

Maternal effect on thermotolerance in lettuce seeds

Abstract – The objective of this work was to evaluate the occurrence of the maternal effect on thermotolerance in lettuce (*Lactuca sativa*) seeds, as well as to find an enzymatic marker for this trait in lettuce seedlings. Seeds from the Everglades thermotolerant cultivar, the Verônica susceptible cultivar, and their reciprocal hybrids were used. For each cultivar and reciprocal hybrid, germination percentage and vigor (germination speed index) tests were carried out at 20 and 32°C. Thermotolerance was defined as the capacity to germinate at 32°C. To obtain markers that could be associated with thermotolerance, enzymatic tests were performed. The bands of the electrophoretic enzymatic extracts were quantified using the ImageJ software. The hybrids behaved similarly to their female genitors. When cultivar Verônica was the female genitor, hybrid seeds did not germinate at high temperatures and showed a lower expression of the esterase enzyme. However, when the female genitor was the Everglades cultivar, hybrids showed a high germination at high temperatures and a greater esterase expression. Therefore, there is a maternal effect on tolerance to thermoinhibition in lettuce seeds, and the esterase enzyme can be used as a selective marker to identify homozygous progenies for this trait.

Index terms: *Lactuca sativa*, esterase, seed dormancy, temperature.

Efeito materno na tolerância à termoinibição em sementes de alface

Resumo – O objetivo deste trabalho foi avaliar a ocorrência do efeito materno na tolerância à termoinibição em sementes de alface (*Lactuca sativa*), bem como encontrar um marcador enzimático para esta característica em mudas de alface. Foram utilizadas sementes da cultivar termotolerante Everglades, da cultivar suscetível Verônica e de seus híbridos recíprocos. Para cada cultivar e híbrido recíproco, foram conduzidos testes de germinação e vigor (índice de velocidade de germinação) a 20 e 32°C. A termotolerância foi definida pela capacidade de germinação a 32°C. Para encontrar marcadores que pudessem ser associados com a termotolerância, foram realizados testes enzimáticos. As bandas eletroforéticas dos extratos enzimáticos foram quantificadas por meio do programa ImageJ. Os híbridos apresentaram comportamento semelhante ao dos seus genitores femininos. Quando a cultivar Verônica foi o genitor feminino, as sementes dos híbridos não germinaram sob alta temperatura e apresentaram menor expressão da enzima esterase. Porém, quando a cultivar Everglades foi o genitor feminino, os híbridos apresentaram alta germinação sob temperatura elevada e maior expressão da esterase. Portanto, há efeito materno na tolerância à termoinibição em sementes de alface, e a enzima esterase apresenta potencial como marcador para identificar progênies homocigotas para essa característica.

Termos para indexação: *Lactuca sativa*, esterase, dormência de sementes, temperatura.

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Introduction

Species, such as lettuce (*Lactuca sativa* L.), which are widely consumed and cultivated worldwide, are subjected to suboptimal environmental conditions for plant development (Barros & Cavalcante, 2021). In tropical regions, for example, lettuce seeds are sown to germinate at average temperatures higher than 25°C, which is above the temperature considered optimal for the species (Nascimento & Cantliffe, 2002). Yoong et al. (2016) added that, lettuce, as other thermosensitive species, does not germinate over 28°C, hindering crop yield.

Regarding the effects of higher temperatures on lettuce plants, several parameters have been analyzed, such as changes in plant morphological characteristics (Zhao et al., 2022), nutritional values (Song et al., 2015; Zhang et al., 2016), and seed germination (Yoong et al., 2016). Regarding seed germination, Catão et al. (2014, 2016), when evaluating lettuce accessions under different temperatures and storage times, reported, among other results, a 70% germination ratio for seeds of the Everglades cultivar at 35°C, indicating its possible thermotolerance.

According to Catão et al. (2014), thermotolerant species produce a higher number of enzymes, such as endo- β -mannanase, which soften the embryo-surrounding endosperm and weaken its cell wall, consequently, allowing of embryo growth and seed germination. In contrast, thermosensitive seeds have more mannose and/or a lower expression of endo- β -mannanase, making seed germination more difficult (Ferreira et al., 2021). In this line, Wei et al. (2020) highlighted the following mechanisms related to thermoinhibition: the arrest of embryo development and consequent radicle protrusion and hardness of the endosperm surrounding the embryo.

