Priming in lettuce seeds

Condicionamento osmótico em sementes de alface

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ABSTRACT

One of the methods used to promote the rapid and uniform germination of vegetable seeds is the use of osmotic conditioning. The technique aims to regulate the osmotic potential of the solution, thus restricting the entry of water into the seeds without them germinating during the process. This work evaluated the germination of lettuce seeds of Grand Rapids, Everglades, and Verônica cultivars, submitted to osmotic conditioning. The seeds were immersed in polyethylene glycol solutions (PEG 6000) at potentials of 0.0; -0.6; -0.8, and -1.0 MPa, and remained in imbibition for 14, 24, and 48 h. After this period, the seeds were germinated on paper, moistened with water at 2.5 times their mass, and kept at 20ºC for seven days. The experimental design used was completely randomized, in a factorial scheme with three cultivars, four osmotic potentials, and three temperatures, with four replications of 25 seeds each. The germination percentage, the germination speed index, the mean speed of germination, and the mean germination time were evaluated. Data were subjected to analysis of residual normality by the Shapiro-Wilk test and the homogeneity of variances using the Bartlett test and submitted to analysis of variance. Data from osmotic potential and time of imbibition were submitted to polynomial regression. The treatment means were compared at 5% probability. Seeds of ‘Grand Rapids’ and ‘Everglades’ showed positive effects after osmoconditiong treatments.

Keywords: *Lactuca sativa* L.

RESUMO
Um dos métodos empregado para promover a germinação rápida e uniforme de sementes de hortaliças é o uso do condicionamento osmótico. A técnica visa a regulação do potencial osmótico da solução, restringindo assim a entrada de água nas sementes sem que germinem durante o processo. Com este trabalho avaliou-se a germinação de sementes de alface das cultivares Grand Rapids, Everglades e Verônica, submetidas a condicionamento osmótico. As sementes foram imersas em soluções de polietilenoglicol (PEG 6000) nos potenciais de 0,0; -0,6; -0,8 e -1,0 MPa, e permaneceram em embebição por 14, 24 e 48 h. Após este período, foram postas para germinar sobre papel tipo mata-borrão, umedecido com água na proporção de 2,5 vezes a sua massa e mantidas a 20ºC, durante sete dias. O delineamento experimental utilizado foi o inteiramente casualizado, em esquema fatorial com três cultivares, 4 potenciais osmóticos e 3 temperaturas, com quatro repetições de 25 sementes cada. Avaliou-se a porcentagem de germinação, o índice de velocidade de germinação, a velocidade média de germinação e o tempo médio de germinação. Os dados foram submetidos à análise de normalidade dos resíduos pelo teste de Shapiro-Wilk e a homogeneidade das variâncias, pelo teste de Bartlett. Em seguida foram submetidas a análise de variância. Os dados de potencial osmótico e tempo de embebição foram submetidos a regressão polinomial. As médias foram comparadas pelo teste de Tukey a 5% de probabilidade. Sementes de ‘Grand Rapids’ e ‘Everglades’ apresentaram efeitos positivos após tratamento de osmocondicionamento.

Palavras-chave: *Lactuca sativa* L., germinação, vigor.

1 INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the most consumed leafy vegetable. Lettuce accounts for 11% of total vegetable production in Brazil, with an estimated 671.509 tons. One hundred eight thousand three hundred eighty-two rural properties produce lettuce commercially (IBGE, 2017).

In the state of Sergipe, lettuce production is 841 tons, and the municipality of Itabaiana is responsible for approximately 65.5 percent (551 t) of the state's total production, moving 437 thousand reais in this city, establishing itself as the largest producer of this vegetable (IBGE, 2006). Because of its importance in human food, techniques to improve cultivation technology are being researched extensively. Seed technology to improve germination is one of the researched approaches. The first stage in the development of higher plants is seed germination. One of the pillars for forming a uniform and vigorous crop is its successful development. The quality of the seeds is only one of many factors in the process. Sowing technology and the necessary environmental conditions (water, oxygen, light, and temperature) are also included. The environment in the field is frequently stressful for the seed, with changes in plant metabolic activities. Under adverse conditions, strategies are recommended to shorten this period and increase germination.

