambas as substâncias se mostrou de modo geral, um método promissor para superar efeitos deletérios abióticos encontrados a campo no momento da germinação. A utilização de nitratos via condicionamento fisiológico é uma alternativa para promover efeitos benéficos perante a situações de estresse. O condicionamento fisiológico proporciona maior velocidade de emergência, independente da substância utilizada, sem interferir no desenvolvimento inicial de plântulas.

Palavras-chave: Nitrato de Cálcio, Nitrato de Potássio, Priming, *Zea mays*.

1. Introduction

In Brazil, corn (*Zea mays* L.) is grown mainly in the off-season or second crop. According to CONAB (2020), it is estimated that in the 2019/2020 harvest, the national production was 100.48 million tons, with an average grain yield of 5.5 t ha⁻¹. Corn cultivation demands care during sowing to avoid adversities that could negatively influence seedling emergence and establishment in the field. Among the most significant adversities that can affect the crop is the water deficit, interrupting and hindering the metabolic processes during the water imbibition process of seeds.

Regarding corn cultivation, there are other stresses such as salt stress, which acts on water absorption and consequently on ion accumulation, damaging cell structures (Pereira et al., 2012). Thermal stress caused by low temperatures also hinders the entry of oxygen and slows down plant metabolism (Carvalho et al., 2009). Hypoxia is a process that reduces respiration and starts fermentation, promoting the formation of toxic substances for cells (Gazola et al., 2014).

In this context, using seeds with high physiological potential is one of the first steps to face possible adversities in the initial crop stages. Zucareli et al. (2015) point out that several characteristics must be adequate to classify the quality of seed, including genetic traits, physical properties, phytosanitary quality, and especially highlighting the physiological quality. However, during production, processing, storage, and transport, seeds are subject to internal and external damage (Smiderle et al., 2003) to reduce their physiological potential, delaying emergence and compromising the final stand.

According to Santos et al. (2008), physiological conditioning is a technique that can result in a better expression of seed vigor, reduce germination and emergence time, standardize the plant stand establishment, and, in some species, it can even promote seed dormancy overcome. In this technique, the seeds are subjected to hydration, and in a controlled way, reach phase I and II of germination (hydration and metabolism reactivation) without getting to phase III (radicle emission), so the seeds can return to initial moisture, be stored, and later sown (Marcos Filho, 2015). Associated with this technique, it is still possible to use some substances that act physiologically in the seeds, such as nitrates.

According to Cardoso et al. (2015), the nitrate action is due to its reduction to nitrite. This process reoxidizes the NAD(P)H molecules, making them available again, providing stimulus to the pentose-phosphate route and the shikimic acid pathway. These substances are responsible for forming essential compounds for germination metabolism. However, the use of calcium nitrate acts similarly; Gouveia et al. (2017) reported in their work that this substance applied to corn seeds promoted greater tolerance to low temperatures, thus

enabling higher vigor expression. Sanz et al. (2015) report that the nitrate to nitrite reduction can later be reduced to nitric oxide (NO), highly influent on enzyme and hormone production in plant tissues. Therefore, it acts mainly in overcoming dormancy in seeds through inhibiting abscisic acid and inducing gibberellin production, providing higher germination speed.

Given the above, the study hypothesizes that using chemical agents (nitrates) in physiological conditioning reduces the effects of possible stresses to which corn seeds are exposed during germination and emergence, favoring the seedling establishment. The study aimed to evaluate different chemical agents used in corn seed physiological conditioning to overcome abiotic stresses during germination.

2. Material and Methods

Corn seeds of two cultivars, Morgan (Lot 1) and Nidera (Lot 2), from the 2016/2017 harvest were used. They were submitted to physiological conditioning by direct immersion, at a constant temperature of 25°C, for 18 hours. After hydration, the seeds were dried in a dry chamber (temperature of 17°C and relative humidity of 45%), dispersed on paper towels until they returned to the initial moisture (the hygroscopic balance during seed storage). This procedure was carried out for 48 hours (accompanied by successive weighings).