Considering the importance of endosperm softening for seed germination, the study of enzymes linked to this process can be of interest. José et al. (2004) and Wang et al. (2019) found that the catalase, esterase, and superoxide dismutase enzymes are linked to tolerance to abiotic stress, such as high temperatures. This is important due to the observed effects of heat stress on lettuce, which changed the expression of 25 transcripts in this species in the study of Wei et al. (2020). In this scenario, enzyme-related tests, which are of low cost and fast, have potential to be applied as markers for identifying

cultivars with thermotolerance (José et al., 2004), speeding the breeding process (Wang et al., 2016; Kosma et al., 2020).

According to Oliveira et al. (2021), thermotolerance is a genetically controlled characteristic linked to the seed endosperm. Since 2/3 of this triploid tissue are constituted by the female genitor, thermotolerance is subjected to the maternal effect (Rosado et al., 2020; Li et al., 2021). This shows the importance of characterizing thermotolerance in the female genitor, which can influence hybrid sensitivity to higher temperatures, aiming to transfer this trait from a thermotolerant cultivar to another of interest in conventional breeding.

Under high temperatures, thermodormancy breaking is a common practice since thermosensitivity impairs seed germination, hindering crop production and leading to financial prejudices (Wang et al., 2019). However, the used treatments mean extra steps and costs for seedling production, directly impacting profit (Kaya, 2022). In this context, using thermotolerant cultivars is of foremost importance during the plant breeding process, in order to avoid the production of cultivars with thermosensitive seeds (Oliveira et al., 2021).

The objective of this work was to evaluate the occurrence of the maternal effect on thermotolerance in lettuce seeds, as well as to find an enzymatic marker for this trait in lettuce seedlings.

Materials and Methods

The following thermotolerant and thermosensitive lettuce cultivars, respectively, were evaluated: Everglades, butterhead lettuce; and Verônica, crispy lettuce. The reciprocal hybrids from the crossings of both cultivars were also analyzed. Seedling production was conducted in a nursery at Universidade Federal de Lavras. Initially, 'Everglades' and 'Verônica' seeds were cultivated in polystyrene trays with 128 cells, with three seeds each. The trays were placed in a greenhouse with a 30% shading screen to reduce solar radiation incidence. After germination and seedling emergence, thinning was performed, and only one plant was kept per cell. Since the Everglades cultivar is characterized by a higher precocity, sowing was staggered at 32 cells per week to certify that the flowering of both cultivars would coincide, allowing

of the crossings. Twenty-five days after germination, the seedlings were transplanted to 10 L pots with a mix of soil, sand, and organic compost at a 2:1:1 ratio, and kept there until seed production for harvest.

During the flowering period, crossings were carried out in the morning before sunrise, at approximately 4:00 a.m., by emasculating the flowers of the feminine genitor without cutting the stigma. Each emasculated flower was identified by placing a piece of colored wool at its peduncle. The flowers from the male genitors were collected after opening at approximately 8:30 a.m. After stigma development and flower opening, the flowers (classified as bifid) were manually pollinated. After seed development and maturation, harvest was performed for both genitors. Harvested seeds were identified as F₁ for two crossings: 'Everglades' x 'Verônica', in which the seeds from the emasculated flowers of 'Everglades' were pollinated by 'Verônica'; and 'Verônica' x 'Everglades', in which the seeds from the emasculated flowers of 'Verônica' were pollinated by 'Everglades'. The seeds from self-pollinated flowers were identified using the respective cultivar names. All seeds were cleaned, dried, and stored in a cold chamber at 15°C and 50% relative humidity.

F₁ seed purity was certified by sowing both cultivars and hybrids in polystyrene trays. To determine contamination, the edges of the leaves were compared with those of 'Verônica' (wrinkled) and 'Everglades' (smooth). The appearance of 100% of the hybrid leaves was intermediary between that of the Verônica and Everglades cultivars.

