

Comparison of methods to evaluate resistance of lettuce genotypes against *Bremia lactucae*

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Abstract

For the lettuce crop, there is no specific or standard methodology to evaluate genotype resistance of lettuce against downy mildew. Therefore, the aim of this work was to compare three methodologies for the identification of resistance to downy mildew. The experiment was carried out at Universidade Federal de Uberlândia, Monte Carmelo Campus between 2017 and 2018. The statistical method used was a completely randomized design with 12 treatments (cultivar Solaris, used as a control; and 11 genotypes of lettuce F5: 6: UFU-Crespa 75 # 2, UFU-Crespa 189 # 2, UFU-Crespa 206 # 1, UFU-Lisa 66 # 3, UFU-Lisa 66 # 7, UFU-Lisa 215 # 3, UFU-Lisa 215 # 6, UFU-Lisa 215 # 10, UFU-Lisa 215 # 12, UFU-Lisa 215 # 13, UFU-Lisa 215 # 14) and four replications. The seeding of genotypes was carried out in transparent boxes and after 15 days the inoculation was performed using a spore suspension (1×10^4 sporangia/ml) and sterile deionized water for the controls. The disease progression was assessed by three methods: Mesquita, Dickinson & Crute and Horsfall & Barrat. The data were standardized and submitted to the univariate analysis of variance by the F test and multivariate analyzes. The univariate clustering results allowed the separation of the genotypes into two distinct resistance groups for the Dickinson & Crute and Mesquita methods. In the multivariate analysis, more formation of groups among genotypes with different levels of resistance to downy mildew in the Mesquita method was observed. The methodology proposed by Mesquita was more efficient in discriminating genotypes with different levels of resistance to downy mildew, separating them in a larger number of groups.

Keywords: Downy mildew; *Lactuca sativa* L.; methodologies; multivariate analyzes; plant breeding.

Introduction

The lettuce downy mildew, caused by the biotrophic oomycete *Bremia lactucae*, is one of the main diseases of lettuce crop, because it reduces the quality of the leaves - the marketable part of the crop - and is often accentuated by post-harvest losses, which occur during transport and storage (Kunjeti et al., 2016; Parra et al., 2016). Symptoms of lettuce downy mildew usually occur as pale-yellow, angular patches delineated by the veins of the leaves. On the abaxial side of the leaves, the presence of white sporangia, consisting of sporangiophores and sporangia is observed (Mieslerová et al., 2013).

The disease management of lettuce downy mildew is mainly done through fungicide application and use of resistant cultivars, carrying Dm genes or resistance factors R, which express hypersensitivity reaction (Castoldi et al., 2014; Tobar-Tosse et al., 2017). However, in the literature there is a lack of standard methodologies for evaluation of resistance, tolerance or susceptibility of genotypes against *B. lactucae*.

There are four basic measures that can be used for quantification of diseases: incidence, severity, intensity and density of the pathogen. However, several studies (Petrželová et al., 2011; Araújo et al., 2014; Castoldi et al., 2014; Tobar-Tosse et al., 2017) use only the percentage of sporulated plants considering: highly resistant cultivars, those with sporulated plant numbers less than or equal to five percent, and cultivars with incomplete resistance, those with sporulated plant numbers between five and twenty percent. However, such methodology may not be the most adequate, or even be prone to subjectivity, and might generate inconsistent results.

The methodology described by Horsfall & Barrat (1945) was selected by Teramoto et al. (2011) to evaluate the resistance of cucumber hybrids to the fungus *Corynespora cassiicola*. However, Jemelková et al. (2015) used the Dickinson & Crute (1974) scale to examine the variation in downy mildew (*B. lactucae*) resistance of *Lactuca aculeata* genotypes from different geographical origins. However, Tobar-Cough et al.

(2017) evaluated the resistance of curly lettuce strains to downy mildew breeds, based on the scale proposed by Mesquita (2008).

Taking into account that the lettuce breeding programs are focused on the selection of materials that combine resistance or tolerance to pests and diseases and high productivity, the disease evaluation should use methods that allows quick and reliable results. Therefore, this study aimed compare three methodologies for identifying resistance to diseases, in order to define the more suitable for evaluation of lettuce genotypes against downy mildew.