Germination is defined, in seed physiology, as the protrusion of the radicle. Physiological viability, appropriate sowing, temperature, water, oxygen, and light are all critical factors in seed germination. Water absorption, enzymatic activation, reserve tissue degradation, embryo growth initiation, and seedling establishment are all critical events in pre- and post-germination. In lettuce, germination occurs at the end of the embryonic axis, close to the micropyle region (NASCIMENTO, 2002).

The lettuce embryo seed is entirely enveloped by the endosperm and must be crossed by the radicle for germination. Consequently, the endosperm is the primary organ that prevents embryo growth acting as a physical barrier to radicle emission, particularly under adverse conditions (SUNG et al., 2008). Germination can be erratic at high temperatures, resulting in seed dormancy (KOZAREWA et al., 2006; KATHPALIA et al., 2018).

The optimal temperature for lettuce seed germination is 20°C, and most cultivars do not germinate at temperatures above 30°C; this is due to its origin in temperate regions Asia (FILGUEIRA, 2003; NASCIMENTO; PEREIRA, 2007; AHMED et al., 2020). One of the causes of lettuce's poor adaptation in hot climate regions is an increase in soil temperature above the ideal for germination (WATT et al., 2011).

Despite impressive production, many Brazilian regions face cultivation restrictions imposed by environmental factors that can disable seed germination. Temperatures above 25°C affect the percentage and speed of germination, water uptake rate, and biochemical reactions, which determine germination (SCHWEMBER; BRADFORD, 2010; BERTAGNOLLI et al., 2003).

According to Nascimento (2003), when high temperatures are present during the imbibition of lettuce seeds, two distinct phenomena can be observed, thermoinhibition as a reversible process in which germination can occur when the temperature is reduced. Otherwise, thermodormancy, also known as secondary dormancy, the seeds do not germinate even after the reduction of temperature. Germination occurs when seeds are treated with growth regulators, subjected to osmotic conditioning, the environment is adjusted, or tolerant germplasm is used.

Thermoinhibition is an ecologically important phenomenon in determining the optimal time for plant seeds to germinate in winter, and it frequently causes crop germination to be delayed (DENG; SONG, 2012; OLIVEIRA et al., 2021). Depending on the variety, lettuce seeds may exhibit thermoinhibition and fail to germinate in the dark or light at temperatures of 26°C or higher (BLACK et al., 2006).

The mechanism of lettuce seed germination at high temperatures can be related to the weakening of the endosperm, which allows the embryo to grow under those conditions. Furthermore, the first hours of imbibition are critical for lettuce to germinate under high-temperature conditions (NASCIMENTO et al., 2004; OUYANG et al., 2020).

Water is one of the most critical factors influencing germination phases because it reactivates metabolism and is involved in all stages, both directly and indirectly. Various metabolic reactions associated with the water content of the seed trigger water uptake until the seeds reach a specific metabolic state (MARCOS FILHO, 2005).

Heydecker and Gibbins proposed the technique of osmoconditioning (1978). It entails immersing seeds in an osmotic solution for specific times and temperatures, during which water entry occurs at a slower and more controlled rate (VARIER et al., 2010). Osmotic conditioning submitted the seeds to hydro imbibition control, sufficient to allow the essential respiratory processes of germination but insufficient to promote radicle protrusion. The seeds would complete phases I and II of imbibition, which is necessary for germination, but would not progress to phase III, characterized by cell elongation and radicle protrusion (CELIA et al., 2008). According to Khan (1992), biochemical and physiological processes are stimulated to the point where the imbibition medium's low osmotic potential inhibits germination.

Furthermore, osmoconditioning induces the accumulation of solutes (sugars, organic acids, and ions) from the beginning of seed metabolism, resulting in significant turgor in rehydration and promoting the emergence of the primary root in a shorter period, as well as a higher percentage of germination (BRADFORD, 1986).

