



A single dominant gene/locus model for control of *Fusarium oxysporum* f. sp. *lactucae* race 1 resistance in lettuce (*Lactuca sativa*)

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Abstract *Fusarium oxysporum* f. sp. *lactucae* (FOLac) is responsible for significant economic losses across major lettuce-producing regions around the world. Thus far, only FOLac race 1 isolates have been reported associated with *Fusarium* wilt outbreaks in Brazil. The most sustainable strategy for disease control is the pyramidization of race-specific resistance factors in lettuce cultivars. The loose-leafy cultivar ‘Vanda’ was found as one of the most promising sources of resistance to FOLac race 1. The genetic basis of this resistance was determined by analyzing the reaction to this pathogen of segregating populations derived from the cross ‘Gisele’ (susceptible) × ‘Vanda’ (pollen donor). A single molecular marker-genotyped F₁ hybrid plant was selfed and individual plants of a segregating F₂ population as well as 63 families F₂:F₃ were inoculated with a FOLac race 1 isolate by using the root-dipping method (3 × 10⁶ conidia/ml). Our results confirmed the high levels of resistance of ‘Vanda’ even under very harsh experimental conditions. Overall, the reaction of the

F₁ plants and the segregating patterns of the F₂ population ($n = 82$) and of the F₂:F₃ families ($n = 838$ plants) fit a single dominant gene/locus model. However, the phenotypic expression of resistance might suffer effects of additional genetic factor(s) (e.g., locus dosage, minor modifying genes, and incomplete penetrance). Notwithstanding, the high levels of FOLac race 1 resistance and its relatively simple genetic control makes ‘Vanda’ a major germplasm source for lettuce-breeding programs aiming to incorporate this trait in a wide array of elite lines from distinct varietal groups.

Keywords *Fusarium* wilt · Genetic control · Penetrance · Inheritance · Breeding

Introduction

The cultivated lettuce (*Lactuca sativa* L.) germplasm encompasses a large number of commercial and landrace cultivars, which are subdivided into groups based mainly upon morpho-agronomic and horticultural traits (Subbarao 1998). Lettuce is the most important leafy vegetable crop in Brazil, with significant economic importance, being cultivated under open-field, plastic house, and hydroponic conditions (Sala and Costa 2012).

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Diseases caused by soil-borne fungi are among the most limiting factors for the lettuce crop across many regions of the world. Vascular wilt, caused by physiological races of *Fusarium oxysporum* f. sp. *lactucae* (FOLac), is one of the main lettuce diseases in tropical and subtropical regions (Cabral et al. 2018). The pathogenic variability of FOLac isolates has been grouped thus far into four races based upon the response of a differential set of lettuce cultivars (Fujinaga et al. 2003; Gilardi et al. 2016; Cabral et al. 2018). Race 1 has the widest geographical distribution, being reported in Japan (Matuo and Motohashi 1967), the United States (Hubbard and Gerik 1993; McCreight et al. 2005), Taiwan (Huang and Lo 1998), Iran (Millani et al. 1999), Portugal (Pasquali et al. 2007), Italy (Pasquali et al. 2005, 2007), and, more recently, in Brazil (Cabral et al. 2014, 2018). Japan has the highest pathogen diversity with the report of three (1, 2, and 3) races (Fujinaga et al. 2001, 2003; Yamauchi et al. 2004). More recently, a new race (= race 4) has been characterized in Europe (Gilardi et al. 2016).

In Brazil, soil-borne pathogens are also important yield-limiting factors for the lettuce crop, especially due to poor management practices often employed by the growers, including the highly intensive use of the same area with consecutive cycles of cultivation (Lopes et al. 2010). In Brazil, *Fusarium* wilt was initially reported in Espírito Santo State (Ventura and Costa 2008), followed by its detection in other states in southeast Brazil (viz. Rio de Janeiro, São Paulo, and Minas Gerais) as well as in the southern region of Brazil (viz. Santa Catarina, Paraná, and Rio Grande do Sul) (Cabral et al. 2014). Nationwide surveys indicated that, currently, only FOLac race 1 isolates are implicated as causal agents of this vascular disease in Brazil (Cabral et al. 2018).

The employment of cultivars with genetic resistance is the most sustainable method for FOLac control in lettuce due to the low efficiency of alternative strategies (Davis et al. 2006; Lopes et al. 2010; Matheron and Porchas 2010). The identification of genetic sources combining phenotypic stability and broad-spectrum resistance (i.e., effective against a wide range of variants of the pathogen) would provide the ideal genetic material for use in breeding programs aiming to produce FOLac-resistant cultivars (Tsuchiya et al. 2004; Scott et al. 2010; Cabral and Reis 2013). However, most of the *Lactuca* germplasm

sources identified so far displayed a typical race-specific resistant reaction (Gilardi et al. 2016).