Results

In general, lettuce genotypes presented distinct behaviors for resistance to *B. lactucae*. The identification of genotypes with more or less resistance varied according to the analysis used (multivariate or univariate analyzes).

Results of univariate clustering by the Scott-Knott test ($p = 0.05$) allowed the separation of the genotypes into two distinct groups for reaction to *B. lactucae* for the Dickinson & Crute and Mesquita methods (Table 1). According to the means of such methods it is possible to separate some genotypes from the susceptible cultivar Solaris and identify genotypes resistant to *B. lactucae* race 1. However, it was not observed when using the methodology of Horsfall & Barrat, in which there was no distinguishing between genotypes in groups, pointing out that all evaluated genotypes present the same reaction to *B. lactucae*, and therefore, all susceptible to mildew.

According to the Mesquita method, five genotypes were resistant, showing no signs of the pathogen in the leaves after inoculation (zero score), whereas by the methodology of Dickinson & Crute, eight genotypes were resistant to downy mildew. However, they formed only two groups resistant and susceptible. Therefore, in addition to univariate analyzes, it is important to use multivariate analyzes to distinguish genotypes with moderate resistance to downy mildew.

Figure 1 represents the graphical dispersion in the two-dimensional space of the 12 genotypes under study, using the first and second canonical variables. The two canonical variables explained 85.53%, 91.48% and 96.33% of the total variation, respectively for Dickinson & Crute methodologies (Figure 1A), Horsfall & Barrat (Figure 1B) and Mesquita (Figure 1C).

In the Dickinson & Crute methodology it was possible to separate the genotypes into three groups, group I(susceptible to mildew): cultivar Solaris; group II(resistant to mildew): UFU-Crespa 189 # 2, UFU-Crespa 206 # 1 and UFU-Lisa 215 # 12 genotypes; and group III (tolerant to mildew): genotypes UFU-Crespa 75 # 2, UFU-Lisa 66 # 3, UFU-Lisa 66 # 7, UFU-Lisa 215 # 3, UFU-Lisa 215 # 6, UFU-Lisa 215 # 10, UFU-Lisa 215 # 13 and UFU-Lisa 215 # 14. By the methodology of Horsfall & Barrat, the genotypes were separated into only two groups, group I(susceptible to mildew): cultivar Solaris; and group II(resistant to mildew): all other genotypes. Already by the methodology of Mesquita, there were the separation of the genotypes in four groups, being group I (susceptible to mildew): cultivar Solaris; group II(resistant to mildew): UFU-Crespa 189 # 2, UFU-Crespa 206 # 1 and UFU-Lisa 215 # 12 genotypes; group III(moderately resistant to mildew): UFU-Crespa 75 # 2, UFU-Lisa 215 # 6 and UFU-Lisa 215 # 13 genotypes; and group IV(tolerant to mildew): genotypes UFU-Lisa 66 # 3, UFU-Lisa

66 # 7, UFU-Lisa 215 # 3, UFU-Lisa 215 # 10 and UFU-Lisa 215 # 14.

Discussion

Cruz and Carneiro (2006) point out that the analysis by canonical variables should only be used when, in two or three canonical variables, more than 80% of the total variation is explained. Therefore, it can be affirmed that there was a satisfactory description of the genetic divergence represented graphically. It is important to point out that when genetic diversity is studied by canonical variables, the purpose is to identify similar genotypes in bi or three-dimensional scatterplots (Stähelin et al., 2011).

