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RESEARCH ARTICLE / *ARTIGO*

Screening of lettuce accessions for resistance to *Fusarium oxysporum* f. sp. *lactucae* race 1

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lactucae* (FOLAC) is one of the most important problems for lettuce growers because it causes major losses in production. To identify cultivars with potential for use in the management of the disease, 102 accessions were evaluated for resistance to isolates of race 1 of FOLAC. Initially, preliminary screening was done, using the isolate Fus-173. Then, 47 materials selected as highly resistant and a susceptible control (Regina) were reevaluated for resistance to other isolates of FOLAC race 1 from different locations: Fus-202 and Fus-205, in October 2011, Fus-219 and Fus-222, in November 2011; and Fus-207, Fus-209, and Fus-220 in December 2011. Inoculation was performed on 25 day old seedlings in the greenhouse by the method of cutting the roots and immersing them in the conidial suspension. The evaluation was performed using a scale ranging from 0 (healthy plant) to 4 (dead plant). Resulting data was transformed into a disease index and submitted to an analysis of variance and means comparison by the Tukey test (P=0.05). Thirty-two accessions were identified with wide resistance spectrum to different isolates of the pathogen in the four periods of inoculation.

Key words: *Lactuca sativa*, Fusarium wilt , genetic resistance.

INTRODUCTION

Lettuce, *Lactuca sativa* L., is one of the most consumed vegetables in Brazil. In terms of economic importance it is the sixth most economically valuable vegetable crop in Brazil, and eighth in volume produced (Costa & Sala, 2005). The most important lettuce variety group in terms of cultivated area and volume of production in Brazil is Crisp Leaf, which corresponds to approximately 60% of the national market. Then comes the crisphead segment, with 25% of the total market, followed by the smooth and other types (mimosa, romaine, colored cultivars, etc) responsible for 15% of the total (Costa & Sala, 2005).

Diseases caused by soil-inhabiting fungi are very limiting for lettuce crops, often due to intensive use of fields for successive crops without crop rotation. These practices have caused a reduction in cultivated area and productivity (Lopes et al., 2010). Among the major fungal diseases of lettuce is Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *lactucae* Matuo and Motohashi. This disease was recently reported in Brazil, initially in the state of Espirito Santo (Ventura & Costa, 2008), and then in other states of the Southern and Southeastern regions of Brazil (Cabral et al., 2009).

Isolates of *F. oxysporum* f. sp. *lactucae* are grouped into three races (1, 2 and 3) according to their reaction on race-differential cultivars of lettuce (Fujinaga et al., 2003). In Japan, the presence of the three races has already been reported (Fujinaga et al., 2001, 2003; Yamauchi et al., 2004). On the other hand, in other countries such as Italy, Portugal, Iran, USA and Brazil only race 1 has been reported (Pasquali et al., 2007; McCreight et al., 2005; Brunelli et al., 2010).

The management of Fusarium wilt of lettuce can be accomplished only by adopting a combination of control measures such as preventing infestation of new cultivation areas, using healthy or treated seeds and seedlings of good quality. The transit of equipment and people from infested fields to new areas should also be avoided. In areas already infested with the pathogen crop rotation should take place at least every three years, as well as soil solarization and the incorporation of manure (Matheron et al., 2005; Lopes et al., 2010; Matheron et al., 2010).

Despite all these measures recommended for the control of Fusarium wilt of lettuce, the most efficient and viable control method for the producer is the use of cultivars with genetic resistance. However, nothing is known about the level of resistance to Fusarium wilt in cultivars from different lettuce segments grown in Brazil. Moreover, there is a constant release of new cultivars by the breeding programs, and it is necessary to evaluate and characterize the new accessions in terms of resistance to root pathogens of economic importance for lettuce, particularly *F. oxysporum* f. sp. *lactucae*.

Thus, the objective of this study was to evaluate a set of lettuce cultivars and lines for resistance to Fusarium wilt, aiming to identify sources of resistance to race 1 of *F. oxysporum* f. sp. *lactucae* and analyze the resistance

to different isolates of this pathogen race among the most promising accessions.