The genetics of resistance against different FOLac isolates of distinct races has been described as either qualitative or quantitative. Inheritance studies as well as the development of genetic maps encompassing the resistance factors to this pathogen were reported using lettuce populations from the United States and Japan (McCreight et al. 2005; Michelmore et al. 2011; Aruga et al. 2012). The results available thus far indicate that resistance to FOLac race 2 isolates is controlled by a single dominant gene/locus (Aruga et al. 2012), whereas the response to FOLac race 1 isolates is controlled by either multiple or recessive genetic factors (McCreight et al. 2005; Michelmore et al. 2011).

A practical problem faced by breeding programs to carry out genetic studies in lettuce is the fact that its floral structure (an inflorescence of multiple cleistogamic flowers) makes controlled crosses difficult and cumbersome (Nagata 1992). The production of *bona fide* F₁ hybrids between lettuce accessions is not entirely reliable, even when involving controlled manual cross-hybridization, since substantial levels of self-fertilization can still occur (Nagata 1992; Sala and Nascimento 2014). In crossings involving phenotypically similar lettuce lines/cultivars, the availability of molecular markers is a powerful tool for reliable identification of hybrid plants. In this respect, the molecular marker systems randomly amplified polymorphic DNA (RAPD) and sequence-characterized amplified regions (SCAR) are simple and widely used tools in the fingerprinting of lettuce germplasm accessions (Dziechciarkova et al. 2004; Silva et al. 2005; Simko et al. 2011).

Stable and highly effective sources of genetic resistance against Brazilian FOLac race 1 isolates were identified in some accessions of the cultivated lettuce germplasm (Cabral and Reis 2013). Among them, the loose-hearted lettuce cultivar ‘Vanda’ was found to be one of the most promising breeding materials, displaying high levels of resistance to FOLac race 1 isolates (Cabral and Reis 2013). So far, no study has been conducted to elucidate the genetic control of the resistant reaction displayed by ‘Vanda’. In this context, our main objective was to study the inheritance of this highly effective resistance to FOLac race 1 isolates derived from this commercial cultivar.

Materials and methods

Crosses between parental lines with contrasting responses to FOLac 1 and development of segregating populations for inheritance studies

Crosses were made between the cultivars ‘Gisele’ (susceptible parental cultivar; Agristar do Brasil) and ‘Vanda’ (resistant parental cultivar; Sakata Seed Sudamerica) following the “clip-and-wash” method of emasculation (Nagata 1992). These two parental lettuce cultivars belong to the varietal group loose-hearted with green leaves. ‘Vanda’ was used exclusively as a male (= pollen donor) parent. Plants resulting from the controlled crosses (= F₁ generation) were sown in trays. A single *bona fide* F₁ individual plant (genotyped with molecular markers; see description below) was previously selected and self-fertilized to constitute the F₂ generation. Contrasting parental lines, *bona fide* F₁ individuals (genotyped with molecular markers; see description below) and plants of the F₂ population and from F₂:F₃ families were used for the inheritance studies. Only F₃ families derived from F₂-resistant plants were used for inoculation due to the fact that the inoculation of the F₂ under our experimental conditions leads to a precocious and total collapse of the susceptible plants.

Genomic DNA extraction of the contrasting parental lines, F₁ plants, and individuals of the segregating populations

The DNA was extracted from the leaf tissue of the two contrasting parental lines, F₁ plants, and from all F₂ individuals using a modified 2X CTAB procedure (Boiteux et al. 1999). DNA quantifications were done using the NanoDrop[®] spectrophotometer apparatus (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The DNA was then purified using the Power Clean[®] Pro DNA Clean-up kit, following the manufacturer’s recommended procedures (MO BIO Laboratories, Carlsbad, CA, USA). This step was employed due to the frequent presence of PCR inhibitors observed under our experimental conditions in most of the genomic DNA samples extracted from lettuce.