The maternal effect was determined by germination and enzymatic tests. The germination tests were carried out at 4 (first count) and 7 days (final count) and to obtain the germination speed index (GSI) for seeds from each F₁ crossing ('Everglades' x 'Verônica' and 'Verônica' x 'Everglades') and from the self-pollinated flowers. The tests were conducted in a completely randomized design with four treatments and five replicates of 50 seeds each. The seeds were laid on germination paper moistened to 2.5 times their weight and, then, placed in germination boxes, which were kept in BOD-type chambers at 32°C with a 12-hour photoperiod (thermoinhibition condition). The GSI was measured through daily germination counts according to Maguire (1962), and germination percentages were calculated at 4 and 7 days. After

this period, temperature was lowered to 20°C to verify the viability of the remaining seeds after 7 more days, i.e., at 14 days after sowing.

To verify the hypothesis that thermoinhibition is linked to endosperm resistance to rupture and consequent radicle protrusion, germination was also evaluated after the endosperm of 'Verônica' and 'Everglades' seeds was removed using a scalpel. The seeds were kept at 32°C with four replicates of 50 seeds each. This way, for each cultivar, whole seeds and seeds with a removed endosperm were evaluated, totaling four treatments (2 cultivars x 2 seed conditions).

For the enzymatic tests, the evaluated enzymes were: catalase and superoxide dismutase, antioxidants linked to stress response, preventing the accumulation of reactive oxygen species in cellular compartments (Pinheiro et al., 2016; Silva et al., 2018; Nandi et al., 2019); and esterase, an enzyme found in cellular membranes that hydrolyzes esters and acts directly on lipid metabolism, indicating lipid peroxidation (Pedó et al., 2016; Oliveira et al., 2020) due to abiotic stresses such as high temperatures (Zhang et al., 2010) and poor storage conditions (Catão et al., 2016). For the enzymatic analyses, samples of 350 seeds from both F₁ crossings and from the self-pollinated flowers were used. For the enzyme electrophoretic analysis, seeds were macerated in liquid nitrogen and polyvinylpyrrolidone and, then, stored at -86°C until use. For enzyme extraction, a buffer with 0.2 mol L⁻¹ Tris HCl pH 8.0 + 0.1% betamercaptoethanol was used at a ratio of 250 µL per 100 mg of seeds. The mix was homogenized in a vortex and kept for 12 hours in a refrigerator. After that, the material was centrifuged at 15,339 g for 60 min at 4°C (Biemelt et al., 1998).

Electrophoresis was conducted in discontinuous acrylamide gels of 7.5% (separating gel) and 4.5% (concentrating gel). The system gel/electrode used was Tris-glycine, pH 8.9. A 50 µL of supernatant were added, and electrophoresis was conducted at 150 volts for 6 hours. After that, the gels were revealed for the catalase, esterase, and superoxide dismutase enzymes (Alfenas et al., 2006). Measurements were conducted with the ImageJ software (Rasband, 2016), resulting in an average density for each treatment according to pixel quantity.

All data were subjected to the analysis of variance and Tukey's test, at 5% probability, when differences among treatments were detected. Data were analyzed using the R software for Windows (R Core Team, 2013). The maternal effect was verified by comparing treatments for each evaluated trait.

Results and Discussion

In the first count, at 32°C, the average seed germination was 68% for 'Everglades', but not more than 16.4% for 'Verônica' and their respective hybrids (Figure 1). At 32°C, the GSI showed a similar pattern, with higher values for 'Everglades', followed by F₁ hybrids from 'Everglades' x 'Verônica' (Figure 2 A). In the final count, seed germination was over 80% only for 'Everglades', with intermediate values for F₁ hybrids from 'Everglades' x 'Verônica', and lower ones for 'Verônica' and F₁ hybrids from 'Verônica' x 'Everglades' (Figure 2 B).

