

Genetic control of thermoinhibition tolerance in lettuce seeds

Abstract – The objective of this work was to assess the genetic control of tolerance to thermoinhibition in lettuce (*Lactuca sativa*) seeds. Seeds of the F₁, F₂, and F_{2.3} generations derived from the cross between cultivars Everglades (tolerant to thermoinhibition) and Verônica (sensitive to thermoinhibition) were used, besides seeds from the parents. Seed germination tests were conducted in a completely randomized design, with four replicates of each parent, eight of F₁, and four of each of the 26 F_{2.3} progenies. The mean of the F₂ population was considered as the mean of the sample of the 26 progenies. The genetic control of thermoinhibition tolerance in lettuce seeds is attributed to one or a few genes. The additive effects are more expressive than the nonadditive ones, and narrow-sense heritability is relatively high, allowing the prediction of success in selection.

Index terms: *Lactuca sativa*, genetic variability, heritability, seed germination.

Controle genético da tolerância à termoinibição em sementes de alface

Resumo – O objetivo deste trabalho foi determinar o controle genético da tolerância à termoinibição em sementes de alface (*Lactuca sativa*). Utilizaram-se sementes das gerações F₁, F₂ e F_{2.3} oriundas do cruzamento entre as cultivares Everglades (tolerante à termoinibição) e Verônica (sensível à termoinibição), além de sementes dos próprios genitores. Realizaram-se testes de germinação das sementes em delineamento completamente casualizado, com quatro repetições para cada um dos genitores, oito para a F₁ e quatro para cada uma das 26 progênies F_{2.3}. A média da população F₂ foi considerada como a média da amostra das 26 progênies. O controle genético da tolerância à termoinibição em sementes de alface é atribuído a um ou poucos genes. Os efeitos aditivos são mais expressivos do que os não aditivos, e a herdabilidade no sentido restrito é relativamente alta, o que permite antever sucesso com a seleção.

Termos para indexação: *Lactuca sativa*, variabilidade genética, herdabilidade, germinação de sementes.

Introduction

Lettuce is a leafy plant of great economic importance in Brazil and is cultivated in almost all the regions of the country (Silva et al., 2019). Temperature can act directly or indirectly on germination, affecting seed dormancy and viability (Deng & Song, 2012). In addition, germination problems can cause poor quality seeds and a delay in

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seedling production, resulting in low yields and direct losses for farmers (Bufalo et al., 2012; Nascimento et al., 2012).

In general, in autogamous species, the estimation of genetic and phenotypic parameters and the testing of the hypothesis of monogenic inheritance have been carried out using data from plants of the P_1 and P_2 contrasting lines, of the F_1 (cross between P_1 and P_2) and F_2 (self-fertilization of F_1 plants) generations, and of RC_1 (backcrosses between F_1 and P_1 individuals) and RC_2 (backcrosses between F_1 and P_2 individuals) (Matos Filho et al., 2014). In interparietal crosses between contrasting genitors, it is possible to identify hybrids by phenotypic characters, although this is better observed in adult plants (Silva et al., 2005). Specifically in lettuce, it is difficult to ensure seeds derived from backcrosses, since these generations have intermediate phenotypic characteristics. In this case, the option is usually to use seeds of $F_{2,3}$ progenies to confirm F_2 segregation (Carvalho Filho et al., 2011).

In the literature, there are few known reports on thermotolerant lettuce genotypes. Argyris et al. (2011), for example, mentioned the UC96US23 accession of *Lactuca serriola* L., which is capable of germinating at temperatures close to 37°C, whereas Yoong et al. (2016) referred to the primitive PI251246 accession of *Lactuca sativa* L., with seed germination capacity even at temperatures above 33°C; this latter accession was obtained by crossing UC96US23 with cultivar Salinas (Schwember & Bradford, 2010). Catão et al. (2014) and Almeida et al. (2019) identified cultivar Everglades of *L. sativa* as tolerant to thermoinhibition.

The study of the genetic control of the thermotolerance trait is important for the implementation of breeding programs. This way, it is possible to better understand the behavior of generations obtained from the crossing of thermosensitive and thermotolerant genotypes, as well as to establish more suitable breeding methods for lettuce.

The objective of this work was to assess the genetic control of tolerance to thermoinhibition in lettuce seeds.

Materials and Methods

The used lettuce (*L. sativa*) seeds were produced in a protected environment, at the experimental facilities of Center for Development and Technology Transfer of Fazenda Palmital, belonging to Universidade Federal

de Lavras, located in the municipality of Ijaci, in the state of Minas Gerais, Brazil (21°09'24"S, 44°55'34"W, at 831 m of altitude).