Development of SCAR markers useful for identification of *bona fide* hybrid plants from ‘Gisele’ × ‘Vanda’ crosses

A total of 520 RAPD primers (Operon series OPA-01 to OPZ-20, Operon Technologies, Alameda, CA, USA) were evaluated in the search for polymorphisms between the susceptible (‘Gisele’) and resistant (‘Vanda’) parental lines. Purified genomic DNA of two genetically unrelated “iceberg” or “crisphead” lettuce cultivars (‘Laurel’ and ‘Raider’) were included as internal controls in this search for polymorphic RAPD markers. The quantified DNA samples were diluted with TE + RNase (concentration = 50 ng/ml) and used in the RAPD reactions. The reaction was composed of 2 µl of genomic DNA (50 ng/µl), 1.25 µl of 10X buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3), 0.6 µl MgCl₂ (50 mM), 5 µl dNTPs (2.5 mM each), 0.2 µl *Taq* DNA polymerase (5 units/µl), 2 µl of each primer, and 5.95 µl of Milli-Q[®] (Millipore Corporation, Bedford, MA, USA) water with a total reaction volume of 12.5 µl. The program used for amplification of the fragments was of an initial cycle of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s for denaturation, 36 °C for 1 min for annealing, and 72 °C for 2 min for extension; the final step consisted of 68 °C for 10 min followed by 4 °C (∞). Only informative RAPD bands with high intensity and stability were considered in further validation analyses. In the second step, stable amplicons were selected and removed from the agarose gel under UV light. Polymorphic amplicons were purified, cloned, and sequenced, with the purpose of designing more specific SCAR primers (Paran and Michelmore 1993). For SCAR marker development, the pGEM-T Easy Vector Systems[®] protocol (Promega, Madison, Wisconsin, WI, USA) was used for the cloning of polymorphic RAPD amplicons. Ligation was done by adding 0.5 µl of the pGEM-T Easy[®] vector, 5 µl of the buffer, 1 µl ligase (T4 DNA), 0.5 µl of Milli-Q water, and 3 µl of the PCR product (purified amplicons) to microtubes, with a total volume of 10 µl. Reactions were incubated at 4 °C overnight. After this period, 50 µl of competent cells (JM 109 High Efficiency Competent Cells; Promega, Madison, WI, USA) was added to 2 µl of the ligation reaction, and this was incubated on ice for 20 min. Thermal shock was induced by removing the tubes from the ice and placing them in a water bath at exactly 42 °C for 45 s.

Immediately, the tubes were removed and conditioned on ice for 2 min and 950 μ l of liquid LB medium was added. The transformations were incubated (for 1 h and 30 min at 37 °C) under constant agitation. Bacterial suspensions (100 μ l) of each clone were spread on Petri dishes containing LB + ampicillin medium (50 μ g/ml). Plates were incubated in an oven at 37 °C for 24 h. Confirmation and selection of the transformed bacteria were done via PCR using the primers of vector M13 (reverse and forward M13 primers). DNA was then extracted from the selected bacterial colonies. Sequencing of the cloned amplicons was carried out in an ABI PRISM 3100 sequencer (Applied Biosystems of Brazil, São Paulo–SP, Brazil) apparatus belonging to the Genomic Analysis Laboratory of Embrapa Vegetable Crops (CNPV). ABI Prism BigDye[®] version 3.1 (Applied Biosystems of Brazil, São Paulo–SP, Brazil) was employed with primers of vector M13 (reverse and forward primers). The SeqMan[®] program (Lasergene, Madison, Wisconsin, WI, USA) was used to analyze sequence quality, remove low-quality segments (Allex 1999), and find the consensus sequences. The obtained sequences were compared to those available in the GenBank database using BLASTn tool (www.ncbi.nlm.nih.gov/blast/).

Pairs of oligonucleotides were designed with the assistance of the PrimerSelect[®] program (Lasergene, Madison, Wisconsin, WI, USA) using the original RAPD amplicon sequences with the addition of 5–10 bases according to the information obtained after sequencing analyses. Three pairs of SCAR primers (*F* = forward/*R* = reverse) were designed: SCAR OP-A17-1-F (5'-GACCGCTTGTCAGCA-3') and SCAR OP-A17-1-R (5'-GACCGCTTGTTGGGGTT-3'); SCAR OP-B11-10-F (5'-AGACCCGTTACTACTGGAAGGAA-3') and SCAR OP-B11-10-R (5'-ACCCGTCCCGGTATGATGATTCC-3') and SCAR OP-W15-3-F (5'-CGGAACGACGGAAATG-3') and SCAR OP-W15-3-R (5'-CACCGGAACCACACC-3'). PCR assays were performed using 2 μ l of genomic DNA (50 ng/ μ l) in a final volume of 12.5 μ l, containing 1.25 μ l of 10X buffer (100 mM Tris–HCl, 500 mM KCl, pH 8.3), 0.6 μ l of MgCl₂ (50 mM), 0.5 μ l of dNTPs (2.5 mM each), 0.2 μ l *Taq* DNA polymerase (5 units/ μ l), 0.3 μ l of each SCAR primer, and 7.35 μ l Milli-Q[®] water (Millipore Corporation, Bedford, MA, USA). The program used for amplification of all three SCAR markers had an initial cycle