The experimental design was completely randomized (CRD) arranged in a 2 x 5 factorial scheme. The first factor consisted of two seed lots (Lot 1 and Lot 2), and the second factor of different chemical agents used in physiological conditioning: Control (unconditioned); Water – immersion in deionized water; Ca(NO₃)₂ – 0.2% calcium nitrate solution; KNO₃ – 0.2% potassium nitrate solution; Ca(NO₃)₂ + KNO₃ – 0.1% calcium nitrate + 0.1% potassium nitrate solution, with four repetitions.

For each abiotic stress, the following assessments were performed: Germination test consisted of four subsamples of 50 seeds per treatment, using three sheets of "Germitest" type paper as the substrate, and moistened with water in a proportion of three times the weight of dry paper, placed in a twinning chamber at a temperature of 25 °C. The counting was performed on the 7th day according to the methodology proposed by Brazil (2009). The first germination count was performed together with the germination test, and the seeds considered germinated on the 4th day of evaluation were computed according to BRASIL/MAPA (2009).

The germination speed index (GSI) was calculated by the sum of the number of seeds germinated each day, divided by the number of days elapsed between sowing and germination, according to the equation proposed by Maguire (1962). The different abiotic stresses evaluated were simulated with the following methodologies: Low

temperature - maintained at 18 °C (Adapted from Vieira & Kryzanowski, 1999) during standard germination test proposed by (2009). Hypoxia - seeds underwent the following conditioning: they were completely submerged in water inside a disposable cup-type container, for 24 hours, at a constant temperature of 25°C (Grzybowski et al., 2015).

Salt stress - directly moistening the germination substrate ("Germitest paper") with a saline solution, three times the weight of the paper, in which to each liter of water was added 4.73 grams of NaCl, reaching an osmotic potential of -0.2 MPa, according to Richards (1980). Water deficit - performed from the simulation of water deficit, in which polyethylene glycol (PEG 6000) solution was used. The level of osmotic potential used was -0.2 MPa, equivalent to a concentration of 119,571 g of deionized PEG L-1H₂O. To calculate the amount of PEG 6000, the equation proposed by Michel and Kaufmann (1973) was used, that is: $\Psi_{os} = (1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7})$ where: Ψ_{os} = osmotic potential (bar); C = concentration of osmotic agent (g of PEG 6000 L⁻¹ H₂O); T = temperature (°C) (MOTERLE et al., 2008). The solution containing PEG 6000 was supplied directly to the germination substrate, three times the weight of the paper.

Data were previously tested for normality by the Shapiro-Wilk test and homogeneity by the Bartlett test, which met the assumptions. Subsequently, the data were subjected to analysis of variance, and the significant effects of the F-test were considered at a 5% significance

level. The Tukey test was applied at a 5% probability level to compare means (Ferreira, 2014).

3. Results and Discussion

An interaction between seed lots and conditioning was observed for the first germination count and emergence speed index under low temperature (Table 1). Under hypoxia, the influence of the interaction between the factors was observed for the first germination count. Under salt stress conditions, there was no influence of the interaction between factors. Seeds subjected to water stress have been influenced by the interaction between factors for the first germination count and total germination (Table 1).

All methods of physiological conditioning proved to be effective in minimizing low-temperature stress effects during germination, considering the final germination percentage. Germination of conditioned seeds had faster germination and showed up to 8% more germination than the control, regardless of substance (Table 2). Grzybowski et al. (2015) report that corn, cultivated and adapted as a summer crop mainly in places with higher temperatures, may have seedling germination and emergence affected due to lower temperatures. Also, Carvalho et al. (2009) stated that low temperatures during the germination hinder the membrane reorganization process, causing high losses of fundamental compounds for this stage, in addition to a germination metabolism decline.

Table 1. Summary of analysis of variance for the first germination count (FGC), total germination (GER), and germination speed index (GSI) of corn seed lots (*Zea mays* L.) in response to abiotic stresses, low temperature, hypoxia, salt stress, and water deficit. Cassilândia/MS, 2017.