In some studies, the technique has positive effects that are more pronounced in adverse conditions such as low or high temperature, water stress, and salinity (BITTENCOURT et al., 2004; BAZ et al., 2020). Osmoconditioning is a recommended for overcoming temperature stress-induced thermoinhibition and thermodormancy. The process is a potential alternative for improving seed performance and achieving satisfactory, rapid, and uniform emergence even in seeds with low vigor or sown in unsuitable conditions.

The study aimed to determine the effectiveness of osmotic conditioning with polyethylene glycol solutions on germinating seeds from three different lettuce cultivars, 'Grand Rapids', 'Everglades', and 'Verônica', at different temperatures.

2 METHODOLOGY

Lettuce seeds of the cultivars Grand Rapids, Everglades, and Verônica were tested. The treatments consisted of soaking the seeds in solutions of (PEG 6000) with osmotic potentials of 0.0; -0.6, -0.8, and -1.0 MPa, for 14, 24, and 48 h. A completely randomized design was used, making up a 3x4x3 factorial scheme (three cultivars x four osmotic potentials x three times) with four replications of 25 seeds. The seeds were placed on paper in germination boxes (Gerbox®), moistened at 2.5 times their weight with polyethylene glycol solutions (PEG 6000) at the different potentials, and times at 20ºC. After, the seeds were washed in running water, germinated in gerbox®, in paper moistened with 2.5 times their weight in distilled water, and kept at 20°C, under continuous light. Data were evaluated until the seventh day.

Evaluations were conducted daily. The germination percentage (%G), germination speed index (GSI), the mean speed of germination (MSG), and mean germination time (MGT) were estimated. The data normality and homogeneity were examined using the Shapiro-Wilk and Bartlett tests. The data were submitted for analysis of variance (ANOVA). Later the information on potential and soaking time was submitted to polynomial regression, and the comparison of means was performed using the Scott-Knott test at 5% probability.

The seeds were kept in the B.O.D growth chamber under the above conditions, with observations every 12 hours. The seeds were considered germinated considering the protrusion of the radicle and growth of aerial structure according to the Rules of Seed Analysis (BRASIL, 2009).

The germination percentage (%G) was estimated using the formula of Labouriau & Valadares (1976), the GSI was estimate according to Maguire (1962).

With the results obtained through daily counts of germination until the seventh day after sowing, the MSG was estimated using the formula proposed by Labouriau (1983), and the results were expressed in days.

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3 RESULTS AND DISCUSSION

When analyzing the effect of osmoconditioning in PEG solutions at different periods (14, 24, and 48 hours), compared with the control (pure water), there was a low significant difference in treatments for seed germination of the cultivars Grand Rapids and Everglades. Seeds of Veronica does not present significant differences for the variables. Seeds of ‘Veronica’ presented low %G, SGI, MSG, and high MGT compared to the other cultivars, with values different by zero in only two treatments, the control and -0.8MPa for 14 h, with no significant differences (TABLE 1). Short periods may not allow the treatment to be successful, while very long periods may favor germination during treatment and impair seed vigor (NASCIMENTO, 2004).

There was no difference among treatments for the variables evaluated in 'Veronica'. This fact may be related to the reduction of water absorption, a factor related to the intensity of respiration and other metabolic activities that generate energy and nutrients for the embryo's development (FRANZIN et al., 2004; TOLEDO RODRIGUES CLAUDIO et al., 2020).

However, for the variables MGT and MGS, the control showed no significant difference between the periods. However, it differed statistically from the potentials when presenting higher values (TABLE 1).

TABLE 1. Germination (G%), germination speed index (GSI), mean germination time (MGT) and mean speed germination (MSG) of seeds of three lettuce cultivars (*Lactuca sativa* L.) at different times and concentrations of polyethylene glycol (PEG) solution.