of 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min for denaturation, 61 °C for 1 min for annealing, and 72 °C for 2 min for extension; the final step consisted of 72 °C for 7 min and a final stage of 4 °C ∞ . PCR conditions were optimized in terms of annealing temperature for each primer used. The newly designed primers were validated in the parental cultivars as well as in 30 putative hybrid plants (*F*₁).

Phenotypic characterization of parental lines, *F*₁ hybrids, and segregating populations for reaction to a FOLac race 1

The fungal isolate 'CMM-3577' was also deposited in the fungal collection named after "Prof. Maria Menezes" in Recife, Pernambuco State–PE, Brazil (Cabral and Reis 2013). The fungal isolate was cultivated in PDA-t culture medium (Potato Dextrose Agar supplemented with 50 ppm tetracycline) for 5 days. The spore suspension was adjusted to a concentration of 3×10^6 microconidia/ml. For seedling production, the seeds were sown in polystyrene trays with 128 cells filled with sterile substrate (Plantmax[®]). Thirty-day-old seedlings were removed from the cells and the roots were washed in running water to eliminate the substrate adhered to the roots. The apical portions of the roots (\approx 2 cm) were cut using sterilized scissors and held in 50 ml of the spore suspension for 3 min. Then, the seedlings were transplanted to 3 L plastic pots containing a sterilized mixture of clay, manure, sand, and carbonized sterilized rice straw. These pots were irrigated previously (1 h before planting). After this, 3 ml of the 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. Plants were maintained in a greenhouse with air temperature varying from 25 to 35 °C and a photoperiod of 12 h. The parental cultivars, 'Vanda' (*n* = 30) and 'Gisele' (*n* = 30), the *F*₁ generation (*n* = 30; composed of true hybrid plants as indicated by SCAR marker analysis). A single marker-genotyped *F*₁ plant was previously selected and selfed to generate the *F*₂ generation (*n* = 82). These populations were simultaneously inoculated in the first bioassay employing the isolate 'CMM-3577' (= Fus-209) of FOLac race 1 (belonging to the Phytopathogenic Fungi Collection of CNPV, Embrapa Vegetable Crops, Brasília–DF, Brazil).

Evaluation of the response of lettuce plants in each generation to FOLac race 1 isolate and statistical analyses

The evaluation was performed 20 days after inoculation using a 1–5 grading scale adapted from Santos (1996), where 1 = plants with no symptoms; 2 = plants with no symptoms of wilting or yellowing leaves, but with vascular browning; 3 = plants with intense vascular browning and early leaf wilting or yellowing; 4 = severely wilted plants, associated with leaf yellowing and necrosis; 5 = dead plants. The plants were arbitrarily classified as resistant ($R \leq 1.99$) and susceptible ($S > 2$). The resistant/susceptible reactions to the FOLac isolate were annotated for each individual plant across all generations/populations. Chi-square (χ^2) analysis at a 5% probability level was used to test for goodness of fit of the observed segregation patterns to predicted Mendelian ratios (Schuster and Cruz 2004; Ramalho et al. 2008) assuming a major gene/locus model with complete dominance (3:1 segregation). Sixty-three individual F_2 plants were selfed to produce $F_2:F_3$ families (total $n = 838$ plants). These $F_2:F_3$ families and the parental cultivars ‘Vanda’ ($n = 10$) and ‘Gisele’ ($n = 10$) were then inoculated and evaluated in a second bioassay essentially as described above. No susceptible F_2 plants produced F_3 seeds for this analysis. Eight to 16 plants of each $F_2:F_3$ family were employed aiming to confirm the inheritance model observed in the F_2 population and also to identify homozygous resistant versus heterozygous resistant plants.

Bulked segregant analysis (BSA) strategy to verify potential linkage of polymorphic markers with FOLac race 1 resistance factor(s)

Genomic DNA was extracted from all F_2 plants before inoculation as described above. After phenotypic resistance evaluation, two contrasting groups (bulks) of DNA were assembled from seven resistant and seven susceptible individuals from the F_2 generation, respectively, as described by Michelmore et al. (1991).

