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Broad-spectrum resistance in lettuce germplasm to the soil-borne pathogens *Berkeleyomyces basicola* and *B. rouxiae*

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ABSTRACT

One of the emerging biotic problems of the lettuce (*Lactuca sativa*) crop in Brazil is the black root rot caused by two *Berkeleyomyces* species, *B. basicola* and *B. rouxiae*. Although sources of resistance have already been reported for *B. basicola*, there is still no information about the response of distinct lettuce accessions to *B. rouxiae*. Herein, we evaluated a diversified germplasm collection, composed of accessions from different *L. sativa* morphotypes, searching for sources of genetic resistance effective against isolates of both causal agents of the black root rot disease complex. Sixty-eight *L. sativa* accessions were initially screened for resistance to one *B. basicola* isolate. Thirty-three accessions (with the highest levels of resistance) were inoculated again with two *B. basicola* and two *B. rouxiae* isolates. Similar levels of resistance were observed for the isolates of *B. basicola* and *B. rouxiae*. The lettuce morphotypes ‘Romaine’, ‘Batavian’, and ‘Crispy loose-leaf’ displayed higher frequency of accessions with resistance to both fungi. Broad-spectrum resistance against both pathogens detected in distinct lettuce morphotypes opens the opportunity for breeding programs to incorporate these genetic factors in a wide range of commercial cultivars.

Keywords: *Lactuca sativa*, cultivar reaction, breeding, resistance, wilting, black root rot.

RESUMO

Resistência de amplo espectro em germoplasma de alface aos patógenos de solo *Berkeleyomyces basicola* e *B. rouxiae*

Um dos problemas bióticos emergentes da cultura da alface (*Lactuca sativa*) no Brasil é a podridão negra das raízes causada por duas espécies de *Berkeleyomyces* (*B. basicola* e *B. rouxiae*). Embora fontes de resistência já tenham sido relatadas para *B. basicola*, ainda não há informações sobre a resposta de diferentes acessos de alface para *B. rouxiae*. No presente trabalho, uma diversificada coleção de germoplasma, composta por acessos de diferentes morfotipos de *L. sativa*, foi avaliada visando identificar fontes de resistência efetivas contra isolados de ambos os agentes causais da podridão negra das raízes. Sessenta e oito acessos de *L. sativa* foram inicialmente avaliados para resistência a um isolado de *B. basicola*. Trinta e três acessos (com os maiores níveis de resistência) foram inoculados novamente com dois isolados de *B. basicola* e dois de *B. rouxiae*. Níveis semelhantes de resistência foram observados para os isolados de *B. basicola* e *B. rouxiae*. Os morfotipos de alface ‘Romana’, ‘Batavia’ e ‘Crespa’ apresentaram maior frequência de acessos com resistência a ambos os fungos. A resistência de amplo espectro detectada contra ambos os patógenos em morfotipos distintos de alface abre a oportunidade para os programas de melhoramento incorporarem esses fatores genéticos em uma ampla gama de cultivares comerciais.

Palavras-chave: *Lactuca sativa*, reação de cultivares, melhoramento genético, resistência, murcha, podridão negra das raízes.

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A wide array of biotic problems can affect the lettuce (*Lactuca sativa* L.) crop in tropical and subtropical Brazilian regions (Sala & Costa, 2012). In addition, the traditional practice of the growers of carrying out

successive production cycles in the same field area has gradually increased the incidence of soil-borne pathogens (Cabral *et al.*, 2019). One of the emerging problems is the black-root rot caused by *Berkeleyomyces basicola* (former *Thielaviopsis basicola*) (Nel *et al.*, 2018). However, novel taxonomic approaches have subdivided the genus *Berkeleyomyces* into two previously undescribed cryptic species: *B. basicola* and *B. rouxiae* (Nel *et al.*, 2018). The two *Berkeleyomyces* species do not show clear-cut host specificities, and they may indistinctly infect ≈170 plant species (Farr & Rossman, 2022). In susceptible hosts, endoconidia and chlamydospores are profusely produced (Pereg, 2013). The persistence of chlamydospores in the soil and the broad host range of the *Berkeleyomyces* species makes difficult to eradicate these pathogens from infested fields (Nel *et al.*, 2018). The infection cycle begins with a short biotrophic phase followed by a necrotrophic phase where the pathogen induces the characteristic dark coloration of the roots, resulting in root rot and foliage wilting (Mims *et al.*, 2000; Pereg, 2013).

