

RESEARCH NOTE

Germination of melon seeds under water and thermal stress¹

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ABSTRACT – Seeds vigor can influence seed performance under stress conditions. The objectives of this study were to evaluate the effect of water and thermal stress on germination and performance of melon seedlings, and to verify if germination under stress conditions is an efficient parameter to evaluate the vigor of these seeds. Four lots of ‘Golden Mine’ melon had their initial quality characterized by germination, first count, accelerated aging and seedling emergence tests. Germination under water stress was performed on a paper moistened with PEG 6000 solution at 0.06, -0.3, -0.6 and -0.9 MPa. The percentage and speed of germination, length and dry mass of the seedlings were evaluated. For the thermal stress experiment, cold test and germination at sub- (15 °C) and supra-optimal (35 °C) temperatures were performed, as well as at the ideal temperature (25 °C). The germination of melon seeds under water stress induced by PEG 6000 at -0.3 and -0.6 MPa is an efficient method to detect differences in the physiological potential of lots of melon seeds, but these differences disappear under severe water stress (-0.9 MPa). Germination under sub-optimal temperatures also allows to identify differences in seeds performance and to classify them according to the vigor level.

Index terms: *Cucumis melo* L., water deficit, temperature, vigor.

Germinação de sementes de melão sob estresse hídrico e térmico

RESUMO – O vigor das sementes pode influenciar seu desempenho sob condições de estresse. Objetivou-se avaliar a influência do estresse hídrico e térmico na germinação e desempenho das plântulas de melão e verificar se a germinação sob condições de estresse é eficiente para avaliação do vigor destas sementes. Quatro lotes de melão ‘Golden Mine’ foram caracterizadas quanto à qualidade inicial pelos testes de germinação, primeira contagem, envelhecimento acelerado e emergência de plântulas. A germinação sob estresse hídrico foi realizada em papel umedecido com solução de PEG 6000 a 0,0; -0,3; -0,6 e -0,9 MPa. Foram avaliadas a porcentagem e velocidade de germinação, comprimento e massa seca de plântulas. Para avaliação do estresse térmico, utilizaram-se os testes de frio e de germinação à temperatura sub (15 °C) e supra ótima (35 °C), além da germinação sob temperatura ideal (25 °C). A germinação das sementes de melão sob estresse hídrico induzido por PEG 6000 a -0,3 e -0,6 MPa é eficiente para detectar diferenças no potencial fisiológico de lotes de sementes de melão, mas estas diferenças desaparecem sob estresse hídrico severo (-0,9 MPa). A germinação sob temperaturas sub-ótimas também permite identificar diferenças no desempenho das sementes e classificá-las quanto ao nível de vigor.

Termos para indexação: *Cucumis melo* L., deficiência hídrica, temperatura, vigor.

Introduction

Germination occurs with the resumption of the metabolic activity of the seed embryo in a coordinated and sequential

way, which includes hormonal regulation, protein metabolism, reserves transportation, and other events (Rajjou et al., 2012). For these processes, seeds need to reach an adequate level of hydration, which allows the reactivation of the metabolism

¹Submitted on 08/29/2017. Accepted for publication on 12/05/2017.

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and, consequently, the growth of the embryonic axis (Marcos-Filho, 2015). Due to the fact that it is highly influenced by environmental conditions, germination is considered the most critical phase in plants life cycle (Ghavami and Ramin, 2007).

In situations of low water availability, the seed absorbs water due to its reduced matric potential. However, if there is not enough water to continue the germination process, the embryo may die (Hobbs and Obendorf, 1972). Thus, water stress is one of the main factors that affect germination, and results in reduced growth and production rates (Ansari et al., 2012). The responses to water stress vary according to the species, intensity and other factors, and in each situation there may be a water potential value in which the germination does not occur (Adegbuyi et al., 1981).

Many studies have been performed relating water potential and seed performance. Torres et al. (1999), when working with water potential of 0, -0.2, -0.4, -0.6 and -0.8 MPa, observed that potentials equal to or lower than -0.4 MPa could be considered critical to germination and development of cucumber seedlings. Abreu et al. (2014) evaluated the behavior of five maize lineages under water stress, and they observed that the potential of -0.9 MPa affected considerably the physiological quality of the seeds. Soares et al. (2015) observed that an increase in osmotic concentration resulted in a considerable decrease in germination and vigor of two soybean cultivars, mostly up from the potential of -0.3 MPa.