Results

Development of SCAR markers for identification of true hybrid (‘Gisele’ × ‘Vanda’) plants

An initial screening was conducted with a total of 512 RAPD primers (Fig. 1). Of these, 13 primers (OPA-01, OPA-17, OPB-03, OPB-11, OPC-04, OPL-12, OPK-03, OPO-17, OPS-01, OPS-08, OPT-14, OPS-06, and OPW-15) were selected for a second round of evaluation due to their well-defined polymorphic amplicons between the two contrasting parents for FOLac race 1 reaction [‘Gisele’ (susceptible) and ‘Vanda’ (resistant)]. Further analysis indicated that only three of these RAPD-derived markers had stable polymorphisms between the contrasting parents. The sequence information of these three polymorphic RAPD amplicons was converted to three pairs of SCAR primers (with the following codes SCAR OPA-17-1, SCAR OP-B11-10, and SCAR OP-W15-3). Additional analyses confirmed that these SCAR markers allow an unequivocal distinction between hybrid versus self-fertilized plants (Fig. 2), indicating their usefulness.

Segregation patterns of the reaction to FOLac race 1 across segregating lettuce populations

The disease incidence and severity in the F_2 and F_3 progenies derived from a cross between a resistant and a susceptible cultivar was used to infer the inheritance of the FOLac race 1 resistance trait of ‘Vanda’ as previously characterized by Cabral and Reis (2013). Under the experimental conditions, ‘Vanda’ displayed 100% resistance (30 out of 30), whereas ‘Gisele’ displayed 100% (30 out of 30) susceptibility (= dead plant) to FOLac race 1. The F_1 generation displayed 25 out of 30 plants (83.33%) with resistant response to FOLac race 1. The reaction to the pathogen in an F_2 population of 82 individuals was distributed in the following classes: 19 plants with grade 5.0 (highly susceptible) and 63 plants with grades 1 or 2 (resistant). The segregation pattern observed in the F_2 generation displayed a good fit at the 3:1 ratio (resistant vs. susceptible) according to the Chi-square test (Table 1).