MATERIALS AND METHODS

Pathogen isolates

In this work eight *F. oxysporum* f. sp. *lactucae* isolates were used. They were characterized at formae specialis level on lettuce, other species from Asteraceae family and from other botanical families. They also were characterized as race 1 by inoculation on a set of differential cultivars of lettuce (Brunelli et al., 2010; Cabral et al., 2011) following the standard methodology (Fijinaga et al., 2001). Race identity of isolates was confirmed by PCR, using a set of specific primers (Cabral et al., 2011). After its characterization, isolates were deposited in the culture collection of plant pathogenic fungi "Professora Maria Menezes" – CMM as: CMM-3573 (Fus-173), CMM-3574 (Fus-202), CMM-3575 (Fus-205), CMM-3576 (Fus-207), CMM-3577 (Fus-209), CMM-3580 (Fus-219), CMM-3581 (Fus-220), and CMM-3583 (Fus-222).

Preliminary screening of lettuce accessions for resistance to *Fusarium oxysporum* f. sp. *lactucae* race 1

In this assay, the isolate Fus.173 of *F. oxysporum* f. sp. *lactucae* race 1 was used. This isolate was obtained from lettuce with wilt symptoms, collected in Antônio Carlos, Santa Catarina state. The fungus was grown in plates containing PDA for five days. After this period, three culture discs (5 mm diameter) were removed from actively growing cultures and placed in Erlenmeyer flasks containing 100 mL of potato-dextrose (PD). The flasks were placed on a shaker (140 RPM) for10 days at 25°C and 12-hour photoperiod. After this process, the culture was filtered through a double layer of cheesecloth and the concentration of spores in the resulting suspension was estimated using a haemocytometer. Subsequently, the spore suspension was adjusted to a concentration of 10^6 microconidia mL^{-1}.

The screening was carried out in May 2011 with 77 cultivars from various seed companies and 25 lines from the lettuce breeding program conducted by Embrapa Hortaliças. Two cultivars identified as highly susceptible in preliminary tests ('Regina' and 'Elisa'), were included as susceptible controls. Twenty five seeds of each accession were sown in polystyrene trays with 128 cells, filled with sterile substrate (Plantmax ®). The seedlings were inoculated 25-30 days after sowing, i.e., when they had four true leaves fully formed.

Inoculation was performed by removing the seedlings from the trays, washing the roots in water to remove the attached substrate, and cutting their ends (approximately 2 cm) using sterile scissors. Subsequently, the roots were immersed for three minutes in a Becker flask containing 50 mL of the spore suspension. After that, the seedlings were transplanted to 3L volume plastic pots containing a sterilized mixture of clay, manure, sand and carbonized sterilized rice straw. These pots were irrigated previously (one hour before planting). After this, 3mL conidial suspension was added in the collar region of each seedling. The plants were not irrigated again on the day of inoculation to prevent loss of the inoculum through runoff. The experimental design was completely randomized with 104 treatments and three replications, represented by three pots containing four plants each. Plants were maintained in a greenhouse with air temperature varying from 23 to 35°C and a photoperiod of 12 hours.

Evaluation was carried out 30 days after inoculation and consisted in the observation of the presence of external (yellowing, necrosis and wilting) and internal (vascular browning) symptoms, which were confirmed by cutting the stem in a vertical direction using a scalpel. To quantify the severity of the disease we adopted a grade scale, ranging from 0 to 4 where: 0 = plants with no symptoms, 1 = plants with no symptoms of wilting or yellowing leaves, but with vascular browning; 2 = plants with intense vascular browning and early leaf wilting or yellowing, 3 = severely wilted plants, associated with leaf yellowing and necrosis, 4 = dead plants (adapted from Santos, 1996).

From the grade values the disease indices (DI) were calculated by the formula of McKinney (McKinney et al., 1923): $DI\ (\%) = 100.\Sigma\ [(fv) / (nx)]$, such that f = number of plants with the same grade; v = grade observed, n = total number of plants evaluated, and x = highest score on the scale. These rates were grouped into classes of reaction to the disease, using the resulting average for each accession: 0.00% = resembling the immune response, (IR); 0.01 to 25.00% = highly resistant (HR); 25.01 to 50.00% = moderately resistant (MR); 50.01 to 75.00% = moderately susceptible (MS); 75.01 to 100.00% = highly susceptible (HS); as adapted from Reis et al. (2004). The accessions that showed a response type similar to immune or highly resistant were selected to be evaluated for resistance to seven other isolates of *F. oxysporum* f. sp. *lactucae* race 1.