Another important issue to consider is that, when seeds are put to germinate in conditions of water deficiency, their response has proved to be dependent on the physiological quality. According to this principle, the water stress test (AOSA, 2002) is based on the fact that more vigorous seeds can tolerate more severe conditions of water stress (Hadas, 1977). In view of this, Vaz-de-Melo et al. (2012) concluded that the germination values showed a high correlation with the vigor levels of maize seeds under water stress.

As stated by Llanes et al. (2016), water stress is a consequence not only of water restriction in soil, but also of the joint action of other deleterious environmental conditions. In this context, temperature is considered an important factor that influences water absorption and several biochemical reactions in seeds metabolism, thus being able to cause direct effects in both percentage and speed of germination (Carvalho and Nakagawa, 2012; Bewley et al., 2013). Although seeds of most species germinate in a wide temperature range, the lower the vigor, the more specific are the requirements for germination. Based on this, cold and germination tests at sub- and supra-optimal temperatures have been performed to evaluate the vigor of seeds of different species (AOSA, 2002; Dias and Alvarenga, 1999; Guissem et al., 2010; Batlla and Benech-Arnold, 2015).

Considering that the conditions that seeds face in the soil in order to germinate are not always ideal, the objective of this work was to evaluate the influence of water and thermal stress on the germination and performance of melon seedlings (*Cucumis melo* L.), and to verify if the germination under stress conditions can be used to estimate the vigor of these seeds.

Material and Methods

The study was carried out at the Laboratory of Seeds Research at Universidade Federal de Viçosa, Brazil. Seeds of four lots of Gold Mine yellow melon (*Cucumis melo* L.) were used in all tests. Initially, the physiological quality of the lots was evaluated by the following tests:

Germination: four replications of 50 seeds were distributed on paper towel rolls, which had been moistened with a volume of water equivalent to 2.5 times the dry paper weight. The rolls were kept in a germinator at 25 °C, and the results were expressed as percentage of normal seedlings obtained on the eighth day after sowing, as determined in Rules for Seed Testing (Brasil 2009).

First germination count: it was performed together with the germination test and then, the percentage of normal seedlings obtained on the fourth day after sowing was calculate (Brasil, 2009).

Seedlings emergence: it was carried out in a greenhouse, on trays containing soil and sand substrate in a 2:1 proportion. Four replications of 50 seeds of each lot were sown at 1.0 cm depth. The seedlings with cotyledons above the substrate surface were counted until count stabilization. The percentage of emergence of seedlings 14 days after sowing was calculated.

Accelerated aging: a sample of 250 seeds was distributed on screens coupled to gerboxes containing 40 mL of distilled water in the bottom. The boxes were capped and maintained in a BOD incubator at 42 °C for 72 hours (Torres et al., 2009). After this period, the seeds were put to germinate according to the germination test. The percentage of normal seedlings was evaluated on the fourth day after sowing.

After the characterization of the initial quality of the lots, seeds germination was evaluated under water and thermal stress conditions, as follows:

Germination under water stress: the same germination test method was used, but the paper was moistened with polyethylene glycol (PEG 6000) solutions with osmotic potentials of 0,0 (only distilled water), -0.3, -0.6, and -0.9 MPa. These solutions were obtained according to the methodology of Villela et al. (1991).

Length of seedlings under water stress: four replications of 25 seeds each were distributed in straight lines on paper

towel moistened with solutions at concentrations 0.0, -0.3, -0.6, and -0.9 MPa (PEG 6000), as described for the germination test. The rolls were kept inside plastic bags in a germination chamber at 25 °C. The normal seedlings were measured eight days after sowing, and the results were expressed in cm.plantula^{-1} .

Dry matter of the seedlings under water stress: the seedlings used for length measurement were kept in a forced air-circulating oven at 75 °C for 48 hours. After drying, the seedlings were weighed, and the results were expressed in mg.plants^{-1} .

Cold test: the germination in this test was performed as described for the germination test, but using distilled water at 10 °C. The rolls were put inside plastic bags in a germination chamber at 10 °C for seven days. After that, they were transferred to germination chamber at 25 °C, where they were kept for five days. The results were expressed as percentage of normal seedlings – those with length equal to or greater than 4 cm after eight days (AOSA, 2002).

Germination at sub- and supra-optimum temperatures: in both conditions, it was used the same methodology of the germination test. For the evaluation at sub-optimum temperature, the rolls were maintained in germination chamber at 15 °C, and the normal seedlings were counted after eight days (Dias and Alvarenga, 1999). For the analyses at supra-optimum temperature, the rolls were kept in germination chamber at 35 °C, and the count of normal seedlings was performed after seven days.