The lettuce market in Brazil is highly segmented with a wide range of commercial morphotypes (Sala & Costa, 2012), including ‘Green crispy loose-leaf’ (market leader), ‘Butterhead’, ‘Iceberg’, ‘Mimosa’ (= ‘Green and Red salad bowl’ or ‘Super crispy’) and ‘Cos/Romaine’. In Brazil, the black root rot was first detected in lettuce in Rio de Janeiro State in the late 1990s (Silva *et al.*, 1999), and it is currently a major limiting factor for the production of ‘Iceberg’ and ‘Butterhead’ morphotypes across all regions (Sala *et al.*, 2008; Souza, 2022). With the expansion of production areas infested with *B. basicola* and/or *B. rouxiae* (Souza, 2022), the implementation of novel management alternatives is required. However, the available options for either cultural or chemical control of these pathogens are scarce, inefficient and/or costly (O’Brien & Davis, 1994; Souza, 2022). In this scenario, the use of resistant cultivars is one of the few sustainable methods of control.

Sources of genetic resistance in *Lactuca* germplasm were reported only to isolates of the former species *T. basicola* (Sala *et al.*, 2008). However, it is difficult to determine precisely against which fungal species these germplasm sources were evaluated due to the recent subdivision of *B. basicola* and *B. rouxiae*. Lettuce cultivation can be equally affected by both fungal pathogens as indicated by the similar levels of incidence of *B. basicola* and *B. rouxiae* across different producing regions of Brazil (Souza *et al.*, 2025). Thus, the objective of the present work was to evaluate a diversified germplasm collection (composed of accessions from different *L. sativa* morphotypes) searching for sources of multiple genetic resistance effective against isolates of the two causal agents of the black root rot disease – *B. basicola* and *B. rouxiae*.

MATERIAL AND METHODS

Berkeleyomyces isolates and inoculation bioassays

All bioassays were carried out in the greenhouses of Embrapa Vegetables (15°56'00"S; 48°08'00"W, 996 m altitude) in Brasília-DF, Brazil. The *B. basicola* and *B. rouxiae* isolates employed in the present study were obtained from symptomatic lettuce plants (Souza *et al.*, 2025). The *B. basicola* isolates (EH-2733 and EH-2740) were collected in Vargem Bonita-DF (15°47'60"S; 47°52'58"W) and Paulínia-SP (22°45'40"S; 47°9'15"W). The *B. rouxiae* isolates (EH-2741 and EH-2743) were collected in Uberlândia-MG (18°55'8"S; 48°16'37"W) and Santa Maria de Jetibá-ES (20°2'27"S; 40°44'45"W), respectively (Souza *et al.*, 2025). This collection of isolates was grown in Petri dishes (9 cm-diameter), containing Potato Dextrose Agar + tetracycline (PDA-t) culture medium, in a BOD incubator at a constant temperature of 23°C (12 hours light and 12 hours dark) for 15 days (Souza, 2022). Conidial suspensions were prepared by adding 10 mL of sterile distilled water to each plate, then the conidia were released with the aid of a soft bristle brush. The spore suspensions were subsequently filtered through a double-layer gauze. The spore concentration was estimated under an optical microscope by counting them with the aid of a Neubauer chamber. In the final step, the suspension was adjusted to concentrations of 7.5×10^5 or 2×10^6 conidia/mL. Seeds of the lettuce accessions were sown in 6.2 cm deep polystyrene trays with 128 cells, containing previously sterilized commercial substrate (Plantmax[®]), sown at 3 mm depth, and kept at a greenhouse (approximately 15-28°C) where they were irrigated twice a day. At 21 days after germination, the seedlings were gently removed from the cells and washed in running water aiming to eliminate the substrate adhered to the roots. Then the root system of each seedling was immersed in 3 mL of the spore suspension (2×10^6 conidia/mL) for three minutes. The residual suspension was placed near the crown area of each transplanted seedling with the aid of a micropipette. The seedlings were then transplanted into 72-cell trays containing 1/3 of the substrate (Plantmax[®]) infested ten days before with a conidial suspension (7.5×10^5 conidia/gram of substrate)

(Sala *et al.*, 2008). Mock-inoculated controls were dipped into sterile distilled water and transplanted to trays containing non-colonized substrate and kept at least one meter away from the inoculated plants to avoid cross contamination.