Breadth of resistance to isolates of *Fusarium oxysporum* f. sp. *lactucae* race 1 of different geographic origins

The lettuce accessions selected in the first trial were evaluated for resistance to seven additional isolates of the pathogen in three assays. In these assays the concentration of the inoculum was increased to $2x10^6$ microconidia mL^{-1}. The first breadth of resistance assay was carried out in October 2011. In this assay, 47 accessions considered HR, plus the cultivar 'Regina', considered HS, and used as control, were tested for resistance to isolates Fus-202 and Fus-205, collected in Muriaé-MG and Santa Cruz do Rio Pardo-SP, respectively. Seedling production, inoculum preparation and inoculation followed the previously described methodology here and as well as for other tests described below. The experimental design was completely randomized in a factorial arrangement of 48 (accessions) x two (isolates) with three replications. Each

replication consisted of a pot with four plants. After inoculation plants were maintained in greenhouse with air temperature varying from 26 to 39°C and a photoperiod of 14 hours. The evaluation was performed 30 days after inoculation and the severity data were also used for calculating the DIs. The DI data were subjected to an analysis of variance and the means were compared by the Tukey test (P=0.05) through the SAS program.

The second breadth assay was carried out in November 2011. In this assay the same accessions and methodology were used as in breadth assay l, but isolates Fus-219 and Fus-222 were used, from Colombo-PR and Itajaí-SC, respectively. After inoculation plants were maintained in greenhouse with air temperature varying from 25 to 40°C and a photoperiod of 14 hours.

The third breadth assay was carried out in December 2011. In this assay the same accessions and methodology were used as in assay 1, but with isolates Fus-207, Fus-209 and Fus-220 from Campinas-SP, Nova Friburgo-RJ, and Colombo-PR, respectively. After inoculation plants were maintained in greenhouse with air temperature varying from 23 to 38°C and photoperiod of 14 hours.

RESULTS AND DISCUSSION

In the preliminary assay none of the accessions included (cultivars or breeding lines) of lettuce showed an immune-type reaction (zero disease), to isolate Fus-173. Of 104 accessions tested, 30 (including the susceptible controls) reacted as susceptible (high or medium susceptibility). From this group, 19 accessions (18.6%) were rated as highly susceptible, with DI ranging from 77.08% to 100%, 25 accessions (24.5%) were rated as moderately resistant with DI ranging from 27.08% to 47.91% and 47 accessions (46.1%) were highly resistant with DI ranging from 2.08% to 25%. These 47 accessions were preliminarily considered here as promising sources of resistance and potentially adequate for planting in areas where the disease is present. In the group of susceptible accessions, 19 cultivars belonged to segment "Butterhead". Likewise Garibaldi et al. (2004) evaluating 32 cultivars of lettuce of different segments found that seven cultivars (including some romaine and mimosa type) were resistant to Fusarium wilt, while the butterhead lettuce cultivars were all susceptible. These results indicate that butterhead cultivars should not be used in areas where Fusarium wilt occurs. Data obtained in this assay also indicated that in general cultivars from the mimosa segment: Mimosa, Green Salad Bowl, Oak Leaf Saladin, Red Salad Bowl, Salad Bowl Roxo, Read Salad Bowl Ultra Rosso, Red Mimosa Vermelha and Roxane were highly resistant to *F. oxysporum* f. sp. *lactucae*. This is in agreement with Scott et al. (2010) who found that two mimosa cultivars (Lolla Rossa and Red Rossa) were highly resistant to Fusarium wilt race 1. These results indicate a low variability within the mimosa lettuce segment and suggest that they may all have a common origin. Therefore resistance to race 1 in this lettuce segment was effective for isolates from USA and from Brazil.