Statistical design and analysis: the experiment was carried out in a completely randomized design (CRD) with four replications. The data were submitted to analysis of variance in a factorial scheme 4 (lots) by 4 (osmotic potential or temperature). The means of the lots characterization and temperature effect were compared by the Tukey test at 5% probability. The means of osmotic potentials were adjusted to regression equations, and the regression coefficients evaluated by the t-test at 1% and 5% probability.

Results and Discussion

It can be seen in Table 1 that the highest germination percentage and germination speed (evaluated by the first count of the germination test) were obtained in seeds from lot 2, in comparison to lots 3 and 4, which did not differ between them. There was no statistical difference in the percentage of germination, germination speed and vigor between seeds from lots 1 and 2, according to the accelerated aging test. In this test, seeds from lots 1, 3 and 4 did not differ from each other. As for seedlings emergence, lots 1, 2 and 3 did not differ among themselves, but the results obtained from lots 1 and 2

were greater than those from lot 4. Thus, in general, it can be noted that the four lots showed differences in physiological quality, with a greater vigor in seeds from lots 1 and 2, and a lower vigor in lots 3 and 4.

Figure 1a shows that seed germination in all four lots decreased with the reduction of the water potential of the solution, and the lowest values were obtained when seeds germinated at -0.9 MPa. At this concentration, germination was practically inexistent, and there was no significant difference between the lots. It is important to emphasize that the percentage of abnormal seedlings significantly increased as the osmotic potential of the solution was reduced.

When the performance under water stress of each lot was evaluated (Figure 1a), it could be observed that higher germination values were obtained at 0.0 and -0.3 MPa. Germination was practically maintained up to this potential in lots 1, 2 and 3, and there was a slight drop in the values observed in lot 4 seeds. These results differ from those observed by Pinheiro et al. (2016), who evaluated saline stress in melon seeds, and observed significant decrease in germination at -0.3 MPa. However, it is important to emphasize that saline stress causes, besides water restriction, toxic effects due to the absorption of ions, and this can cause variance in the responses (Ibrahim, 2016). Higher potentials usually cause a drastic reduction in germination, as stated by Torres et al. (1999), who observed a decrease of 50% in germination of cucumber seeds at the potential of -0.8 MPa.

Up from the concentration of -0.3 MPa, there was a significant reduction in germination of the four lots, which reached values below 20% (it was practically zero at the potential -0.9 MPa) (Figure 1a). According to the results of the initial quality evaluation tests (Table 1), it was verified that, in general, lots 3 and 4 had been classified as showing less vigor than lots 1 and 2. When the germination under water stress was considered, at the potential of -0.3 MPa, seeds from lot 4 had a worse performance than those from lot 3, with a reduction in germination, in comparison to the potential 0.0

Table 1. Means values obtained in the germination, first count, accelerated aging and seedling emergence tests from four seed lots of 'Golden Mine' melon.

Lots	Germination (%)	First count (%)	Accelerated Aging (%)	Emergence (%)
1	86 AB	66 AB	75 AB	88 A
2	91 A	70 A	82 A	93 A
3	81 B	60 BC	70 B	84 AB
4	82 B	56 C	68 B	78 B

*Means followed by the same letter in the line do not differ statistically according to the Tukey test at 5% probability.