As the temperature was lowered to 20°C, non-germinated seeds from both cultivars and hybrids finished the germination process. Higher germination percentages were observed for 'Everglades' and F₁

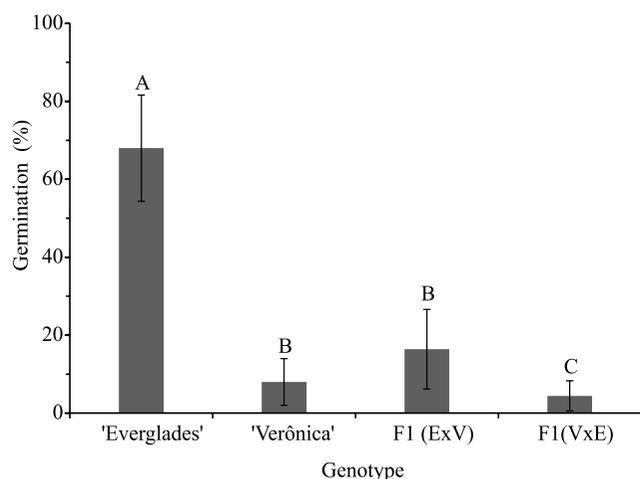


Figure 1. Seed germination of the Everglades and Verônica lettuce (*Lactuca sativa*) cultivars and their reciprocal hybrids at 32°C after four days. Means with equal letters do not differ by Tukey's test, at 5% probability. ExV, 'Everglades' (female genitor) x 'Verônica' (male genitor); and VxE, 'Verônica' (female genitor) x 'Everglades' (male genitor).

hybrids from 'Everglades' x 'Verônica', whereas lower percentages were found for 'Verônica' and F₁ hybrids from 'Verônica' x 'Everglades' (Figure 2 B). Similarly to germination, the GSI also increased (Figure 2 A).

Therefore, seed germination was significantly affected by higher temperatures, as reported by Nascimento & Cantliffe (2002). This finding has led

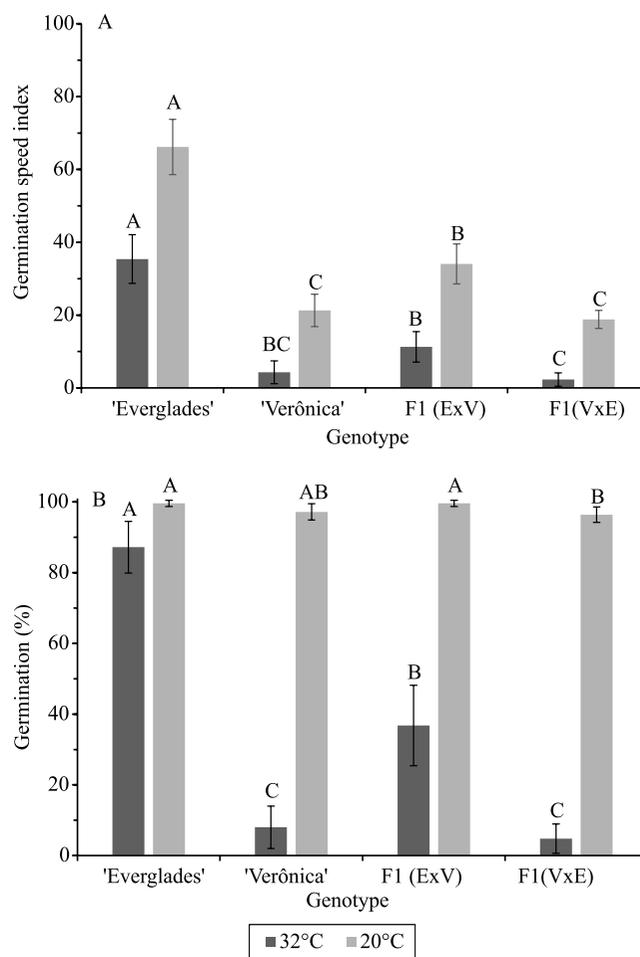


Figure 2. Germination speed index (A) and final germination percentage (B) for seeds from the Everglades and Verônica lettuce (*Lactuca sativa*) cultivars and their reciprocal hybrids. Final germination percentage was counted at 32°C after seven days, and, then, the temperature was lowered to 20°C for seven more days for final germination evaluation. Means with equal letters do not differ by Tukey's test, at 5% probability. ExV, 'Everglades' (female genitor) x 'Verônica' (male genitor); and VxE, 'Verônica' (female genitor) x 'Everglades' (male genitor).