Osmotic potential	% G			GSI			MGT			MSG		
Time	14	24	48	14	24	48	14	24	48	14	24	48
Grand Rapids												
0	97.00Aa	97.00Aa	97.00Aa	26.34Aa	26.34Aa	26.34Aa	5.01Aa	5.01Aa	5.01Aa	0.20Ac	0.20Ac	0.20Ac
-0.6	19.00Bd	96.00Aa	21.00Bd	3.10Be	25.94Aa	2.54Be	4.13Bb	5.01Aa	6.03Ba	0.14Ac	0.20Ac	0.17Ac
-0.8	76.00ABb	91.00Aa	94.00Aa	18.20Bc	24.64Aa	25.19Aa	5.19Aa	4.01Bb	5.03Aa	0.19Bc	0.40Ab	0.20Bc
-1.0	97.00Aa	90.00Aa	77.00Ab	26.27Aa	24.03Bb	19.79Cc	5.01Aa	3.01Bb	5.09Aa	0.20Bc	0.60Aa	0.20Bc
Everglades												
0	83.00Aa	83.00Aa	83.00Aa	22.01Ab	22.01Ab	22.01Ab	5.04Aa	5.04Aa	5.04Aa	0.20Ac	0.20Ac	0.20Ac
-0.6	54.00Bc	96.00Aa	86.00Aa	8.28Bd	22.92Ab	22.32Ab	5.69Aa	4.05Bb	5.03Aa	0.18Bc	0.40Ab	0.20Bc
-0.8	78.00ABb	82.00ABb	86.00Aa	15.83Bc	22.32Ab	23.02Ab	5.36Aa	5.02Aa	5.02Bb	0.19Ac	0.20Ac	0.20Ac
-1.0	31.00Bd	90.00Aa	85.00Aa	4.06Be	23.98Ab	22.91Ab	3.13Bb	5.03Aa	5.01Aa	0.08Bd	0.20Ac	0.20Ac
Verônica												
0	5.00Ae	5.00Ae	5.00Ae	0.18Af	0.18Af	0.18Af	7.00Aa	7.00Aa	7.00Aa	0.11Ac	0.11Ac	0.11Ac
-0.6	0.00Ae	0.00Ae	0.00Ae	0.00Af	0.00Af	0.00Af	0.00Ad	0.00Ad	0.00Ad	0.00Ad	0.00Ad	0.00Ad
-0.8	3.00Ae	0.00Ae	0.00Ae	0.48Af	0.00Af	0.00Af	2.22Ad	0.00Ad	0.00Ad	0.12Ad	0.00Ad	0.00Ad
-1.0	0.00Ae	0.00Ae	0.00Ae	0.00Af	0.00Af	0.00Af	0.00Ad	0.00Ad	0.00Ad	0.00Ad	0.00Ad	0.00Ad

CV (%)	20.70	18.70	33.70	86.90

Means followed by the same lowercase letter in the column and uppercase in the row do not differ by the Scott-Knott test at 5% probability.

'Grand Rapids' and 'Everglades' showed no significant difference for %G in all potentials, and the mean remained above for the other times. For 'Everglades,' osmotic conditioning in the period of 14 h, in -0.6 and -0.8 and -1.0 MPa, negatively affected the %G and GSI compared to the control, mainly in the potentials -0.6 and 1.0 MPa. Thus, as demonstrated by Zhang et al. (2013), the treatment of lettuce seed with PEG solution increases the percentage of seed germination, but very low or very high PEG concentrations were responsible for the decrease in seed germination (PAWAR; LAWARE, 2018).