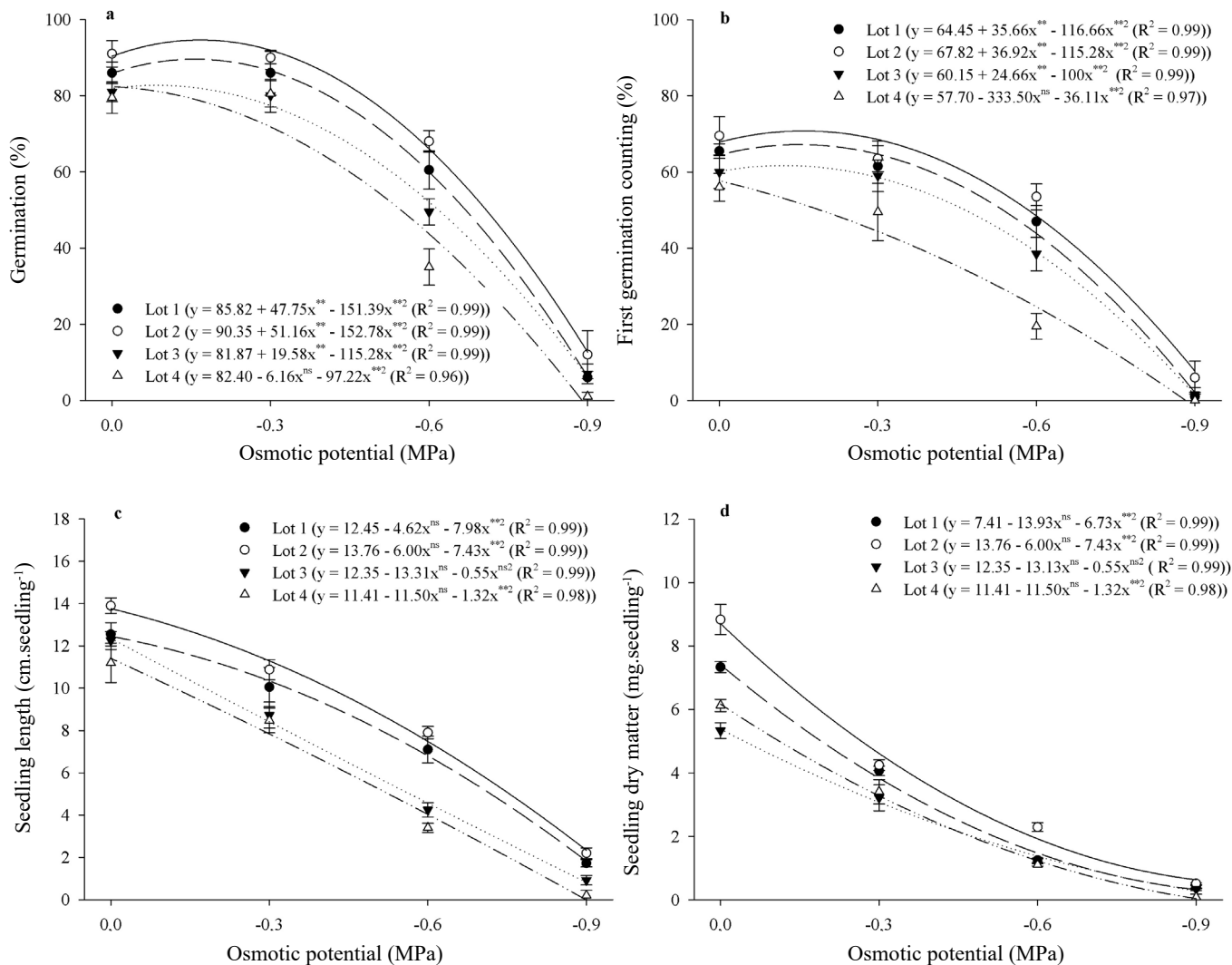


Figure 1. Mean values of germination, first germination count, length and dry mass of seedlings of the four lots of Golden Mine melon seeds under different osmotic potentials.

^{ns}: non-significant, ^{**} significant by the t-test at 5% probability. Bars: standard deviation.

MPa. The same practically did not occur in the other lots. These results indicate a lower vigor of these seeds than the ones from the other lots, considering their performance under water stress at -0.3 MPa.

At the potential of -0.6 MPa, there was stratification in the physiological potential of the lots. Lots 1 and 2 had the best performance, with no significant difference between them; and the lot 4 had the worst performance. This trend was also observed at -0.9 MPa potential (Figure 1a).

As observed in the first germination count, seed germination speed of lots 1, 2 and 3 remained steady up to the potential of -0.3 MPa. It decreased up from this value, and practically reached zero when water restriction was

higher (-0.9 MPa) (Figure 1b). As observed in germination, seed performance reduction was more accentuated up from -0.6 MPa. This can be explained by the decrease in water absorption of the seeds as the osmotic concentration of the PEG solutions increased. Rossetto (1997) also verified that as the water potential reduced, there was a loss of soybean seeds performance in the first germination test count. In flax seeds, Stefanello et al. (2017) observed that osmotic potentials equal to or lower than -0.30 MPa were harmful to germination, and that there was no normal seedling development up from -0.50 MPa concentration.

By comparing the lots (Figure 1b), it could be verified that seeds from lot 4 were more sensitive to water stress at

the potentials -0.3 and -0.6 MPa than those from other lots. Besides, at the potential -0.9 MPa, this lot did not differ from lot 3, which, in its turn, did not differ from lots 1 and 2. It is worth mentioning that the germination speed of all lots at -0.9 MPa potential was very low. These results indicate that this potential had very drastic effects on melon seeds, and did not allow the detection of differences among lots performance. Similarly, Zhang et al. (2013) observed a significant decrease in germination of cucumber seeds submitted to water stress.