Thirty-two accessions featuring a wide spectrum of resistance to different isolates of the pathogen were identified along the four periods of inoculation. In the first breadth assay of resistance for isolate Fus-202, 38 out of the 47 accessions selected in the first test were highly resistant. However, seven accessions were only intermediately resistant and two were moderately susceptible. As for isolate Fus-205, one accession (Grand) showed an immune-type reaction, 41 accessions were highly resistant, three were intermediately resistant and two were moderately susceptible (Table 1). In this assay, considering the two isolates used, it was observed that the resistance classes, established to differentiate the accessions of lettuce in the preliminary screening, did not fit perfectly with the separation of accessions made by Tukey test at 5% (Table 1). In this case, the highly resistant accessions did not differ statistically from the intermediately resistant and moderately susceptible. It was also observed that there was no consistency in the results of nine accessions in relation to their resistance to two isolates of *F. oxysporum* f. sp. *lactucae*. The accessions Amélia, Crespa Repolhuda, Red Star, and CNPH-54 were considered moderately resistant to isolate Fus-202 and highly resistant to Fus-205, while the cultivars Scarlet and Kaiser were moderately susceptible to Fus-202 and highly resistant to Fus-205. The cultivar Mônica was moderately resistant to Fus-202 and moderately susceptible to Fus-205. The cultivar Lavínia was highly resistant to Fus-202 and moderately susceptible to Fus-205, while Green Salad Bowl was highly resistant to Fus-202 and moderately resistant to Fus-205. The accessions Mimosa, Simpson, Red Salad Bowl Ultra Rosso, Mimosa Vermelha, AC-5009, Sabrina, Angelina, Vanda, AMX1-140, Hanson, Cinderela, Vanessa, Cubana, Donna, Grand, Red Salad Bowl, Itapuã, Salad Bowl Roxo, Lavínia, Maravilha das Quatro Estações, Green Salad Bowl, Romana Paris, Romana New, AMX1-133, Raider Plus-pl.04, Irene, AMX1-99, Saia Veia, Lucy Brown, Sofia, AMX1-30, AMX1-96, Elba, Pira Roxo, AMX1-108, Roxane, Laurel-pl.01, and Roxa 01 were highly resistant to both isolates (Table1).

These differences in resistance to each isolate are probably because some cultivars and lines may have a quantitative resistance, which can be influenced by the environment. Moreover, the more concentrated inoculum must have influenced the different result of this assay in relation to the preliminary screening. A higher inoculum level allowed a better separation of accessions with different resistance levels in this assay. Scott et al. (2010) observed that cultivars considered susceptible were more affected than the cultivars with intermediate resistance, when they were challenged with a higher concentration of inoculum. However, when the inoculum concentration was low there were no apparent differences between susceptible and resistant plants. Therefore, in this work the highest concentration of inoculum allowed a better resolution for

TABLE 1 - Resistance levels of preliminarily selected lettuce accessions to four isolates of *Fusarium oxysporum* f. sp. *lactucae* race 1