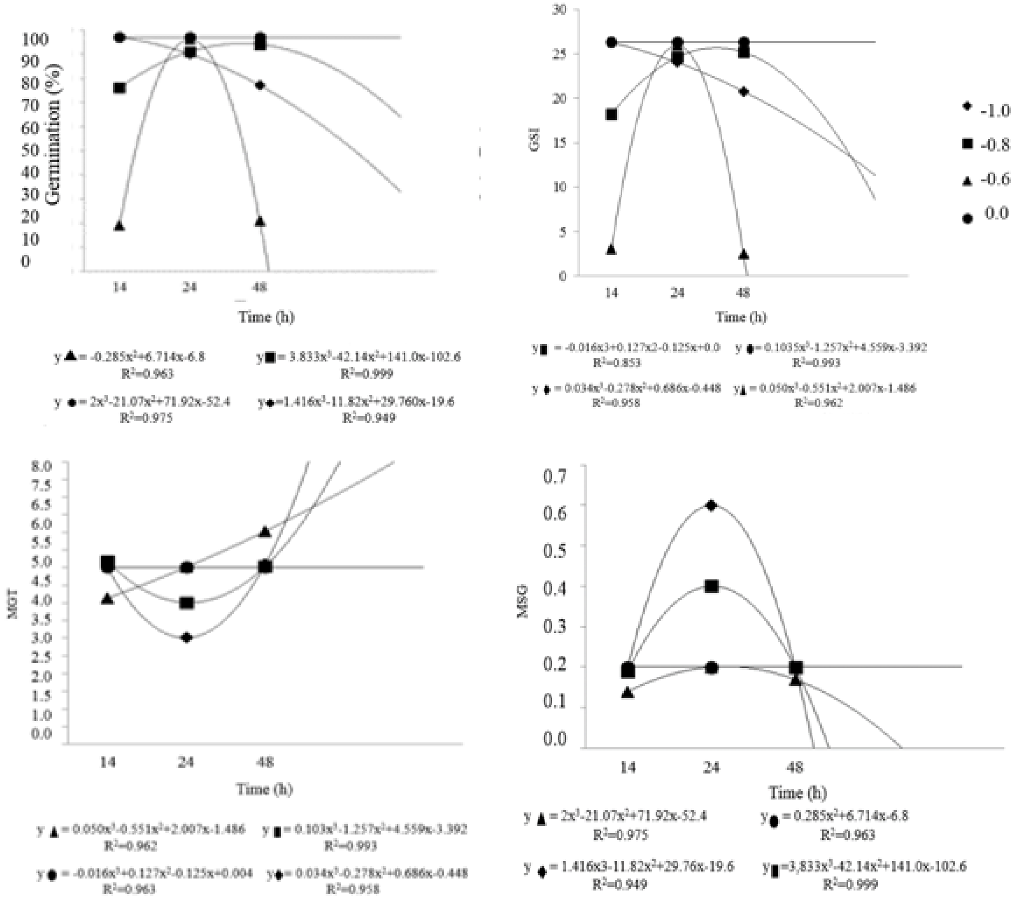
For 'Everglades,' variable %G, in the period of 48 h, the control showed no significant differences among the treatments. However, for 24 h, there was a significant difference between the control and the potential -0.8 MPa. There was no significant difference among treatments in the SGI in the 24 h and 48 h.

There were no significant differences among the treatments for the variables MTG and MSG for 'Grand Rapids and 'Everglades'.

Imbibition of 14 h at -0.6 and -1.0 MPa showed negative responses to the control. Therefore, as demonstrated by Zhang et al. (2013), the treatment of lettuce seed with PEG solution increases the percentage of seed germination, but very low or very high PEG concentrations were responsible for the decrease in germination (PARK et al., 2022).

Bertagnolli et al. (2003) studied the performance of naked and pelleted lettuce seeds (cv. Karla) under different temperatures and water potentials. They observed that -0.3 MPa using naked seeds and -0.6 MPa for pelleted presented a reduction in germination, which became more accentuated with the decreasing water potential. When the seeds were moistened with osmotic solutions of -0.9 MPa, the values reached zero, demonstrating that water stress limits germination in this potential.