Two lettuce cultivars, Everglades and Verônica, were crossed. The Everglades cultivar has smooth, tender leaves and is tolerant to thermoinhibition (Catão et al., 2014; Almeida et al., 2019), while Verônica has wavy, puckered, and loose leaves and is sensitive to thermoinhibition (Kano et al., 2011; Villela et al., 2010).

To obtain the seeds, cultivars Everglades and Verônica were initially sown in polystyrene trays with 128 cells, each of them with three seeds and filled with an organomineral substrate. After germination and seedling emergence, thinning was done, leaving only one plant per cell.

The seedlings were placed on concrete benches, with a 30% shading screen. Then, the seedlings were irrigated as needed and, 25 days after germination, they were transplanted into 10 L pots, containing a 2:1:1 mixture of soil, sand, and organic compost, respectively.

During the conduction of the plants until seed harvesting, spraying, fertilization, and irrigation were performed, according to the needs of the culture (Ribeiro et al., 1999).

At the time of flowering, crossings were carried out in the morning, with the emasculation of the flowers of the 'Everglades' female parent before sunrise, at 4:00 a.m., to avoid cutting the stigmas. The flowers from the 'Verônica' male parent were collected for pollination after flower opening at around 8:30 a.m.

The stigmas were pollinated after being fully developed, i.e., when they became bifid with open flowers. Pollination was done manually with an open flower of the Verônica cultivar, which was rubbed directly on the stigmas of the emasculated flower of the female parent. After their development and maturation, seeds were harvested and identified as F_1 . The F_1 seeds were sown and cultivated as previously described, to generate the F_2 and $F_{2,3}$ seeds.

After obtaining the seeds of the F_2 generation, new seeds from all generations were needed for the study, but they had to be collected at the same time and under the same conditions. This was done to avoid any effect of different environmental conditions, especially temperature at the time of seed formation.

These seeds were sown simultaneously with those of the parents and of the F_1 and F_2 generations,

and were harvested 15 days after anthesis, at the physiological maturity point (Clemente et al., 2013). During the seed production process, the maximum and minimum average temperatures were estimated, being, respectively, 44.3 and 31.5°C.

Afterwards, the seeds from the 'Everglades' and 'Verônica' parents and from the F₁ generation, with F₂ in bulk, were collected. The 26 F_{2:3} progenies were also collected individually. The purity of the F₁ seeds was assessed by sowing the parents and the F₁ generation in a polypropylene tray, in which the occurrence of contamination was determined by differences in leaf edge: frilled and curled edges for cultivar Verônica (Silva et al., 2008), smooth edges for cultivar Everglades (Catão et al., 2016), and intermediate edges for F₁ (Silva et al., 2005). The seeds of the F_{2:3} progeny were used to detect the proportion of segregation observed in F₂, which allows inferring about the number of genes involved in the trait (Ramalho et al., 2012).

For the first count and germination (%) tests (Brasil, 2009), an experiment was carried out in a completely randomized design with replicates of 50 seeds per treatment, with four replicates of each parent, eight replicates of F₁, and four replicates of each of the 26 F_{2:3} progenies, totaling 120 plots. To estimate the mean of the F₂ population, the sample mean of the 26 progenies was used.

For the germination tests, the seeds were spread on blotting paper moistened with distilled water and placed in BOD chambers, at 35°C, under a 12 hour photoperiod (Brasil, 2009). The first and second germination counts were carried out four and seven days after sowing, respectively. To ensure that the remaining seeds were in thermoinhibition and not thermodormancy, temperature was reduced to 20°C after the second count. New counts were done four and seven days later, in order to assess the thermoinhibition effect, using seed germination data at 20°C. Both counts were performed as recommended by Brasil (2009).

After the test was completed, the seeds that failed to germinate, even at 20°C, had their integuments removed and were subjected to the tetrazolium test (2,3,5-triphenyltetrazolium chloride) (Brasil, 2009). The results were expressed as percentage of viable seeds.

Data were subjected to the analysis of variance according to the F-test using the Sisvar software

(Ferreira, 2011), and the R, version 3.1.3 (R Core Team, 2015), software was used to estimate the components of mean and variance.