Accession	Varietal Segment	DI* October 2011		DI November 2011	
		Fus-202	Fus-205	Fus-219	Fus-222
Regina**	Butterhead	89.58 aA	89.58 aA	89.58 aA	89.58 aA
Scarlet	Crisp leaf	66.66 abA	18.75 d-gA	4.16 bcA	18.75 bcA
Kaiser	Crisphead	58.75 bcA	6.25 fgB	6.25 bcA	6.25 bcA
Isabela	Crisp leaf	48.33 b-dA	45.83 b-eA	6.25 bcB	25.00 bA
Amélia	Crisphead	37.91 c-eA	4.167 fgB	2.08 cB	22.91 bcA
Crespa Repolhuda	Crisphead	35.83 c-eA	14.58 d-gB	12.50 bcA	8.33 bcA
Red Star	Crisp leaf	27.50 deA	18.75 d-gA	6.25 bcB	22.91 bcA
Oak leaf Saladin	Mimosa	27.08 deA	37.50 b-fA	6.25 bcB	25.00 bA
CNPH-54	Crisphead	27.08 deA	25.00 c-gA	18.75 bcA	18.75 bcA
Mônica	Crisp leaf	27.08 deA	54.16 bcA	20.83 bcA	22.91 bcA
Mimosa	Mimosa	25.00 deA	25.00 c-gA	18.75 bcA	25.00 bA
Simpson	Crisp leaf	25.00 deA	25.00 c-gA	16.66 bcA	20.83 bcA
Red Salad Bowl U. Rosso	Mimosa	25.00 deA	25.00 c-gA	14.58 bcA	6.25 bcA
Mimosa vermelha	Mimosa	25.00 deA	25.00 c-gA	4.16 bcA	16.66 bcA
AC-5009	Crisphead	25.00 deA	4.17 f-gB	4.16 bcA	6.25 bcA
Sabrina	Crisp leaf	25.00 deA	25.00 c-gA	16.66 bcA	25.00 bA
Angelina	Crisphead	25.00 deA	25.00 c-gA	6.25 bcB	25.00 bA
Vanda	Crisp leaf	25.00 deA	20.83 c-gA	12.50 bcB	25.00 bA
AMX1-140	Crisp leaf	25.00 deA	18.75 d-gA	6.25 bcB	25.00 bA
Hanson	Crisphead	25.00 deA	8.33 f-gB	4.16 bcA	4.16 cA
Cinderela	Crisp leaf	25.00 deA	25.00 c-gA	14.58 bcA	25.00 bA
Vanessa	Crisp leaf	25.00 deA	14.58 d-gA	6.25 bcA	14.58 bcA
Cubana	Crisp leaf	25.00 deA	25.00 c-gA	18.75 bcA	22.91 bcA
Donna	Romaine	25.00 deA	22.91 c-gA	12.50 bcA	20.83 bcA
Grand	Crisp leaf	25.00 deA	0.00 gB	6.25 bcA	10.41 bcA
Red Salad Bowl	Mimosa	25.00 deA	14.58 d-gA	12.50 bcA	14.58 bcB
Itapuã	Crisp leaf	25.00 deA	20.83 c-gA	18.75 bcA	25.00 bA
Salad Bowl Roxo	Mimosa	25.00 deA	10.41 f-gA	8.33 bcB	25.00 bA
Lavínia	Mimosa	25.00 deA	70.83 abA	20.83 bcA	25.00 bA
Maravilha das 4 estações	Butterhead	25.00 deA	25.00 c-gA	14.58 bcB	25.00 bA
Green Salad Bowl	Mimosa	25.00 deB	47.91 b-dA	6.25 bcB	25.00 bA
Romana Paris	Romaine	25.00 deA	22.91 c-gA	2.08 cA	8.33 bcA
Romana New	Romaine	25.00 deA	25.00 c-gA	12.50 bcB	25.00 bA
AMX1-133	Crisp leaf	25.00 deA	25.00 c-gA	2.08 cB	25.00 bA
Raider Plus pl.04	Crisphead	12.50 eA	12.50 e-gA	12.50 bcA	12.50 bcA
Irene	Crisphead	25.00 deA	20.83 c-gA	6.66 bcA	6.66 bcA
AMX1-99	Crisp leaf	25.00 deA	18.75 d-gA	4.16 bcB	25.00 bA
Saia Veia	Butterhead	25.00 deA	20.83 c-gA	10.41 bcA	20.83 bcA
Lucy Brown	Crisphead	25.00 deA	22.91 c-gA	25.00 bA	25.00 bA
Sofia	Romaine	25.00 deA	25.00 c-gA	16.66 bcB	25.00 bA
AMX1-30	Crisp leaf	22.91 deA	25.00 c-gA	8.33 bcB	25.00 bA
AMX1-96	Crisp leaf	22.91 deA	22. 91c-gA	8.33 bcB	25.00 bA
Elba	Crisp leaf	22.91 eA	22.91 c-gA	10.41 bcB	22.91 bcA
Pira Roxa	Crisp leaf	20.83 eA	2.08 gB	16.66 bcA	6.25 bcA
AMX1-108	Crisp leaf	20.83 eA	20.83 c-gA	12.50 bcA	20.83 bcA
Roxane	Mimosa	18.75 eA	18.75 d-gA	6.25 bcA	14.58 bcA
Laurel pl.04	Crisphead	16.66 eA	16.66 d-gA	2.08 cA	16.66 bcA
Roxa 01	Crisp leaf	14.58 eA	10.41 fgA	10.41 bcA	6.25 bcA

*DI (Disease index) values followed by the same uppercase letter within a row or lowercase letter within a column do not differ statistically according to the Tukey test (P=0.05).