The germination speed, evaluated by the first count, was more affected than the percentage of germination (Figures 1a and 1b). Torres et al. (1999) found that the vigor of cucumber seeds was more affected than their germination as the osmotic potential was reduced. This became more evident at potentials equal to or lower than -0.4 MPa.

Reducing water availability for the seeds resulted in a reduction in seedlings length and dry matter mass (Figures 1c and 1d). Seedlings length decreased with the water restriction in all four lots, as of the potential 0.0 MPa, and the most reduced values were obtained at the potential -0.9 MPa, which ranged from 2.78 (lot 2) to 0, 40 cm.plantula⁻¹ (lot 4). Therefore, a significant reduction in seedling size at the potential -0.9 MPa was observed, especially in comparison to the potential 0.0 MPa, in which values were 13.9 (lot 2) and 11.2 cm.plantula⁻¹ (lot 4), respectively.

It was also observed that lots 1 and 2 (greater vigor) were less sensitive to water stress than lots 3 and 4 (lower vigor) at both the potentials -0.3 MPa and -0.6 MPa, and that the lowest rate of seedling development occurred at -0.6 MPa potential. In the condition of highest water restriction (-0.9 MPa), seedlings growth was practically zero in seeds from lot 4, which did not differ from lot 3. At this potential, all lots had a marked reduction in seedling length, and all of them showed a poor performance. At the potential 0.0 MPa, lot 2 was superior to the others.

Therefore, water restriction caused by the osmotic potentials -0.3 and -0.6 MPa allowed to identify differences in seedlings length among the lots that could not be observed at 0,0 (without water stress) nor at -0.9 MPa (severe water stress). Kappes et al. (2010) evaluated the germination and performance of maize seedlings under different osmotic potentials, and they verified reductions in seedling length up from the potential -0.3 MPa, and also different responses among the lots.

There was a reduction in the dry mass of seedlings of all lots as the water restriction increased, and the most significant reduction was observed in seeds from lot 2, which decreased up to 8 percentage points in comparison to the control treatment (0.0 MPa) and the severe stress condition

(-0.9 MPa) (Figure 1d). At the potential 0.0 MPa, there was a significant difference among the lots concerning the dry mass of the seedlings, and the highest value was obtained in lot 2, and the lowest in lot 3. On the other hand, at the potentials of -0.3 and -0.9 MPa, there was no difference among the lots.

In summary, the effect of water stress was more expressive on seedlings length and germination speed (Figures 1c and 1d) than on germination (Figure 1a), and it was less drastic for higher vigor seeds (lots 1 and 2). Thus, at potentials lower than -0.3 MPa, there was a sharp decrease in water uptake by the seeds, which resulted in a reduction in both germination percentage and germination speed, and also in the development of seedlings, in comparison to the control. This evidences the harmful effect of low water availability on melon seedlings growth.

It is important noticing that the germination test performed under water deficit in substrate at -0.3 MPa and at -0.6 MPa showed differences in the performance of the lots that were not observed in the treatment with no water restriction (0.0 MPa) and with a more severe restriction (-0.9 MPa). According to Rossetto (1997), the differences between germination and emergence of soybean seedlings at -0.04 and -0.10 MPa were small; however, they became more pronounced as the water restriction increased. Therefore, subjecting melon seeds to water stress simulated by PEG 6000 at -0.3 or -0.6 MPa can be a viable alternative to evaluate their vigor, in order to detect differences in lots performance that cannot be verified at the potential 0.0 MPa.

In general, for all water deficiency situations, lot 2, followed by lot 1, had the best performance, which shows that high vigor seeds are less affected by water stress than those of lower vigor. Rossetto (1997) observed a restriction on the development of soybean seedlings at the potential -0.4 MPa. In this condition, the emergence speed of seedlings from the lowest vigor seeds lot was the most impaired.

Regarding the cold test, lot 2 presented the highest germination value. In lot 1, germination was greater than in lots 3 and 4, which, in their turn, did not differ from each other (Figure 2). In the evaluation of thermal stress (Figure 2), in all lots, the highest germination values were observed at 25 °C, which is the temperature indicated by the Rules for Seed Testing (Brasil, 2009) as the ideal for germination of melon seeds. The lots with greater physiological quality (1 and 2) exhibited no difference in germination of the seeds submitted to all three thermal stress conditions. On the other hand, in lots 3 and 4, the germination at sub-optimal temperature (15 °C) was greater than that obtained in the cold test (Figure 2).