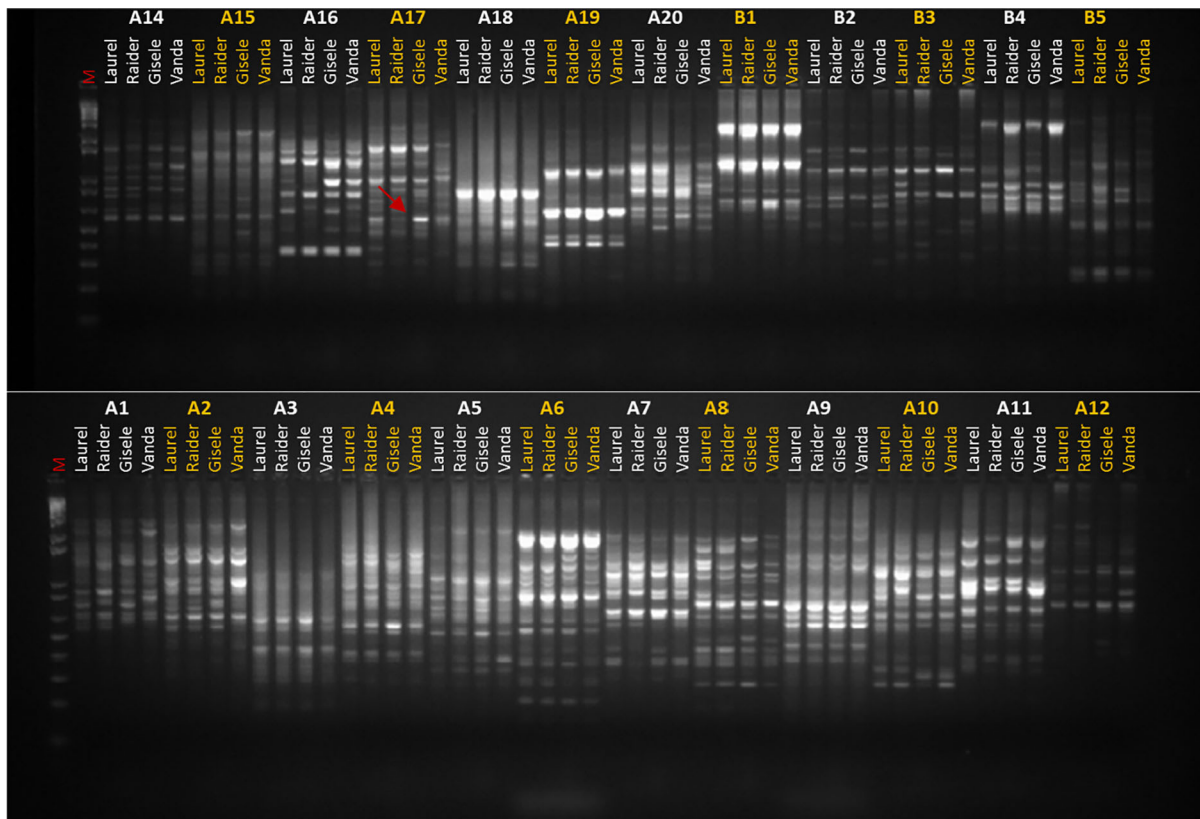


Fig. 1 Profile of amplicons generated with a subset of randomly amplified polymorphic DNA (RAPD) primers (Operon Technologies, Alameda, CA, USA) using as template the genomic DNA from the contrasting lettuce parental cultivars for the reaction to *Fusarium oxysporum* f. sp. *lactucae* race 1 [‘Vanda’, resistant and ‘Gisele’ susceptible]. Purified genomic DNA of two genetically unrelated “iceberg” lettuce

cultivars (‘Laurel’ and ‘Raider’) were included (as internal controls) in this search for polymorphic markers. The lane with the ‘M’ code indicates the 1-kb plus molecular ladder marker (Invitrogen). The *arrow* indicates a polymorphic RAPD amplicon between the contrasting parents ‘Vanda’ and ‘Gisele’ obtained with the primer OPA-17

Segregation pattern of the F₂:F₃ families

Only 63 resistant individuals (or potential escapes) were able to generate F₃ progenies because FOLac isolate race 1 induces the death of susceptible F₂ plants. Therefore, considering that resistance is expected to follow a single monogenic gene model, we should get approximately 1/3 of the F₃ progenies breeding true for resistance and approximately 2/3 of the F₃ progenies (obtained from heterozygous plants) displaying segregating reactions. In our assay, 23 out of the 63 F₃ progenies displayed plants with uniform resistant reactions whereas 40 families segregated, confirming the expected (1:2) proportion (Table 1). F₃ families composed only by susceptible plants were not

detected in the present study, indicating that none of the progenies were derived from potential disease-escaped F₂ plants.

Linkage of polymorphic SCAR markers and FOLac race 1 resistance factor(s) assessed by BSA

All three pairs of SCAR primers (named SCAR OP-B11-10, SCAR OP-W15-3, and SCAR OP-A17-1) displayed stable and polymorphic amplicons between contrasting parents but not within contrasting bulks. No strong deviation of the expected 3:1 ratio of these three SCAR markers and the resistant/susceptible phenotype was observed in a sample of 69 F₂

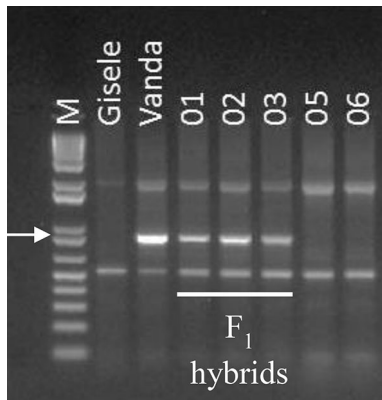


Fig. 2 Profile of PCR amplicons (obtained with the primer pair SCAR OPW-15-3) of contrasting parental lines and putative F₁ plants evaluated for reaction to an isolate of *Fusarium oxysporum* f. sp. *lactucae* (FOLac) race 1. The agarose gel electrophoretic (1.5%) illustrates the amplicon patterns of ‘Gisele’ (susceptible) and ‘Vanda’ (resistant) as well as for five putative hybrid F₁ plants (codes 01, 02, 03, 05, and 06). The putative F₁ hybrid plants were obtained from crosses using ‘Gisele’ as female parent and ‘Vanda’ as pollen donor. The letter ‘M’ is the lane with the 1-kb plus molecular ladder marker line (Invitrogen). The arrow points to a polymorphic ‘Vanda’-derived sequence-characterized amplified region (SCAR) marker, which was employed to distinguish true hybrid F₁ plants (e.g., those indicated by a white bar) from self-fertilized ‘Gisele’ plants (e.g., lanes 05 and 06)

individuals showing resistance and 13 F₂ individuals classified as susceptible, indicating that markers and the FOLac race 1 reaction segregated independently (i.e., no linkage).