several authors, such as Nascimento et al. (2012), Catão et al. (2014), and Almeida et al. (2019), to determine the thermotolerance of lettuce cultivars by characterizing seed germination at a range of temperatures. In the present study, although none of the hybrids reached a ratio close to that of 'Everglades', germination was higher for F₁ hybrids from 'Everglades' x 'Verônica' and lower for F₁ hybrids from 'Verônica' x 'Everglades' and the self-pollinated 'Verônica'. These results evidence that thermotolerance in lettuce is, in fact, influenced by the female genotype, as also found for tomato (*Solanum lycopersicum* L.) by Nascimento et al. (2016). This information is important for crossings between genotypes with or without thermotolerance in breeding programs, aiming to obtain cultivars with a better performance.

As to the seeds with a removed endosperm, at 32°C, germination was similar for 'Everglades', but higher for 'Verônica', being above 67% when the endosperm was removed, but only 12% when it was not (Figure 3), evidencing that thermotolerance is linked to the endosperm. According to Sung et al. (1998),

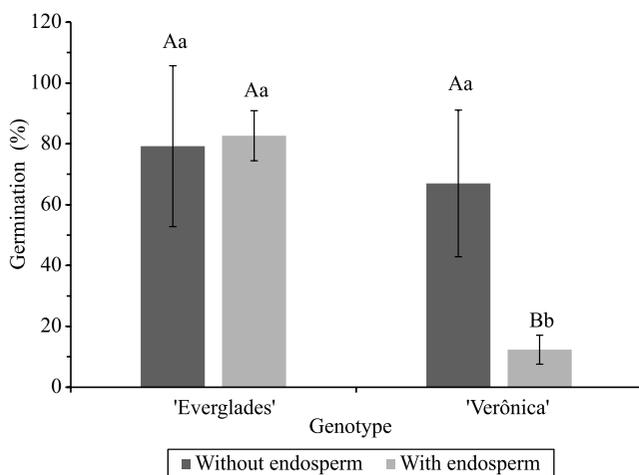


Figure 3. Germination of seeds from the Everglades and Verônica lettuce (*Lactuca sativa*) cultivars with and without the endosperm at 32°C. Lowercase letters compare treatments with seeds with and without the endosperm, and uppercase letters compare cultivars in the same treatment with seeds with and without the endosperm. Means with equal letters do not differ by Tukey's test, at 5% probability.

the higher the thermosensitivity of a cultivar, the harder the seed endosperm, whereas, the higher the thermotolerance of a cultivar (including Everglades), the softer the seed endosperm. Nascimento & Cantliffe (2002) added that the endosperm acts as a physical barrier, which is stronger the harder the tissue is, preventing embryo growth and consequent seed germination. These findings explain why the effect of endosperm removal was only observed for the thermosensitive cultivar, Verônica.

The obtained results evidence that the endosperm is the tissue with the greatest influence on thermotolerance. Moreover, considering that the endosperm is a triploid tissue with 2/3 of genetic material from the female genitor, the maternal effect on lettuce thermotolerance was high (Rosado et al, 2020; Li et al., 2021). This shows the importance of carefully choosing the female genitor in lettuce breeding programs, aiming for new cultivars with these traits and others of interest.

As to the evaluated enzymes, no differences were found for catalase and superoxide dismutase when comparing the different cultivars and hybrids (Figure 4 A and C). Therefore, there is no evidence of a link between these enzymes and thermotolerance in lettuce.

For esterase, the expression of the enzyme was higher in seeds from 'Everglades' and F₁ hybrids from 'Everglades' x 'Verônica' (Figure 4 B), intermediate for F₁ hybrids from 'Verônica' x 'Everglades', and low for 'Verônica'. Therefore, seeds from thermotolerant cultivars, such as Everglades, show a higher production of this enzyme than those from thermosensitive cultivars, as Verônica. These results also confirm the maternal effect, since higher values were observed for the hybrids whose female genitor was 'Everglades'. In addition, the potential of esterase as a marker for lettuce seed quality was confirmed in the present study by the higher expression of esterase in the thermotolerant cultivar and its F₁ hybrids, as well as the lower one in the thermosensitive cultivar and its F₁ hybrids. Likewise, Catão et al. (2016), evaluating the effect of storage conditions and different germination temperatures on lettuce, reported a higher esterase expression in seeds with a higher vigor (thermotolerance) during storage.