SV	Probability F ($p < 0.05$)					
	Low temperature			Hypoxia		
	FGC	GER	GSI	FGC	GER	GSI
Seed Lots (L)	<0.00*	<0.00*	<0.00*	<0.00*	<0.00*	<0.00*
Conditioning (C)	<0.00*	<0.00*	<0.00*	<0.00*	0.19 ^{ns}	<0.00*
Interaction (L x C)	<0.00*	0.14 ^{ns}	<0.00*	<0.00*	0.36 ^{ns}	0.11 ^{ns}
Error	15.61	11.12	0.07	8.28	18.22	0.15
CV (%)	11.46	4.26	3.05	4.21	5.76	3.58
SV	Salt Stress			Water deficit		
	FGC	GER	GSI	FGC	GER	GSI
	FGC	GER	GSI	FGC	GER	GSI
Seed Lots (L)	<0.00*	<0.00*	<0.00*	<0.00*	<0.00*	<0.00*
Conditioning (C)	<0.00*	0.05*	<0.00*	<0.00*	<0.00*	<0.00*
Interaction (L x C)	0.34 ^{ns}	0.63 ^{ns}	0.23 ^{ns}	<0.00*	<0.00*	0.15 ^{ns}
Error	15.41	29.98	0.19	7.42	13.08	0.14
CV (%)	5.98	6.92	3.98	7.37	4.85	4.47

* = statistically differ by F test at $p < 0.05$; ns = not significant. SV = sources of variation; CV = Coefficient of variation.

Table 2. Effect of conditioning with nitrated chemical agents and corn seed lots (*Zea mays* L.) on the first germination count, total germination, and germination speed index under low-temperature conditions. Cassilândia/MS, 2017.

Conditioning	Seed Lots		Mean
	Lot 1	Lot 2	
	1 st germination count (%)		
Control	00 bD	12 aC	06
Water	13 bB	81 aA	47
Ca(NO ₃) ₂	17 bB	69 aB	43
KNO ₃	49 bA	68 aB	58
Ca(NO ₃) ₂ + KNO ₃	02 Bc	59 aB	30
Mean	16.2	57.8	
	Germination (%)		
Control	86	94	90 B
Water	93	100	96 A
Ca(NO ₃) ₂	92	98	96 A
KNO ₃	96	98	97 A
Ca(NO ₃) ₂ + KNO ₃	93	99	96 A
Mean	92 b	98 a	
	Germination Speed Index		
Control	6.14 bD	7.35 aC	6.74
Water	7.30 bBC	11.44 aA	9.37
Ca(NO ₃) ₂	7.48 bB	10.67 aB	9.07
KNO ₃	9.47 bA	10.64 aB	10.05
Ca(NO ₃) ₂ + KNO ₃	6.82 bC	10.22 aB	8.52
Mean	7.44	10.06	

Means followed by the same letter, lower case in line and upper case in the column, are not statistically different by Tukey test ($p < 0.05$)

According to Marcos Filho (2015), low temperatures make imbibition difficult, delaying the germination process, thus prolonging the environmental exposure period of seeds. The author also reports that low temperatures effects are aggravated in lots with lower physiological potential, corroborating the current study, as observed in lot 1 (lower force) compared to lot 2 (Table 2).

The conditioning carried out with potassium nitrate in lot 1 provided faster germination under low-temperature conditions. On the other hand, the conditioning carried out with water was superior for lot 2. However, the beneficial effect of physiological conditioning is demonstrated in the first germination count, compared to the control treatment, regardless of the substance used for lot 2 (Table 2). The superior result of lot 1 in the first count, conditioned with KNO₃ (Table 2), agrees with Binotti et al. (2014), who reported that potassium nitrate could increase germination speed when used in pre-germinative treatments. According to Marcos Filho (2015), this is due to this substance action in the pentose-phosphate pathway cycle, in which NADPH is oxidized, resulting in the NADP release, ultimately initiating metabolism.