Evaluation criteria implemented in the bioassays

The disease assessments were performed 21 days after inoculation, using a visual scale based upon the degree of symptom severity on the lettuce roots as proposed by O'Brien & Davis (1994) where: **1** = absence of symptoms, **2** = traces of necrosis in the root system, **3** = up to 50% of the root system with necrosis, **4** = more than 50% and less than 90% of necrotic root system, and **5** = more than 90% of the root system severely affected (**Figure 1**). The average grade reaction of each material was calculated, expressed by the arithmetic mean of the scores. This assessment was used to classify the lettuce accessions into three arbitrary categories of reaction namely: **resistant** (average severity scores between 1.0 and 2.0), **intermediate resistant** (scores between 2.01 and 4.0) and **susceptible** (scores between 4.01 and 5.0) (Sala *et al.*, 2003). A disease severity index (DSI) was calculated from the data of the average grade reaction of each cultivar according to McKinney (1923), where $DSI = [\sum (\text{reaction grade} \times \text{frequency}) / (\text{total number of units} \times \text{maximum scale grade})] \times 100$. After obtaining the DSI for each germplasm accession, the data were submitted to analysis of variance. The DSI was transformed into a square root of $x+1$ to normalize its distribution. The DSIs were compared and grouped using the Scott-Knott test ($P \leq 0.05$) using the SISVAR package (Ferreira, 2011).



Figure 1. Grading scale for quantifying the severity of the symptoms induced by *Berkeleyomyces* species in lettuce (*Lactuca sativa*) roots: **1** = absence of symptoms, **2** = traces of necrosis in the root system, **3** = up to 50% of the root system with necrosis, **4** = more than 50% and less than 90% of necrotic root system and **5** = more than 90% of the root system severely affected (O'Brien & Davis, 1994). Brasília, Embrapa Vegetables, 2021.

Bioassay #1: Initial screening of lettuce accessions to *B. basicola* isolate EH-2733

In order to simplify the screening process, the 68 *Lactuca* accessions were initially evaluated (August and September 2021) for reaction to only a single fungal isolate under greenhouse conditions (**Table 1**). The *B. basicola* EH-2733 isolate was chosen for this initial screening due to its aggressiveness to major commercial lettuce cultivars (data not shown). The experiment was carried out in a completely randomized design with 68 lettuce accessions (with and without inoculation) with three replications, each consisting of four seedlings. The

cultivars ‘Elisa’ (‘Butterhead’ morphotype) and ‘La Brillante’ (‘Batavian’ morphotype) were used as susceptible and resistant controls, respectively (Sala *et al.*, 2008).

Bioassay #2: Search for sources of resistance to four isolates of two *Berkeleyomyces* species in a subset of accessions identified with a resistant reaction in the bioassay #1

From the initial screening of bioassay #1 (Table 1), 33 of the most promising accessions within the resistant reaction category (grades 1.0–2.0) were selected and evaluated in a second bioassay. These accessions were separately inoculated with two *B. basicola* isolates (EH–2733 and EH–2740) and two *B. rouxiae* isolates (EH–2741 and EH–2743) (Souza, 2022). The experiment was carried out in a greenhouse in a completely randomized design with 33 accessions \times four isolates and three replications (with four plants each). Due to lack of seed availability, the cultivars ‘Romaine Balão’, ‘Penlake’, and ‘Blonde de Paris’, grouped in bioassay #1 as resistant (Table 1), were not reevaluated in bioassay #2. The accession ‘PI 342444’ was used as a resistant control (Sala *et al.*, 2008). The cultivars ‘Branca de Paris’, ‘Vanguard 75’, ‘Aurélia’, and ‘Elisa’ were used as susceptible controls.

Table 1. Reaction of 68 lettuce (*Lactuca sativa*) accessions to the *Berkeleyomyces basicola* isolate EH–2733 under greenhouse conditions. Brasília, Embrapa Vegetables, 2021.