The mean components, mean estimator (m), deviations of the homozygote from the mean (\hat{a}), and deviation of the heterozygote from the mean (\hat{d}) were estimated using the method of the weighted least squares, considering the additive and dominant model without epistasis (Ramalho et al., 2012), according to the expression:

$$\hat{\beta} = (C'NS^{-1}C)^{-1}(C'NS^{-1}Y)$$

$$\hat{\beta} = \begin{vmatrix} m \\ \hat{a} \\ \hat{d} \end{vmatrix} \quad C = \begin{vmatrix} 1 & -1 & 0 \\ 1 & 1 & 0 \\ 1 & 0 & 1 \\ 1 & 0 & 0.5 \end{vmatrix} \quad Y = \begin{vmatrix} \bar{P}_1 \\ \bar{P}_2 \\ \bar{F}_1 \\ \bar{F}_2 \end{vmatrix}$$

$$N = \begin{vmatrix} n_1 & 0 & 0 & 0 \\ 0 & n_2 & 0 & 0 \\ 0 & 0 & n_3 & 0 \\ 0 & 0 & 0 & n_4 \end{vmatrix} \quad S = \begin{vmatrix} VP_1 & 0 & 0 & 0 \\ 0 & VP_2 & 0 & 0 \\ 0 & 0 & VF_1 & 0 \\ 0 & 0 & 0 & VF_2 \end{vmatrix}$$

where $\hat{\beta}$ is the vector of the estimates, in which m is the mean estimator, \hat{a} is the estimator of deviations of the homozygote from the mean, and \hat{d} is the deviation of the heterozygote from the mean; C is the model matrix referring to populations P₁, P₂, F₁, and F₂; Y is the vector of the means of each population; N is the matrix associated with the number of plants evaluated, in which n₁, n₂, n₃, and n₄ are the number of plants from populations P₁, P₂, F₁, and F₂, respectively; and S is the matrix of variances associated with the populations, in which VP₁, VP₂, VF₁, and VF₂ are the variances among the plants of generations P₁, P₂, F₁, and F₂, respectively.

The components of phenotypic variance $-\hat{V}_E, \hat{V}_A,$ and \hat{V}_D – were estimated using the weighted least squares method, according to Ramalho et al. (2012) and Cruz et al. (2012), considering the same populations and according to the following expression:

$$\hat{\beta} = (C'NS^{-1}C)^{-1}(C'NS^{-1}Y)$$

$$\hat{\beta} = \begin{vmatrix} \hat{V}_A \\ \hat{V}_E \\ \hat{V}_D \end{vmatrix} \quad C = \begin{vmatrix} 1 & -1 & 0 \\ 1 & 1 & 0 \\ 1 & 0 & 1 \\ 1 & 1 & 1 \end{vmatrix} \quad Y = \begin{vmatrix} VP_1 \\ VP_2 \\ VF_1 \\ VF_2 \end{vmatrix}$$

$$N = \begin{vmatrix} DF P_1 & 0 & 0 & 0 \\ 0 & DF P_2 & 0 & 0 \\ 0 & 0 & DF F_1 & 0 \\ 0 & 0 & 0 & DF F_2 \end{vmatrix} \quad S = \begin{vmatrix} VP_1 & 0 & 0 & 0 \\ 0 & VP_2 & 0 & 0 \\ 0 & 0 & VF_1 & 0 \\ 0 & 0 & 0 & VF_2 \end{vmatrix}$$

where $\hat{\beta}$ is the vector of the estimates, in which \hat{V}_A , is the additive genetic variance, \hat{V}_E , is the environmental variance, and \hat{V}_D , is the dominance variance; C is the model matrix; Y is the vector of the variances estimated

from the observations of the different populations; N is the matrix constructed according to the model matrix, with DF corresponding to the degrees of freedom associated with each source of variation; and S is the weighting matrix that corresponds to the variances observed for each population.

Narrow-sense heritability (h^2_r) was obtained for the trait germination percentage, using the estimates of the variance components:

$$h^2_r = \frac{\hat{V}_A}{\hat{V}_A + \hat{V}_D + \hat{V}_E}$$

Accuracy (rgg') was estimated by the formula:

$$rgg' = \sqrt{1 - (1/F)}$$

where F (Snedecor) is the value of the variance ratio for the effects of treatments associated with the analysis of variance (Resende, 2002).

Finally, incomplete dominance was given by d, which was obtained by the following formula:

$$d = mF_1 \frac{(P_1 + P_2)}{2}$$

where m is the mean of the F_1 generation.

Results and Discussion

Initially, the F-test of the analysis of variance showed differences among the genotypes at 1% probability, confirming variability for the trait thermoinhibition tolerance in seeds – germinated at 35°C – of the Everglades and Verônica lettuce cultivars and of the

generations derived from the cross between them (Table 1).