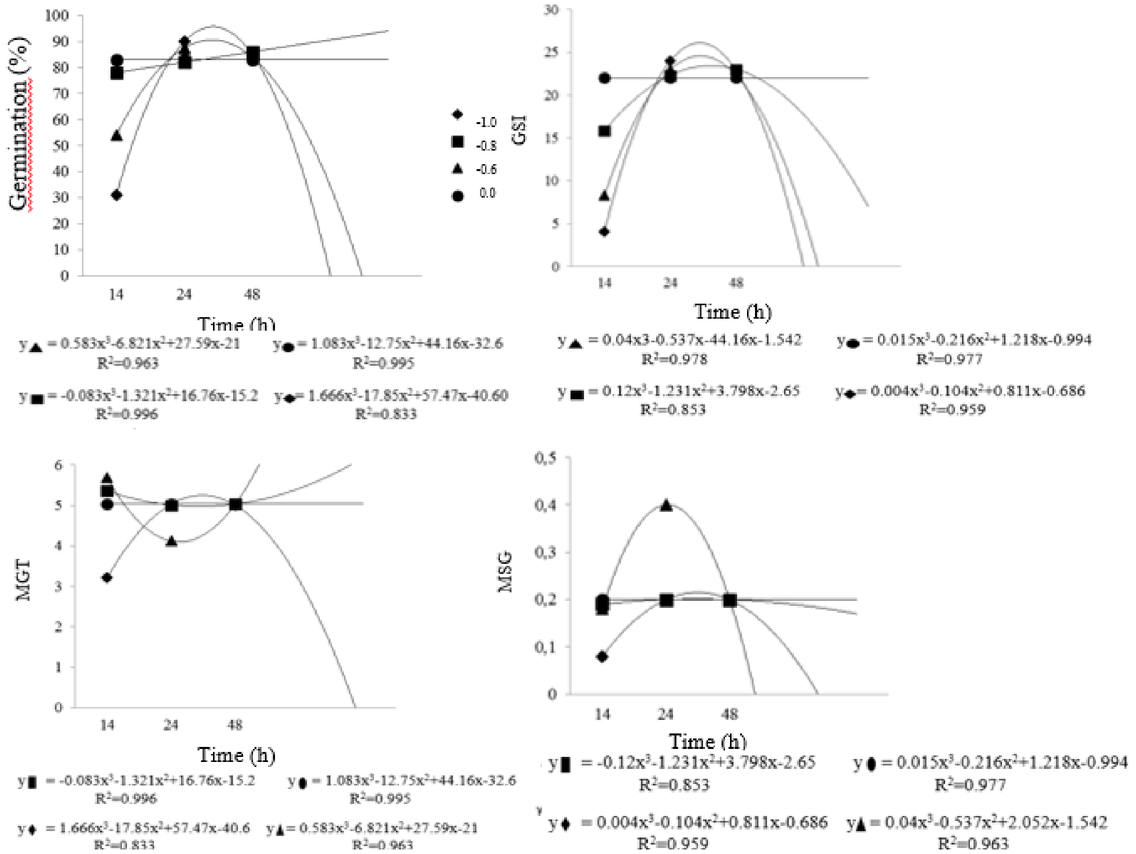
FIGURE 2. Germination of seeds of cv. Grand Rapids at different osmotic potentials, and and time of treatment.



As shown in Figure 2, the MGT is inversely proportional to %G, GSI, and MSG. For 24 h in most potentials, the mean of the variables was positively affected. More clearly seen in germination, where the percentage increased dramatically from the potential -0.6 from 14 h to 24 h; and decreased in the same amplitude from 24 h to 48 h.