** Cultivar Regina included as highly susceptible control.

differentiation of degrees of susceptibility between some cultivars. Another factor that may explain the differences between the results of this assay as compared with the results of the preliminary selection would be the difference in aggressiveness among isolates; for example, isolate Fus-202 was more aggressive than Fus-205 toward six accessions.

Isolates belonging to the same race, coming from different geographical regions, can show differences in their ability to cause disease. These differences between isolates may also explain the disparities of the response of resistance observed in some accessions in this assay.

In the second breadth assay, all 47 accessions were classified as highly resistant to isolates Fus-219 and Fus-222. Accessions classified as highly resistant differed significantly (Table 1) from the susceptible control, according to Tukey test (P=0.05). Comparing the two *Fusarium* isolates, it was observed that isolate Fus-222 was more aggressive toward 16 accessions. Although this experiment had been carried out at another time of the year and with a higher inoculum concentration, it was observed that the tested accessions kept the same level of resistance presented in the preliminary screening. The results of this assay indicate that the differences in resistance, as presented by the accession in the preliminary selection and in the third assay, are more due to the differences in aggressiveness among isolates of the pathogen than to the inoculum concentration.

In the third assay 46 out of 47 accessions were classified as either highly resistant or moderately resistant to isolate Fus-207. For isolate Fus-209, 46 accessions were classified as highly resistant and one accession as moderately resistant. In relation to isolate Fus-220, 41 accessions of lettuce were classified as highly resistant and six as moderately resistant. In this experiment plants classified as intermediately resistant and highly resistant differed significantly (Table 2) from the susceptible control, according to Tukey test (P=0.05). The phenotypic classes of resistance also did not show a good match to the grouping of accessions obtained using the Tukey test (P=0.05), because the plants classified as intermediately resistant did not differ significantly from those classified as highly resistant. In this third assay, it was also observed that there were no consistent results from seven accessions for the three pathogen isolates. The cultivar Maravilha das Quatro Estações was highly resistant to isolates Fus-209 and Fus-220 and moderately resistant to Fus-207, while accessions AMX1-99, Lucy Brown, Crespa Repolhuda, Donna, and Amélia were highly resistant to isolates Fus-207 and Fus-209 and moderately resistant to Fus-220. The cultivar Red Star was considered highly resistant to Fus-207 and moderately resistant to Fus-209 and 220. Accessions AMX1-133, CNPH-54, AMX1-30, Mimosa, Angelina, Green Salad Bowl, Red Salad Bowl, Isabela, Itapuã, Mimosa Vermelha, Lavínia, Sabrina, Sofia, Laurel-pl.04, Romana Paris, Elba, Cinderela, AMX1-106, Scarlet, Simpson, Oak leaf Saladin, AMX1-140, Mônica, Hanson, Salad Bowl Roxo, Saia Veia, Raider Plus-pl.04, Vanda, Irene, Cubana, Vanessa, Red Salad Bowl Ultra Rosso, Kaiser, Romana New, Roxane, Pira Roxa, Grand, AC-5009, AMX1-108, Amélia, and Roxa 01 were highly resistant to all three fungal strains (Table 2).