Accessions	Morphotype	Disease reaction		Severity index (%) ³
		Grade ¹	Class ²	
BRS Mediterrânea	CGL	1.42	R	28.33 a ⁴
La Brillante	BAT	1.42	R	28.33 a
Flashy Trouts Back	ROM	1.50	R	30.00 a
Litte Gem PI 617959	ROM	1.50	R	30.00 a
BRS Lélia	CGL	1.56	R	31.66 a
Romana Balão	ROM	1.58	R	31.66 a
Crespa Itapuã Super	CGL	1.60	R	31.66 a
Argeles	CGL	1.75	R	35.00 a
Maravilha 4 Estações	BHD	1.75	R	35.00 a
Salinas 88	ICE	1.75	R	35.00 a
Salvius	ROM	1.75	R	35.00 a
Vitoria de Santo Antão	BHD	1.75	R	35.00 a
Prado Mimosa	MIM	1.82	R	36.66 a
Balesta	CGL	1.83	R	36.66 a
Betania	CPL	1.83	R	36.66 a
NUM DM 17	CGL	1.83	R	36.66 a
Veneranda	CGL	1.83	R	36.66 a
Crespa Repolhuda	CGL	1.92	R	38.33 a
Crespa Verão	CGL	1.92	R	38.33 a
Hanson	CGL	1.92	R	38.33 a
BRS Leila	CGL	1.92	R	38.33 a
Penlake PI 536753	ROM	1.92	R	38.33 a
Simpson	CGL	1.92	R	38.33 a
Valmaine PI 543959	ROM	1.92	R	38.33 a
Blonde de Paris	BAT	2.00	R	40.00 a
Crespa Verde	CGL	2.00	R	40.00 a
Joker	CPL	2.00	R	40.00 a
Salad Bowl	MIM	2.00	R	40.00 a
Regina de Verão	BHD	2.00	R	40.00 a
Rubi Crespa	CPL	2.00	R	40.00 a
UCO 7107	MIM	2.00	R	40.00 a
Bourguignonne	BHD	2.08	I	41.66 a
Fenke	BHD	2.08	I	41.66 a
Lednický PI 674756	ICE	2.08	I	41.66 a

Babá de Verão	BHD	2.17	I	43.33 a
UCO 7105	CGL	2.17	I	43.33 a
Ninja	CGL	2.25	I	45.00 a
Versaii	BHD	2.25	I	45.00 a
CGDM 16	ICE	2.33	I	46.67 a
Hilde	BHD	2.33	I	46.67 a
Pavane PI 667705	ICE	2.42	I	48.33 a
Tiffany	ICE	2.42	I	48.33 a
Sem Rival	BHD	2.58	I	51.67 b
Grand Rapids TBR	CGL	2.60	I	51.67 b
Iceberg	ICE	2.67	I	53.33 b
Belford	ICE	2.67	I	53.33 b
Capitan	BHD	2.75	I	55.00 b
Green Towers PI 601336	ROM	2.75	I	55.00 b
Vanda	CGL	2.83	I	56.67 b
Gallega de Invierno	BHD	2.90	I	58.33 b
Crocantela	CGL	3.00	I	60.00 b
Patriot	ROM	3.00	I	60.00 b
Samira	CGL	3.00	I	60.00 b
Grand Rapids	CGL	3.08	I	61.66 b
Great Lakes 659	ICE	3.08	I	61.66 b
Vanguard 75 PI 536852	ICE	3.08	I	61.66 b
Sabine	ICE	3.10	I	61.66 b
Ithaca PI 536844	ICE	3.17	I	63.33 b
UCO 2206	CGL	3.17	I	63.33 b
R4T57D	CGL	3.25	I	65.00 b
RYZ 2164	ICE	3.25	I	65.00 b
Cohban Green PI 612637	ICE	3.40	I	68.33 b
Hilde II	BHD	3.42	I	68.33 b
Aurélia	BHD	4.08	S	81.67 c
Dandie	ICE	4.08	S	81.67 c
Vanguard PI 536812	ICE	4.08	S	81.67 c
Branca de Paris	ROM	4.17	S	83.33 c
Elisa	BHD	4.25	S	85.00 c
CV				19.75

¹Mean disease reaction according to a rating scale ranging from 1 to 5. ²Disease reaction categories: Resistant (R), Intermediate (I), Susceptible (S) according to Sala et al. (2003). ³Disease severity, calculated by the McKinney index (1923), using the frequencies of disease classes considering a scale of grades from 1 to 5. ⁴Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test (P<0.05). **To obtain the letters and CV, the disease index was transformed by the square root of X+1. *Lettuce morphotypes: Crispy Green Loose-Leaf (CGL), Crispy Purple Loose-Leaf (CPL), Iceberg (ICE), Butterhead (BHD), Cos/Romaine (ROM) Mimosa (MIM) and Batavian (BAT).