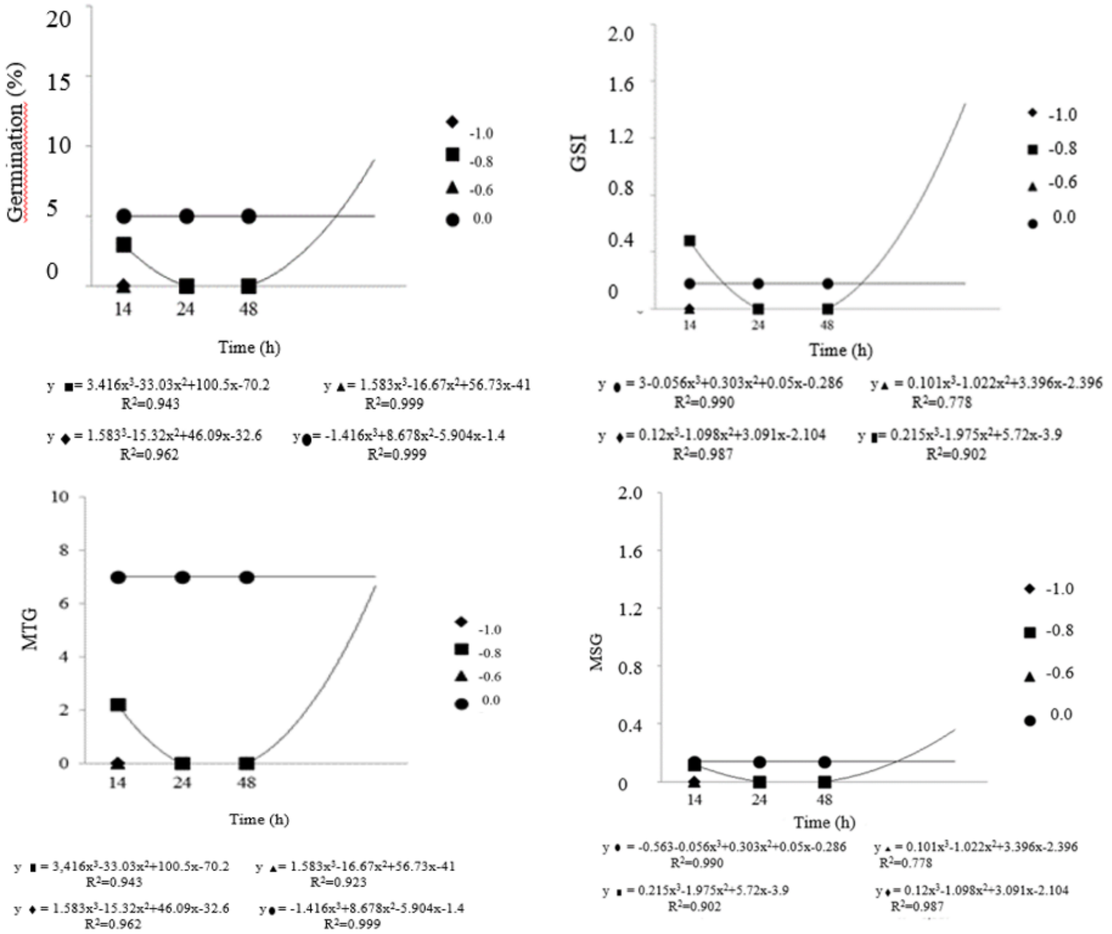
The MGT at -0.6 MPa shows an upward trend for the Grand Rapids cultivar with increasing imbibition time.

FIGURE 3. Seeds of the cultivar Everglades in different osmotic potentials and time of treatment.



The osmoconditioning for 24 h time presented positive for control and the other imbibition times in the two cultivars, Grand Rapids and Everglades, with %G and SGI being lower than the control at 14 h and potentials of -0.6 and -1.0 MPa.

FIGURE 4. Seeds of cultivar Verônica submitted to different osmotic potentials and time of treatment.



The ‘Grand Rapids’ seeds have higher vigor than the Everglades and Verônica when subjected to osmotic conditioning at -0.8 MPa for 24 h. The treatment using -0.6 MPa, negatively affects germination for 14 h and 48 h. The imbibition for 24 h independently of the potential positively affects the variables.

The osmotic conditioning in the three studied concentrations does not interfere in the germination of lettuce for ‘Grand Rapid’ and ‘Everglades’. The osmotic conditioning negatively affects the germination of ‘Veronica’, which presented low viability and vigor even for the control.

4 CONCLUSION

Osmotic conditioning negatively affects seed germination of the cultivar Verônica.

Osmotic conditioning in seeds of ‘Grand Rapids’ and ‘Everglades’ with high viability and vigor does not contribute positively to most of the variables tested, presenting a positive response only in the evaluations of MGT and MSG.

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