The Everglades and Verônica cultivars, corresponding to genotypes 1 and 2, respectively, had a germination percentage of 93 and 17% at 35°C (Figure 1). These results are indicative of the divergence between both parents for thermoinhibition tolerance, which may be attributed to the activity of the endo- β -mannanase enzyme that was greater in Everglades than in the thermosensitive cultivar. Therefore, the highest germination percentage in Everglades may be explained by the higher enzymatic activity in the seeds of this cultivar (Catão et al., 2014, 2018).

Compared with the parents, generations F_1 , represented by genotype 3, and F_2 , represented by genotype 4, had intermediate germination percentages of 81 and 65%, respectively, indicating that the predominant allelic interaction, which conditions thermoinhibition tolerance, is additive. Additive allelic interactions are common for several traits in autogamous species (Baldissera et al., 2014).

The analysis of variance showed a significant difference for germination among the plants of generation F_2 ($F_{2:3}$ progenies) (Table 2). The germination means of the $F_{2:3}$ progenies, corresponding to the 26 genotypes numbered from 5 to 30 (Figure 1), ranged from 9 to 96%, with an overall mean of 65%, which is considered the mean of the F_2 generation. This variation in germination within the progenies is due to segregation, which is fundamental for the selection of genetic parameters of interest, such as heritability, reflecting the proportion of phenotypic variance that can be inherited (Baldissera et al., 2014).

Additive effects ($\hat{a} = 38.78$) are more important than nonadditive ones ($\hat{d} = 26.28$) for the thermoinhibition

Table 1. Analysis of variance of the germination percentage of lettuce seeds (*Lactuca sativa*) at 35°C⁽¹⁾.

Source of variation	DF	SS	MS	F-test	Mean
Genotype	29	110703.5577	3817.3641	62.99**	-
Residual error	90	5454.3506	60.6039	-	-
Total	119	116157.9083	-	-	-
Within parent 1	3	36.6656	12.2219	-	93
Within parent 2	3	212.5025	70.8342	-	17
Within F_1	7	295.6821	42.2403	-	81
Within F_2	103	100975.0404	980.3402	-	65
CV (%)				-	11.89
Overall mean				-	66

⁽¹⁾DF, degree of freedom; SS, sum of squares; MS, mean square; and CV, coefficient of variation. **Significant at 1% probability.

tolerance trait (Table 3). Since the additive effect is related to characteristics that can be passed on to subsequent generations, its greater magnitude means that a large part of the traits that confer thermotolerance will be passed on to other generations (Labroo et al., 2021). The same happens for resistance to the *Meloidogyne javanica* nematode in lettuce seeds, considering the predominance of the additive effect in relation to the nonadditive effects (Maluf et al., 2002).

The estimate of the mean degree of dominance (MDD) indicates a greater dominance regarding thermotolerance (Table 3), which, however, is still lower than that of the additive effects found to control this characteristic (Cruz et al., 2012). According to Cheverud & Routman (1995), the biologically dominant or epistatic gene action is largely captured by statistical average effects, because both statistical dominance and epistasis are residual deviations from

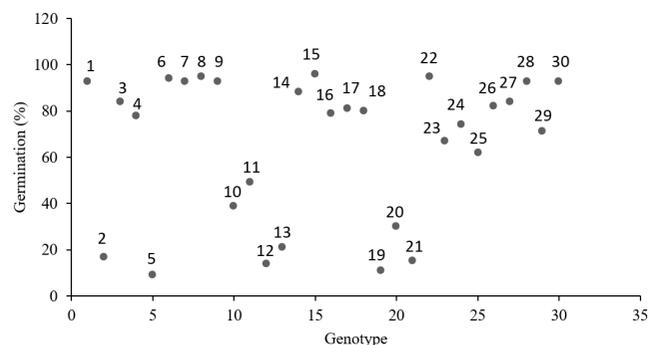


Figure 1. Dispersion of the germination percentage of lettuce (*Lactuca sativa*) seeds at 35°C for: cultivars Everglades (1) and Verônica (2) as parents; generations F₁ (3) and F₂ (4); and 26 F_{2:3} progenies (5 to 30).

Table 2. Analysis of variance of the germination percentage of lettuce (*Lactuca sativa*) seeds collected from plants of generation F₂ (progenies F_{2:3} of 'Everglades' x 'Verônica') at 35°C⁽¹⁾.