Among the consistently highly resistant cultivars in all experiments is the cultivar 'Pira Roxa', a bright red lettuce cultivar belonging to the crisp leaf segment. This cultivar was bred in Brazil, and is resistant to other major lettuce pathogens such as *Thielaviopsis basicola*, *Bremia lactucae* and lettuce mosaic virus, pathotype II. This is acknowledged as the first tropical red crisp leaf lettuce cultivar carrying multiple resistance to diseases in Brazil (Sala & Costa, 2005; Sala et al., 2008). Now we have the evidence that this cultivar is also resistant to Fusarium wilt and hence a good choice for Brazilian producers of lettuce and a source of resistance to various diseases which can be used in lettuce breeding programs in Brazil and elsewhere. Another important result was the observation of resistance to Fusarium wilt found in cultivars such as Lucy Brown and Vanda. The cultivar Lucy Brown has been the most widely planted crisphead lettuce cultivar in Brazil for more than a decade, because of its high tolerance to summer conditions. This cultivar is also tolerant to bacterial spot (*Xanthomonas campestris* pv. *vitians*). However, its cultivation in areas with incidence of *T. basicola* and *B. lactucae* has been limited due to its susceptibility to these pathogens (Costa & Sala, 2005). Another cultivar that was resistant in all tests and is of particular interest is Vanda. This is the most cultivated crisp leaf lettuce cultivar in Brazil, because it is very uniform, it has great commercial value due to its resistance to transport and handling and is resistant to LMV (Costa & Sala, 2005).

Considering all the assays, conducted in different months of the year, it was observed that cultivars from the romaine segment showed good levels of resistance to Fusarium wilt. The cultivars Sofia, Romana New, and Romana Paris were highly resistant to all pathogen isolates, while Donna was intermediately resistant to Fus-220 and highly resistant to the other isolates. The results obtained here were similar to those reported by Matheron et al. (2005) in trials conducted in fields that were naturally infested with *F. oxysporum* f. sp. *lactucae* in Arizona. Those authors identified two lettuce cultivars of the romaine segment (Slugger and King Louie), out of 16 cultivars tested, showing low levels of disease, and therefore considered them to be resistant or tolerant to the pathogen. A similar result was obtained in California by Scott et al. (2010, 2012), who identified three resistant romaine cultivars (Caeser, Forest Green and King Henry) in field trials. As observed for cultivars of the mimosa segment, these results also indicate a low genetic variability within this lettuce segment and suggest that they all have a common origin.

As observed before, all cultivars of the romaine segment and most cultivars of mimosa segment showed excellent resistance levels in this study. However, this is not always the case, since Scott et al. (2010, 2012) demonstrated that some romaine and mimosa segment cultivars were severely attacked by the pathogen when tested under controlled greenhouse conditions. Therefore, there is not always a consistent association between phenotypic type and cultivar susceptibility. This was also observed in the case of butterhead lettuce. Although there are known limitations for cultivating butterhead lettuce in places where Fusarium wilt occurs, two cultivars from this segment behaved as resistant

TABLE 2 - Resistance levels of preliminarily selected lettuce accessions to three isolates of *Fusarium oxysporum* f. sp. *lactucae* race 1 (trial performed in December 2011)