RESULTS AND DISCUSSION

Among the 68 lettuce accessions evaluated in bioassay #1, 31 were classified as resistant, 32 as intermediate resistant, and five as susceptible to *B. basicola* EH-2733 (**Table 1**). All accessions belonging to the morphotypes ‘Mimosa’, ‘Batavian’, and ‘Crispy purple loose-leaf’ were resistant to *B. basicola* (**Figure 2**). Six out of the nine ‘Cos/Romaine’ accessions were classified as resistant. Thirteen out of the 22 accessions of the ‘Crispy green loose-leaf’ morphotype were classified as resistant and nine displayed intermediate reaction. In the ‘Butterhead’ morphotype, only three out of the 14 accessions were classified as resistant to *B. basicola*. From the ‘Iceberg’ morphotype, only one out of 15 accessions was classified as resistant, 12 displayed intermediate reactions and two were susceptible. Therefore, at least one accession with superior levels of resistance was detected within each morphotype.

The disease severity, calculated by the McKinney (1923) index, indicated in the bioassay #1 a wide range of responses (**Table 1**) varying from 28.33 (for the cultivars ‘BRS Mediterrânea’ and ‘La Brillante’) to 85.00 (for the cultivar ‘Elisa’). Our results are in overall agreement with previous screening assays that indicated a heterogeneous response to *B. basicola* isolates among the different lettuce morphotypes. Cultivars of the ‘Butterhead’ group have shown high levels of susceptibility to *B. basicola*. Conversely, most cultivars of the ‘Crispy green loose-leaf’, ‘Crispy purple loose-leaf’, and ‘Batavian’ morphotypes displayed resistant reaction, whereas the ‘Iceberg’ accessions showed heterogeneous reaction to *B. basicola* isolates (Sala *et al.*, 2008). Similarly, we also observed low frequency of resistant accessions within the ‘Butterhead’ and ‘Iceberg’ accessions, although few exceptions were detected. Similar susceptibility variation among morphotypes was also observed for *B. basicola* under field and greenhouse conditions in California (Koike, 2008).