Table 1 Reaction to a *Fusarium oxysporum* f. sp. *lactucae* (FOLac) race 1 isolate from Brazil of the parental lines, F₁ and F₂ generations, and F₂:F₃ families obtained from a controlled

Parental inbred lines, F ₁ , F ₂ and F ₂ :F ₃ generations	Total	Resistant (R)	Susceptible (S)	Expected segregating pattern (R:S)	χ ²
‘Gisele’(P ₁)	30	00	30	–	–
‘Vanda’(P ₂)	30	30	0	–	–
F ₁ (P ₁ × P ₂)	30	25	5	1:0	–
F ₂ population	82	63	19	3:1	0.10 ^{ns}
F ₂ :F ₃ families	63	23	40 ^a	1:2 ^b	0.29 ^{ns}

Individual plants of the parental lines as well as plants of each generation were inoculated with a FOLac isolate via root dipping technique with a spore suspension of 3 × 10⁶ microconidia/ml

^aF₂:F₃ families with segregating resistant and susceptible plants

^bExpected segregating pattern (R and S) among F₂:F₃ families assuming a major gene/locus model with complete dominance

^{ns}Non-significant Chi-square values at the 5% probability level

Discussion

In the cultivated lettuce germplasm, there is an intrinsic difficulty in generating true hybrid plants, since even under controlled crossing systems, some plants of the progeny may in fact be originated from self-fertilization of the mother plant. This reinforces the importance of the use of molecular markers as an auxiliary tool for genetic and breeding works (D’Andrea et al. 2008; Simko et al. 2011; Uwimana et al. 2012a, b). The two contrasting parental lettuce cultivars (‘Gisele’ and ‘Vanda’) employed in the present study belong to the varietal group loose-hearted leaves, and they are phenotypically quite similar. To ensure that true hybrid plants were obtained for the subsequent genetic studies, a large collection of RAPD primers was used in an initial screen for polymorphic markers between these two parental lines. Of these, only three primers were able to produce stable polymorphisms between these two parental lines. The genetic diversity of cultivated lettuce has been investigated by several research groups with different molecular marker systems with variable results in terms of the levels of polymorphisms among accessions (Kesseli and Michelmore 1986; Kesseli et al. 1991; Hu et al. 2005; Simko 2009). The relatively low number of polymorphisms identified in our study is probably due to the narrow genetic base of *L. sativa* (Kesseli and Michelmore 1986; Kesseli et al. 1991; Hu et al. 2005; Simko 2009), which was further aggravated by the fact that the two

cross between the contrasting parental lettuce (*Lactuca sativa* L.) lines ‘Gisele’ (susceptible) × ‘Vanda’ (resistant)

parental lines belong to the same morpho-agronomic group. Despite these limitations, stable polymorphic amplicons were identified for discrimination of the parental lines, allowing the genotyping of *bona fide* hybrid plants. These hybrid plants and their progenies were then used in the subsequent inheritance studies of FOLac 1 resistance. A single SCAR-genotyped F₁ hybrid plant (Fig. 2) was selfed to obtain the F₂ population and individual F₂ plants were selfed to obtain the corresponding F₂:F₃ families.

All segregation patterns fit to one single dominant gene/locus model. The presence of a small fraction of susceptible F₁ plants (five out of 30 inoculated plants) also suggested that the phenotypic expression of the ‘Vanda’-derived resistance might suffer influence of other (yet uncharacterized) genetic factor(s) (e.g., locus dosage effect, minor modifying gene(s), or incomplete penetrance). In fact, gene dosage effect has been observed in previous studies carried out in tomato for resistance to *F. oxysporum* f. sp. *lycopersici* (Alon et al. 1974). It also is important to highlight that, in the present study, the plants were submitted to a harsh inoculation method consisting of dipping the root systems (after mechanical injury) into a suspension with a high concentration of spores (3×10^6 conidia/ml). In addition, to monitor the development of symptoms, the plants were exposed to air temperatures ranging from 25 °C to 35 °C, which are highly favorable for the pathogen and conducive for disease onset and progression. Therefore, our experimental/environmental conditions may have contributed to an increase in the frequency of susceptible F₁ plants as well as in the segregating F₂ and F₃ generations, inhibiting the full expression of the resistance factor(s).