Source of variation	DF	SS	MS	F-test	P-value > F-test
Plants of generation F ₂	25	95,978.2286	3,839.1291	59.93**	0.00
Error	78	4,996.8119	64.0617		
Total	103	100,975.0404			
CV (%)	12.29				

⁽¹⁾DF, degree of freedom; SS, sum of squares; MS, mean square; and CV, coefficient of variation. **Significant at 1% probability.

these effects. Since lettuce is an autogamous species, with a high cleistogamous effect, the high value of the MDD estimate is an important information for the genetic improvement of the crop.

The dominant additive model without epistasis explained the observed means, since the chi-square test was not significant and the model fit (R²) estimate between the observed and estimated means was practically 1.0 (Table 4). Likewise, high values of R² were verified by Oliveira et al. (2019) in a study about pigment genetic parameters and agronomic traits in lettuce, in which the authors also proposed a dominant additive model. Therefore, when superior genotypes for resistance to thermotolerance in lettuce seeds are selected in one generation, the next would have similar results regarding this trait.

The estimate of heritability in the narrow sense was also high (Table 4). This means that the phenotypic variability of thermoinhibition can be attributed to additive variance, that is, to the proportion of genetic variance that can be fixed with selection. Furthermore, it has been reported that the studied trait must be controlled by few genes (Ramalho et al., 2012). Therefore, selection based on phenotype is promising for breeding programs that aim to obtain thermotolerant lettuce cultivars, since the genetic control of this trait is known and the additive effects can be fixed.

Table 3. Estimation of the mean components of the germination percentage of lettuce (*Lactuca sativa*) seeds at 35°C⁽¹⁾.

Model parameter	Estimate	Standard error	Probability > t
m	53.98	8.38	0.0000
â	38.78	8.63	0.0000
â ²	26.28	12.60	0.0199
MDD	0.68	-	-
Population	Germination (%)		
	Observed	Estimated	
'Everglades'	92.92	92.76	
'Verônica'	16.14	15.19	
F ₁	80.82	80.26	
F ₂	65.12	67.12	
χ ²	0.54	p(0.4624)	
R ²	99.99	-	

⁽¹⁾m, mean estimator; â, deviations of the homozygote from the mean; â², deviation of the heterozygote from the mean; MDD, values of the mean degree of dominance; F₁ and F₂, plant generations; χ², chi-square; and R², model fit.

Table 4. Estimation of the variance components of the germination percentage of lettuce (*Lactuca sativa*) seeds at 35°C.

Parameter ⁽¹⁾	Estimate	Error	t-test	Probability t > 0
Genetic variance	966.423846			
Additive variance	938.0999	15.289921	61.35414	1.5879e-75
Dominance variance	28.323946	2.3835914	11.88289	1.9405e-20
h ²	98.5805			
rgg ²	0.99			
R ²	97.318982	0	0	0
χ ²	1.662231	0	0	0

⁽¹⁾h², heritability; rgg², accuracy; R², model fit; and χ², chi-square.

The germination percentage, at 35°C, for the F₁ generation (81%) was considered intermediate, between those obtained for the 'Everglades' (93%) and 'Verônica' (17%) female and male parents, respectively, but tending to approach that of the tolerant mother, as it was higher than the parental mean (55%) (Table 3). This result differs from incomplete dominance, possibly due to a maternal effect, as the F₁ mean is close to that of the female parent (Ramalho et al., 2012).

The deviation of the heterozygote from the parental average () was 26.28, corresponding to the deviation of the mean of the F₁ generation (81%) from the mean of the two parents (55%) (Table 3), indicating incomplete dominance. When the additive effects for a trait are high in a given cross, the mean of the parents does not differ from that of the progeny, differently from the results found in the present work. However, with nonadditive effects (dominance and/or epistasis) in the control of a characteristic, the mean of the parents differs from that of the F₁ progeny (Allard, 1999). In addition, there is a possibility of maternal effect in the F₁ generation, as the germination percentage is close to that of the 'Everglades' female parent (Table 3).

It was found that there was a predominance of the gene action of additivity in controlling the thermoinhibition trait. However, there is also the possibility of the gene action of nonadditive effects, such as the maternal effect. Therefore, to detect the action of the maternal effect, it is necessary to carry out reciprocal crossings (Nascimento et al., 2016).

Conclusions

1. Thermoinhibition tolerance in lettuce (*Lactuca sativa*) seeds is a trait controlled by one or a few genes.

2. Additive effects are more significant than nonadditive effects for the trait thermoinhibition tolerance in lettuce seeds.

3. Narrow-sense heritability is relatively high for the trait thermoinhibition tolerance in lettuce seeds, allowing the prediction of selection gain.

4. Conventional breeding of lettuce aiming at thermoinhibition tolerance is feasible from crosses between contrasting genotypes.

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