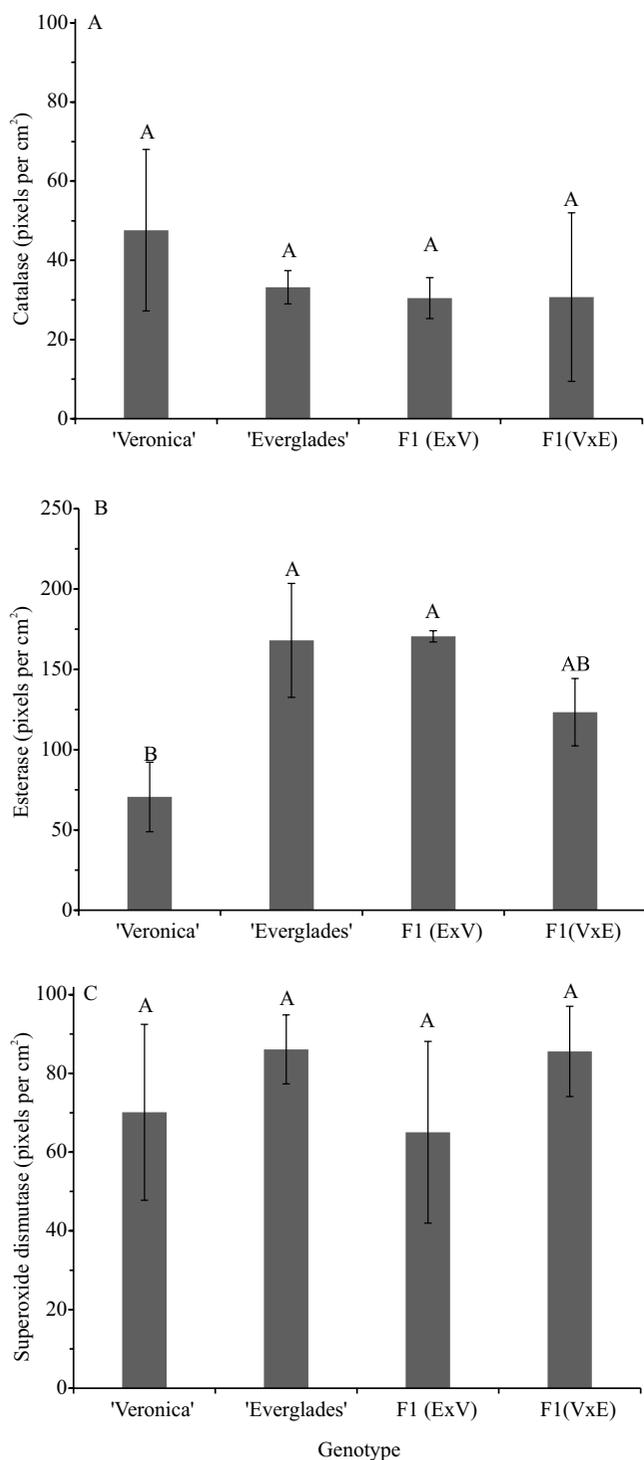


Figure 4. Expression of the catalase (A), esterase (B), and superoxide dismutase (C) enzymes in the Everglades and Verônica lettuce (*Lactuca sativa*) cultivars and their reciprocal hybrids. Means with equal letters do not differ by Tukey's test, at 5% probability. ExV, 'Everglades' (female genitor) x 'Verônica' (male genitor); and VxE, 'Verônica' (female genitor) x 'Everglades' (male genitor).

Conclusions

1. Thermotolerance in lettuce (*Lactuca sativa*) seeds is affected by the female genitor.

2. The expression of esterase is higher in the Everglades thermotolerant lettuce cultivar.

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