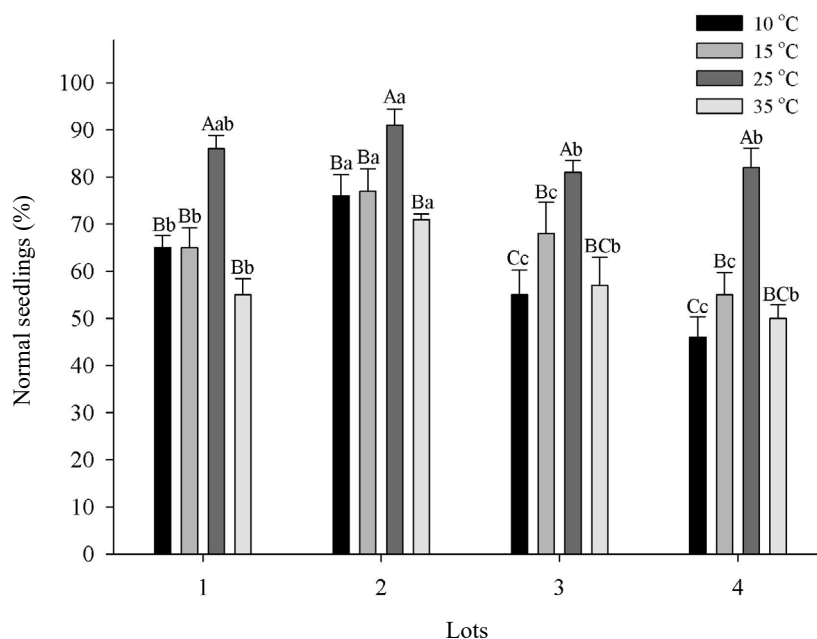


Figure 2. Mean values of germination of 'Golden Mine' melon seeds at cold test (10 °C) and at different temperatures (15, 25 and 35 °C).

The same uppercase letters for temperatures in each lot and the same lowercase letters for lots in each temperature did not differ statistically by the Tukey test at 5% probability. Bars: standard deviation.

There was no significant difference between the germination in sub and supra-optimal temperatures, and the values obtained at 35 °C did not differ from those obtained in the cold test. As stated by Guan et al. (2009), low temperatures induce damage to the cell membrane and limit the germination process, which makes seeds more susceptible to adverse factors. In opposition, high temperatures, particularly at the beginning of imbibition, cause deleterious effects on germination and seedling development (Dias et al., 2011).

The comparison among lots (Figure 2) showed that seeds from lots 3 and 4 had a higher sensitivity to low temperatures than those from lots 1 and 2, and lot 2 had a better performance than lot 1. The low temperatures allowed to classify the lots in three levels of physiological potential: best potential, for lot 2; intermediate potential, for lot 1; and worst potentials for lots 3 and 4. However, at the temperature of 35 °C, lots 1, 3 and 4 did not differ from each other, but their physiological potentials were lower than lot 2. So, actually, the lots could be separated into only two levels of quality.

Therefore, the germination test at 15 °C and the cold test were the most efficient methods to detect differences in the physiological potential of the lots. Thus, seeds performance in each lot was related to the stress temperature, which confirms the assertions of Sbrussi and Zucareli (2014), who said that low vigor seeds are more susceptible to low temperatures during germination. Demir and Van de Venter (1999), working with

watermelon seeds under water and thermal stress, verified that the percentage of germination and the speed of germination reduced extremely at 15 °C, in comparison to the temperatures of 25 °C and 38 °C.

Therefore, as observed in the water stress tests, melon seeds showed a sensitivity caused by low temperatures, and there were marked differences between the lots regarding this. In synthesis, the physiological quality affects the performance of seeds under water and thermal stress, since germination and seedlings growth are directly related to water availability in the substrate and to the temperature. In addition, water stress and germination at low temperature are viable alternatives to evaluate the performance of melon seeds.

Conclusions

Germination of melon seeds under water stress induced by PEG 6000 at -0.3 and -0.6 MPa is an efficient method to detect differences in the physiological potential of melon seed lots. However, these differences disappear under severe water stress (-0.9 MPa).

Germination under sub-optimal temperatures also allows to identify differences in the performance of melon seeds, and to classify them according to the level of vigor.

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