The groups formed by means of the graphic dispersion follow a distinct tendency of the groupings formed through the means test, for all the methodologies studied. This disagreement between multivariate and univariate methods was also observed by other authors, working with different species, such as Nunes et al. (2011a, b), and Azevedo et al. (2013). Regardless of the methodology used, it can be seen that the graphic dispersion of the canonical variables clearly demonstrated the high genetic dissimilarity between the commercial cultivar Solaris and the lettuce genotypes $F_{5,6}$, in which the cultivar Solaris is susceptible to race 1 of *Bremia lactucae* and the evaluated genotypes have different degrees of resistance. These results corroborate with the work of Castoldi et al. (2012), who state that the cultivar Solaris is susceptible to all breeds of *B. lactucae* identified so far. Through the multivariate analysis, it was verified that the method proposed by Mesquita (scale of notes) was more efficient in discriminating the genotypes, separating them in a larger number of groups. The same occurred with the Scott Knott test, in which the methods of Mesquita and Dickinson and Crute allocated the genotypes in distinct groups. The notes scale of the Mesquita method refers to the observation of the presence or absence of mildew on the leaves, so that the note is attributed considering the day the symptom was identified. This is a simple measurement method, in which the severity of the disease is not considered, being a less subjective and error-prone evaluation. There is no scale of notes or a specific method for the analysis of lettuce mildew. In the present study, a number of authors (Petrželová et al., 2011; Araújo et al., 2014, and Tobar-Tosse et al., 2017) only the percentage of sporulated plants, which are: highly resistant cultivars which present sporulated plant numbers less than or equal to five percent; and cultivars with incomplete resistance - those that present sporulated plant numbers between five and twenty percent. The results of this study reinforce the need to standardize a methodology for evaluation of lettuce mildew, suggesting the Mesquita method as the most efficient to discriminate lettuce genotypes with different levels of resistance to downy mildew, between the compared methods in this study.

Materials and methods

Experiment location and plant material

The experiment was conducted at the LAGEN (Laboratory of Seed Analysis and Genetic Resources) located at the Universidade Federal de Uberlândia, Monte Carmelo campus between 2017 and 2018.

Table 1. Reaction of lettuce genotypes to *Bremia lactucae*, based on the Dickinson & Crute methods (area below the disease progress curve); Horsfall & Barrat (disease severity); and Mesquita (scale of notes).

Groups ¹	Number of genotypes and severity (%) ²		
	Dickinson & Crute	Horsfall & Barrat	Mesquita
I	8 (4.65 ± 7.25) a	12 (1.64 ± 1.05)	5 (0.00 ± 0.00) a
II	4 (44.32 ± 16.00) b	-	7 (88.75 ± 11.55) b

¹Means followed by distinct letters in the column differ from each other by the Scott-Knott test at 0.01 and 0.05 significance. ²Enter parentheses, means of the genotypes in each grouping.