In Table 3, none of the physiological conditioning methods proved effective in overcoming the hypoxia-caused stress, having a more harmful effect on the lot with the lowest physiological potential (Lot 1). Dantas et al. (2000) showed that corn seeds had around a 10% germination reduction after 24 hours of submersion, proving the damage caused by this type of stress.

In the stress caused by hypoxia, the damage caused to the seeds is mainly due to the compromised cell metabolism, forcing the cells to seek alternatives such as fermentation to overcome the stress. Consequently, harmful compounds are formed, resulting in losses of seed physiological potential, making them unfeasible in severe cases (Gazola et al., 2014).

There was a reduction in the first germination count percentage in lot 1. These results were inferior to lot 2 and may be related to the lower physiological potential, making the lot more susceptible to stress-caused damage (Table 3). In lot 2, the water conditioning method may have aggravated the situation. However, the percentage did not differ from the control in the presence of chemical agents (Table 3).

The conditioning with calcium nitrate and its association with potassium nitrate overcame salt stress in the first germination count compared to the control (Table 4). Martins et al. (2009) emphasize the importance of rapid and uniform germination and that shorter exposure time ensures greater germination efficiency due to the numerous factors that can compromise the germination process.

The excess of salt in the substrate reduces the osmotic potential of the hydration solution, besides causing damage by toxicity, interfering with the water absorption of seeds directly reflecting on the metabolism reduction and impairment since this is dependent on the amount of water (Sangoi et al., 2009).

Table 3. Effect of conditioning with chemical agents with nitrate, and corn seed lots (*Zea mays* L.), on the first germination count, total germination, and germination speed index, submitted to hypoxia. Cassilândia/MS, 2017.

Conditioning	Seed lots		Average
	Lot 1	Lot 2	
	1 st germination count (%)		
Control	74 bA	95 aAB	84
Water	80 bA	87 aC	83
Ca(NO ₃) ₂	82 bA	95 aAB	88
KNO ₃	79 bA	96 aA	87
Ca(NO ₃) ₂ + KNO ₃	75 bA	90 aBC	82
Average	78	92.6	
	Germination (%)		
Control	86	98	92 AB
Water	84	92	88 B
Ca(NO ₃) ₂	89	98	93 A
KNO ₃	86	98	92 AB
Ca(NO ₃) ₂ + KNO ₃	83	96	90 AB
Average	86 b	97 a	
	Germination speed index		
Control	10.07	12.06	11.06 AB
Water	10.26	11.23	10.74 B
Ca(NO ₃) ₂	10.71	12.11	11.41 A
KNO ₃	10.34	12.08	11.21 AB
Ca(NO ₃) ₂ + KNO ₃	9.88	11.67	10.78 B
Average	10.25 b	11.83 a	

Means followed by the same letter, lower case in line and upper case in the column, are not statistically different by Tukey test ($p < 0.05$).

Table 4. Conditioning effect with nitrated chemical agents and corn seed lots (*Zea mays* L.) on the first germination count, total germination, and germination speed index, submitted to saline stress. Cassilândia/MS, 2017.

Conditioning	Seed Lots		Average
	Lot 1	Lot 2	
	1 st germination count (%)		
Control	73	84	78 B
Water	69	85	77 B
Ca(NO ₃) ₂	81	91	86 A
KNO ₃	76	89	83 AB
Ca(NO ₃) ₂ + KNO ₃	78	95	87 A
Average	75 b	89 a	
	Germination (%)		
Control	94	98	96 AB
Water	86	98	92 B
Ca(NO ₃) ₂	96	100	98 A
KNO ₃	91	97	94 AB
Ca(NO ₃) ₂ + KNO ₃	93	99	96 AB
Average	92 b	98 a	
	Germination Speed Index		
Control	10.56	11.43	11.00 AB
Water	9.81	11.55	10.68 B
Ca(NO ₃) ₂	11.16	11.95	11.55 A
KNO ₃	10.48	11.66	11.07 AB
Ca(NO ₃) ₂ + KNO ₃	10.82	12.12	11.47 A
Average	10.56 b	11.74 a	

Means followed by the same letter, lower case in line and upper case in the column, are not statistically different by Tukey test ($p < 0.05$).