Acession	DI* Fus-207	DI Fus-209	DI Fus-220
Regina**	87.5 aA	79.16 aA	81.25 aA
Maravilha das 4 estações	29.16 bA	22.91 bA	25.00 c-eA
AMX1-133	25.00 bA	25.00 bA	25.00 c-eA
CNPH-54	25.00 bA	25.00 bA	25.00 c-eA
AMX1-30	25.00 bA	18.75 bA	25.00 c-eA
Mimosa	25.00 bA	25.00 bA	25.00 c-eA
AMX1-99	25.00 bA	25.00 bA	27.08 b-eA
Angelina	25.00 bA	18.75 bA	25.00 c-eA
Green Salad Bowl	25.00 bA	25.00 bA	25.00 c-eA
Red Salad Bowl	25.00 bA	20.83 bA	22.91 c-eA
Isabela	25.00 bA	25.00 bA	25.00 c-eA
Itapuã	25.00 bA	25.00 bA	25.00 c-eA
Mimosa Vermelha	25.00 bA	18.75 bA	25.00 c-eA
Lavínia	25.00 bA	25.00 bA	25.00 c-eA
Sabrina	25.00 bA	25.00 bA	25.00 c-eA
Sofia	25.00 bA	22.91 bA	25.00 c-eA
Laurel pl.04	25.00 bA	25.00 bA	25.00 c-eA
Romana Paris	25.00 bA	16.66 bA	25.00 c-eA
Elba	25.00 bA	25.00 bA	25.00 c-eA
Red Star	25.00 bA	29.16 bA	41.66 bA
Cinderela	25.00 bA	18.75 bA	25.00 c-eA
AMX1-96	25.00 bA	22.91 bA	25.00 c-eA
Scarlet	25.00 bA	12.50 bB	25.00 c-eA
Simpson	25.00 bA	20.83 bA	25.00 c-eA
Oak leaf Saladin	25.00 bA	25.00 bA	25.00 c-eA
AMX1-140	22.91 bcA	25.00 bA	25.00 c-eA
Mônica	22.91 bcA	25.00 bA	25.00 c-eA
Hanson	22.91 bcA	25.00 bA	25.00 c-eA
Salad Bowl Roxo	22.91 bcA	25.00 bA	20.83 c-eA
Saia veia	22.91 bcA	20.83 bA	25.00 c-eA
Crespa Repolhuda	22.91 bcA	25.00 bA	27.08 b-eA
Raider Plus pl.04	22.91 bcA	18.75 bA	25.00 c-eA
Vanda	22.91 bcA	20.83 bA	22.91 c-eA
Irene	22.91 bcA	22.91 bA	25.00 c-eA
Lucy Brown	22.91 bcA	25.00 bA	33.33 bcA
Cubana	22.91 bcA	25.00 bA	25.00 c-eA
Donna	22.91 bcA	25.00 bA	29.16 b-dA
Vanessa	20.84 bcA	25.00 bA	12.50 eA
Red Salad Bowl U. Rosso	20.83 bcAB	12.50 bB	25.00 c-eA
Kaiser	20.83 bcA	22.91 bA	25.00 c-eA
Romana New	20.83 bcA	25.00 bA	25.00 c-eA
Roxane	20.83 bcA	20.83 bA	25.00 c-eA
Pira Roxa	18.75 bcA	12.50 bA	25.00 c-eA
Grand	18.75 bcA	22.91 bA	25.00 c-eA
AC-5009	18.75 bcA	22.91 bA	16.66 deA
AMX-108	14.58 bcA	25.00 bA	22.91 c-eA
Amélia	14.58 bcA	16.66 bA	35.41 bcA
Roxa 01	8.33 cB	25.00 bA	25.00 c-eA

*DI (Disease index) values followed by the same uppercase letter within a row or lowercase letter within a column do not differ statistically according to the Tukey test (P=0.05).

** Cultivar Regina included as highly susceptible control.

in all tests. Saia Veia was highly resistant to all fungal isolates and Maravilha das Quatro Estações was also highly resistant to all isolates, except for Fus-207 for which it was moderately resistant.

Out of 47 inoculated accessions, 32 consistently showed broad resistance spectrum to different isolates of the pathogen in four inoculation seasons (Mimosa, Simpson, Red Salad Bowl Rosso, Mimosa Vermelha, AC-

5009, Sabrina, Angelina, Vanda, AMX1-140, Hanson, Cinderela, Vanessa, Cubana, Grand, Red Salad Bowl Ultra Rosso, Itapuã, Salad Bowl Roxo, Romana Paris, Romana New, AMX1-133, Irene, Raider Plus-pl.04, Saia Veia, Sofia, AMX1-30, AMX1-96, Elba, Pira Roxa, AMX1-108, Roxane, Laurel pl.04 and Roxa 01). These cultivars and breeding lines of lettuce are promising sources of resistance to Fusarium wilt. These resistance sources can be used in future breeding programs and are probably adequate for planting in areas where the disease occurs.

The identification of resistance to Fusarium wilt in existing commercial cultivars of lettuce is another positive result of the present work as these are readily available for use by the lettuce growers without the need of a long breeding process. In addition, the cultivation of cultivars from different varietal segments with resistance to the pathogen, in a crop rotation, could be an alternative to promote the reduction of the inoculum, since this practice can minimize the incidence/severity of disease. Moreover, since most cultivars showed no immune-type resistance (no disease) it is still necessary to combine genetic resistance with other disease control measures. In addition, constant monitoring of the variability of the pathogen is needed, to prevent the emergence of new pathogen races.

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