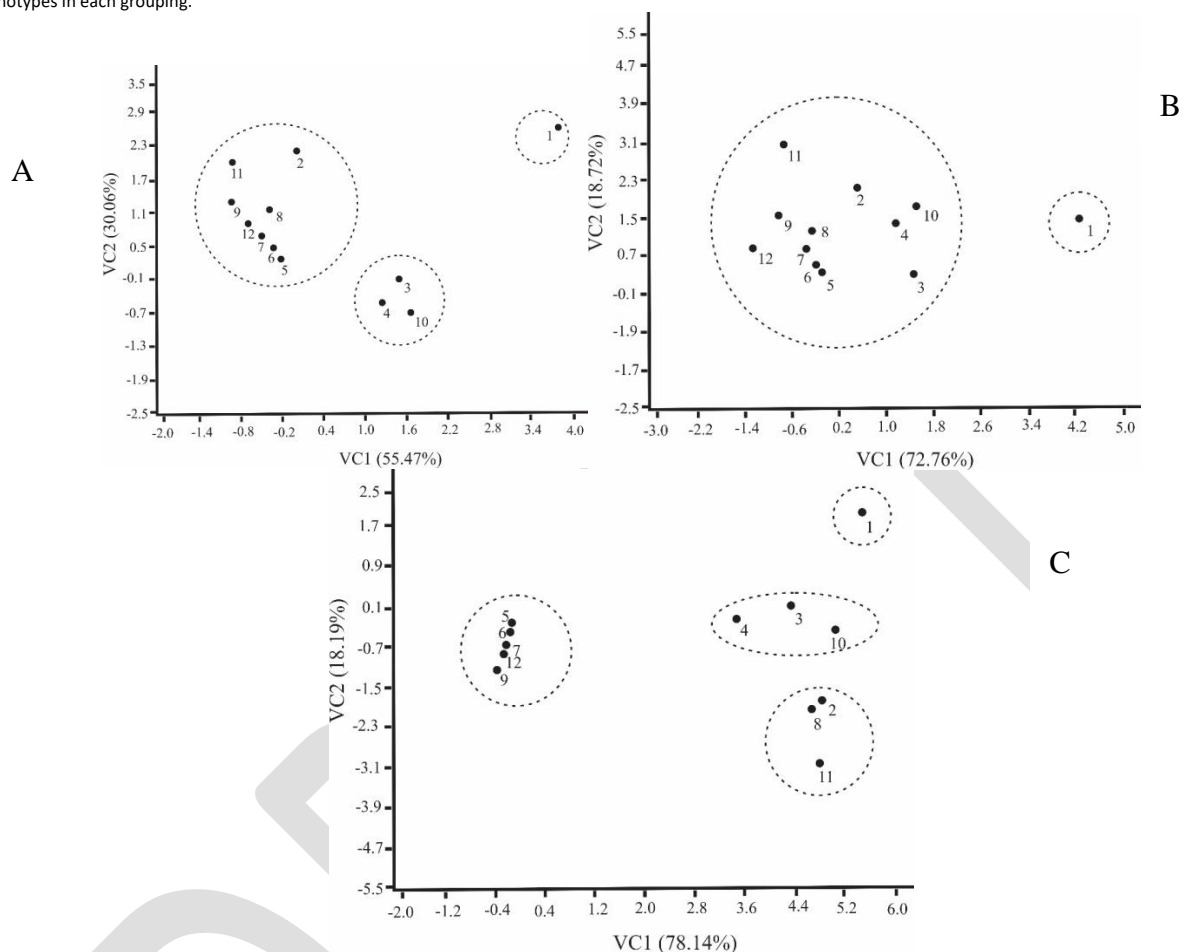


Fig 1. Graphical dispersion of the scores in relation to the two representative axes of the first two Canonic Variables (VC1 and VC2), by Dickinson & Crute (A), Horsfall & Barrat (B) and Mesquita (C) methods. Numerals indicate lettuce genotypes, where 1 = Solaris; 2 = UFU-Lisa 66 # 3; 3 = UFU-Lisa 66 # 7; 4 = UFU-Crespa 75 # 2; 5 = UFU-Crespa 189 # 2; 6 = UFU-Crespa 206 # 1; 7 = UFU-Lisa 215 # 3; 8 = UFU-Lisa 215 # 6; 9 = UFU-Lisa 215 # 10; 10 = UFU-Lisa 215 # 12; 11 = UFU-Lisa 215 # 13; 12 = UFU-Lisa 215 # 14.

To compare the different methodologies for evaluation of genotype resistance, the commercial cultivar Solaris was used as a control, being susceptible to all *B. lactucae* strains (Castoldi et al., 2012) and 11 genotypes of lettuce F5: 6 (UFU-Crespa 75 # 2, UFU-Crespa 189 # 2, UFU-Crespa 206 # 1, UFU-Lisa 66 # 3, UFU-Lisa 66 # 7, UFU-Lisa 215 # 3, UFU-Lisa 215 # 6, UFU-Lisa 215 # 10, UFU-Lisa 215 # 12, UFU-Lisa 215 # 13, UFU-Lisa 215 # 14). The F5:6 genotypes used came from the cross between the cultivar Uberlândia 10,000 (Sousa et al., 2007) x cv. Pira 63 (Tecnoseed®) belonging to the Program for Genetic Improvement of Biofortified Lettuce of UFU.

Inoculum production and inoculation of *B. lactucae*

The multiplication of the *B. lactucae* race 1 was carried out in the susceptible cultivar Solaris until sufficient amount of inoculum was obtained for the inoculations. After multiplication, 30 seeds of each genotype previously disinfested (using 70% alcohol and 1:1 water + hypochlorite)

were seeded in plastic boxes (11 x 11 x 3.5 cm), lined with two substrate sheets of paper moistened and maintained for 15 days in incubation chamber type BOD (Biochemical Oxygen Demand) at 13°C temperature and photoperiod of 12 h, to favor emergence of seedlings and expansion of cotyledonary leaves. When the seedlings presented two cotyledonary leaves completely expanded, the inoculation with the isolate of *B. lactucae* race 1 was carried out, according to the technique of Ilott et al. (1987), using suspension of sporangia removed from infected tissues of the host in distilled water containing surfactant (Tween 20), in order to increase the sporangia adhesion in cotyledonary leaves. The spore suspension was calibrated to 5x10⁴ sporangia/ ml and a Pasteur-type pipette was used for each isolate for inoculation until runoff. After inoculation, the boxes were placed in a BOD incubation chamber with a temperature of 13 °C, and for the first six hours they were kept in a darkroom and after that time the photoperiod was adjusted to 12 hours.