Air and soil temperatures are known to modulate the expression of resistance in a wide array of host–pathogen interactions. High soil and air temperatures can often inhibit the expression of resistance or enhance the susceptible reaction (Schoeneweiss 1975). However, the genetic and molecular basis of temperature sensitivity of many resistance genes are still poorly characterized (Zhu et al. 2010). Classical examples include *Arabidopsis* plants that are more susceptible to *Pseudomonas syringae* pv. *tomato* at 28 °C than at 22 °C (Wang et al. 2009); resistance to *Tobacco mosaic virus* (TMV) conferred by the *N* gene in *Nicotiana* accessions is effective at 22 °C but is not expressed at 30 °C (Whitham et al. 1996). Resistance

to root-gall nematodes conferred by the *Mi* gene in tomato is inactivated at soil temperatures above 28 °C (Hwang et al. 2000).

Previous inheritance and mapping studies of resistance in distinct lettuce germplasm sources and to distinct FOLac race 1 isolates (McCreight et al. 2005; Michelmore 2010; Michelmore et al. 2011) are in contrast with our monogenic dominant model. For example, the segregation patterns of F₁ and F₂ generations to North American isolates of FOLac race 1 from crosses between the susceptible cultivar ‘Vanguard’ and the resistant cultivars ‘Salinas 88’ and ‘Costa Rica No.4,’ indicated that the expression of this trait is under control of multiple recessive factors of resistance (McCreight et al. 2005). In a different set of studies, resistance to FOLac race 1 was mapped in two segregating populations. In one population derived from the crossing [(‘Valmaine’ × ‘Salinas 88’) × ‘Salinas’], three resistance QTLs were detected in the linkage groups LG1, LG2, and LG7. The resistance alleles located in the linkage groups LG1 and LG2 were originated from the resistant parent ‘Valmaine,’ while the allele present in LG7 was derived from the cultivar ‘Salinas’ (Michelmore 2010). Three QTLs associated with resistance in the linkage groups LG1, LG4, and LG8 were detected in the analysis of 112 F₂ individuals derived from the cross between ‘Red Tide’ × ‘Lolla Rosa’ (Michelmore et al. 2011).

The ‘partial resistance’ phenotype observed here is more likely due to semi-dominance and/or incomplete penetrance (i.e., percentage of individuals from a population with a given genotype expressing the corresponding phenotype). This also seems to be involved in the lettuce-FOLac race 1 reaction in ‘Vanda.’ In fact, some susceptible plants (five out of 30) were observed in the F₁ generation and at least one symptomatic plant was found in the majority of the F₂:F₃ progenies derived from individual F₂ plants characterized as resistant. Penetrance is affected, among other factors, by interaction between host genotype, pathogen isolate, inoculum concentration, seedling age, and soil and air temperature. With a general trend, an increase in the concentration (pressure) of the inoculum also induces a reduction in the levels of penetrance, that is, an increase in the incidence of plants expressing symptoms of the disease.

In agreement with our present work, previous inheritance studies of race-specific resistance to vascular pathogens of lettuce, such as *Verticillium dahliae* race 1 (Sandoya et al. 2017) and FOLac race 2 (Aruga et al. 2012), indicated single gene/locus control. Resistance to FOLac race 2 was mediated by the action of a single semi-dominant locus (named as RRD2), since the expected segregation ratios for dominant markers for an F₂ population were in the expected ratio 1:2:1 (resistant to intermediate to susceptible). Similar to our work, a previous study also suggested the presence of potential effects associated with minor loci that may also contribute to some modifications observed in the segregation patterns (Aruga et al. 2012).

The parental cultivars with contrasting responses to FOLac race 1 isolates were chosen because of their agronomic potential and traits of interest, with the aim to obtain segregating populations with high yield, early cycle, adaptation to Brazilian tropical and subtropical conditions, rusticity, and disease resistance traits. The three SCAR markers developed to distinguish hybrid plants derived from crosses between these cultivars are excellent tools to verify the occurrence of hybridization, proving to be a reliable and efficient method that allows their use in the early stages of the development of the putative hybrid plants. The BSA strategy (Michelmore et al. 1991) has often been used in the genetic improvement of lettuce to identify markers associated with contrasting phenotypes. However, in the present study, polymorphisms between parents did not show consistent linkage with the potential FOLac resistance 1 factor(s). In summary, the high levels of resistance and the relatively simple genetic control of FOLac race 1 resistance in ‘Vanda’ (primarily determined by a dominant monogenic locus) makes this cultivar a promising germplasm for use in breeding programs aiming to incorporate this trait in a wide array of elite lettuce lines from distinct groups.

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Compliance with ethical standards

Conflict of interest The authors do not have any conflicts of interest.

Informed consent All authors have reviewed the manuscript and approved its submission to Euphytica.

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