Moterle et al. (2006) demonstrated the adverse effects of salinity on the popcorn seeds germination through the reduced percentage when the proportion of salt in the substrate solution was increased. They also obtained different results in different lots, demonstrating, as, in this study, that physiological potential and genotypes can define the stress response.

The first germination count (FGC) differed in the response of lots to the treatments used. Lot 1 obtained a response in overcoming water stress when conditioned only with water or with calcium nitrate + potassium nitrate, superior to the control. However, in lot 2, potassium nitrate or its association with calcium nitrate had higher means (Table 5). These results agree with Gouveia et al. (2017), which showed that potassium nitrate promoted increments in the first germination count when the seeds were subjected to simulated water stress with PEG 6000 via physiological conditioning in corn seeds.

For germination (GER), when applied in lot 2, the conditioning methods reported results lower than the control, regardless of the substance used. In lot 1, the conditioning effect with calcium nitrate + potassium

nitrate had similar behavior to the control, however with a higher percentage than with calcium nitrate, regarding germination (GER).

The germination speed index (GSI) during water stress (Table 5) showed better results for lot 2 compared to lot 1. According to Moterle et al. (2008), water stress damage seeds by compromising the metabolism. Therefore, the results obtained by these authors show the harmful effects of this type of stress. The germination of popcorn seeds placed at a potential of -0.3 MPa was compromised; however, some cultivars stood out over others.

Kappes et al. (2010) demonstrated in their work the negative influence caused by water deficit on germination, vigor, and initial growth of seeds of different corn hybrids, corroborating the results of the current work. However, the GSI (Table 5) for conditioning carried out with potassium nitrate and association between calcium and potassium nitrate were effective to overcome this type of stress, with values superior to the control, leaving seeds less time exposed to different kinds of damage.

Table 5. Conditioning effects with nitrated chemical agents and corn seed lots (*Zea mays* L.) on the first germination count, total germination, and germination speed index under water deficit. Cassilândia/MS, 2017.

Conditioning	Seed Lots		Average
	Lot 1	Lot 2	
1 st germination count (%)			
Control	28 aB	28 aB	28
Water	40 aA	35 aB	37
Ca(NO ₃) ₂	29 aB	30 aB	29
KNO ₃	31 bAB	51 aA	41
Ca(NO ₃) ₂ + KNO ₃	38 bA	51 aA	44
Average	33.2	39	
Germination (%)			
Control	90 bAB	100 aA	95
Water	88 bAB	96 aB	92
Ca(NO ₃) ₂	83 bB	94 aB	88
KNO ₃	91 aAB	95 aB	93
Ca(NO ₃) ₂ + KNO ₃	92 aA	93 aB	92
Average	88.8	95.6	
Germination Speed Index			
Control	7.89	8.63	8.26 BC
Water	8.39	8.72	8.55 AB
Ca(NO ₃) ₂	7.44	8.31	7.87 C
KNO ₃	8.15	9.34	8.82 A
Ca(NO ₃) ₂ + KNO ₃	8.59	9.34	8.96 A
Average	8.09 b	8.89 a	

Means followed by the same letter, lower case in line and upper case in the column, are not statistically different by Tukey test ($p < 0.05$).

4. Conclusions

Under low-temperature stress conditions, all methods of physiological conditioning proved to be effective in minimizing this type of stress during germination, on the final germination percentage and germination speed.

The use of conditioning with calcium nitrate or its association with potassium nitrate is an alternative to overcome salt stress when a germination percentage higher at four days is desired. During water deficit-, conditioning with potassium nitrate and calcium nitrate + potassium nitrate provided a higher average germination speed.

Authors' Contribution

Caio Cesar Burin, Flávio Ferreira da Silva Binotti and Elijanara Raissa da Silva conceived the experiment, interpreted the data, and wrote the manuscript. Fernando Lourenço Santana Silva carried out lab analyses and data collection. Edilson Costa contributed to the interpretation of results. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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