Experimental design and comparison of methods

The statistical design was completely randomized, with 12 treatments (commercial cultivar susceptible to mildew + 11 biofortified lettuce genotypes F5:6) and four replications.

The progress of the disease was evaluated by means of three patometric methods described below:

a) Methodology proposed by Mesquita (2008), with modifications, so that the seedlings that showed the first signs of *B. lactucae*, received higher grades, as described: 12 = plants that showed signs of the pathogen on the 7th day after inoculation; 11 = plants showing signs of the pathogen on the 8th day after inoculation; 10 = plants showing signs of the pathogen on the 9th day after inoculation; 9 = plants showing signs of the pathogen on the 10th day after inoculation; 8 = plants showing signs of the pathogen on the 11th day after inoculation; 7 = plants showing signs of the pathogen on the 12th day after inoculation; 6 = plants showing signs of the pathogen on the 13th day after inoculation; 5 = plants showing signs of the pathogen on the 14th day after inoculation; 4 = plants showing signs of the pathogen on the 15th day after inoculation; 3 = plants showing signs of the pathogen on the 16th day after inoculation; 2 = plants showing signs of the pathogen on the 17th day after inoculation; 1 = plants showing signs of the pathogen on the 18th day after inoculation; 0 = plants that did not show signs of the pathogen after inoculation.

b) Methodology described by Dickinson & Crute (1974), where: 0 = absence of sporangiophores visible on leaves; 1 = limited sporulation, sporadic sporangiophores present; 2 = less than 50% of the cotyledons covered with sporangiophores and 3 = more than 50% of the cotyledons covered with sporangiophores. The plants were evaluated over 18 days, and the intensity of the disease was then calculated, which was expressed as percentage, according to the following formula: $ID (\%) = \sum [(v) / (n * x) 100]$. From the disease intensity data, the area under the disease progress curve (AUDPC) was calculated using the formula $AUDPC = \sum [(y_1 + y_2) / 2] * (t_2 - t_1)$, where y_1 and y_2 are two consecutive evaluations performed at times t_1 and t_2 , respectively (Campbell e Madden, 1990).

c) Methodology proposed by Horsfall & Barrat (1945), based on classes, according to the severity percentage of the disease, where: class 0 = 0%; class 1 = greater than 0 up to 3%; class 2 = greater than 3 to 6%; class 3 = greater than 6 to 12%; class 4 = greater than 12 to 25%; class 5 = greater than 25 to 50%; class 6 = greater than 50 to 75%; class 7 = greater than 75 to 87%; class 8 = greater than 87 to 94%; class 9 = greater than 94 to 97%; class 10 = greater than 97 to less than 100%; class 11 = 100%.

On the 18th day after inoculation, when all the evaluations were finished by means of the three patometric methods, the percentage of necrotic and sporulated seedlings was also evaluated. The evaluation of the percentage of necrosis and sporulated plants was carried out only with the purpose of performing the multivariate analysis, since their values were fixed among the methods. In the determination of dissimilarity, only the established criteria of each method were varied: area below the disease progression curve (Dickinson e Crute, 1974), disease severity (Horsfall e Barrat, 1945) or grading scale (Mesquita, 2008).

Statistical analysis

The data were standardized and then submitted to univariate analysis of variance by the F test ($p = 0.05$). In the standardization, the values obtained in each of the different note scales were divided by the highest note of the respective scaled scale and then multiplied by 100. Subsequently, the means were grouped and compared by the Scott-Knott test ($p = 0.05$).

In addition, multivariate analyzes were carried out to determine the dissimilarity between the genotypes, obtaining the matrix of dissimilarity by the generalized distance of Mahalanobis ("D"). Multivariate analysis was performed for each method and, also, correlation analysis among its matrices of dissimilarity. The groupings were visualized through a Cartesian graph by the analysis of Canonic Variables (Cruz et al., 2012). All the data obtained were analyzed using the software Genes (Cruz, 2013).

Conclusions

The methodology proposed by Mesquita is more efficient in discriminating genotypes with different levels of resistance to downy mildew, because it allows grouping the genotypes into five classes: susceptible; tolerant moderately resistant and resistant to mildew.

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