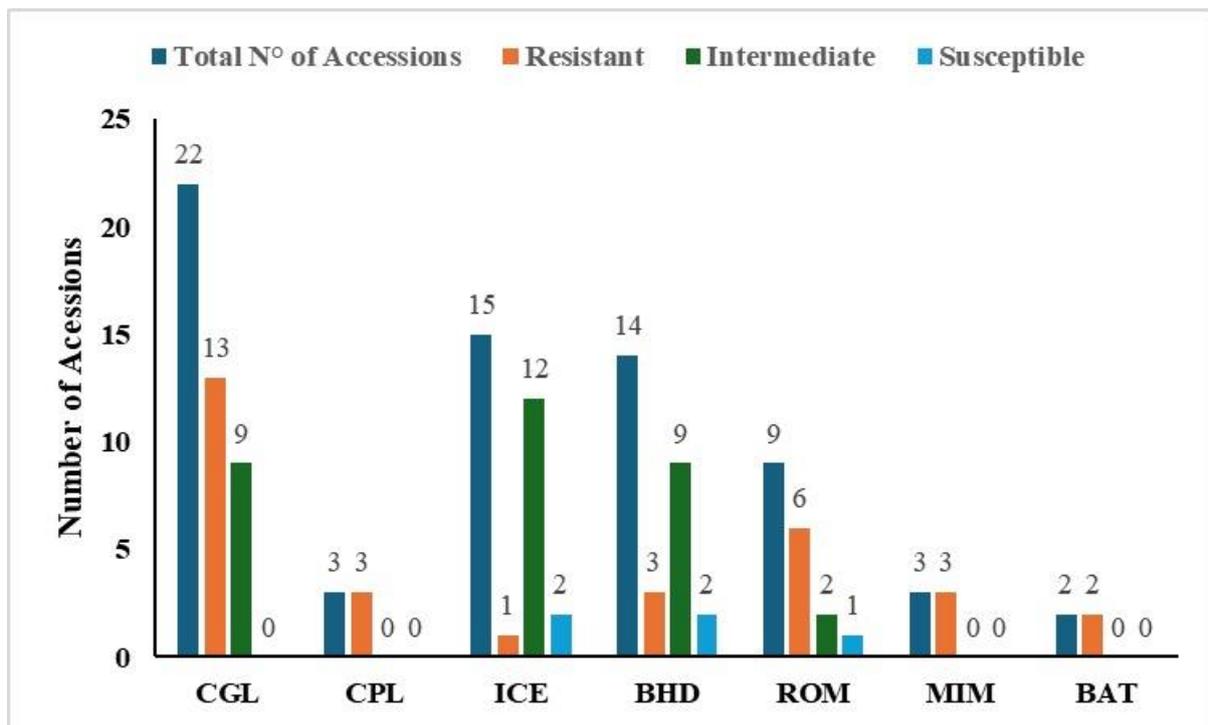


Figure 2. Reaction classes of 68 lettuce (*Lactuca sativa*) accessions from distinct morphotypes to the *Berkeleyomyces basicola* isolate EH-2733. *Lettuce morphotypes: Crispy Green Loose-Leaf (CGL), Crispy Purple Loose-Leaf (CPL), Iceberg (ICE), Butterhead (BHD), Cos/Romaine (ROM), Mimosa (MIM), and Batavian (BAT). Brasília, Embrapa Vegetables, 2021.

In the bioassay #2, significant differences in the DSI values were observed among the 33 accessions of the most promising accessions detected in the bioassay #1 in response to the two isolates of each *Berkeleyomyces* species. However, similar responses were observed for DSIs to *B. basicola* and *B. rouxiae*, even though some exceptions were detected. The subgroup of accessions, including ‘Little Gem’, ‘Maravilha 4 Estações’ (‘Butterhead’), ‘Salvius’ (‘Cos/Romaine’), ‘Argeles’ (‘Crispy green loose-leaf’) and ‘La Brillante’ (‘Batavian’) displayed a phenotypically stable resistance reaction against all four isolates of the two *Berkeleyomyces* species (**Table 2**). The identification of accessions with contrasting reactions for the two *Berkeleyomyces* species will allow the employment of them as parental lines in additional inheritance as well as in genetic mapping studies of the resistance factor(s) for both pathogens.

A low frequency of susceptible accessions to *B. basicola* and *B. rouxiae* isolates was observed in the morphotypes ‘Mimosa’, ‘Batavian’, and ‘Crispy green loose-leaf’. This observation is relevant from the breeding standpoint. ‘Crispy green loose-leaf’ is the most economically important morphotype under Brazilian conditions (Sala & Costa, 2012). The morphotypes ‘Cos/Romaine’, ‘Batavian’, and ‘Mimosa’ are increasing their market share in the country over the last few decades. However, the most impressive cultivation and consumption increase

was observed in the ‘Iceberg’ morphotype (Sala & Costa, 2012). In fact, the ‘Iceberg’ is the most challenging morphotype in terms of genetic improvement for resistance to *Berkeleyomyces* species since it displayed a low frequency of accessions with adequate levels of resistance. The cultivar ‘Salinas 88’ (‘Iceberg’) showed specific resistance against *B. basicola* isolates, confirming previous data of Sala *et al.* (2008). However, this accession did not show adequate levels of resistance to *B. rouxiae* isolates.

Table 2. Reaction of 33 lettuce (*Lactuca sativa*) accessions evaluated under greenhouse conditions against two isolates of *Berkeleyomyces basicola* (EH-2733 and EH-2740) and two *B. rouxiae* isolates (EH-2741 and EH-2743). Brasília, Embrapa Vegetables, 2021.

Accession	Morphotype*	Disease severity index/isolate ¹				Mean
		EH-2733	EH-2740	EH-2741	EH-2743	
Litte Gem (PI 617959)	ROM	30.00 Aa	20.00 Aa	30.00 Aa	30.00 Aa2	27.50
Maravilha 4 Estações	BHD	23.33 Aa	26.67 Aa	30.00 Aa	30.00 Aa	27.50
Salvius	ROM	33.33 Aa	26.67 Aa	23.33 Aa	26.67 Aa	27.50
Argeles	CGL	33.33 Aa	30.00 Aa	30.00 Aa	23.33 Aa	29.17
La Brillante	BAT	30.00 Aa	26.67 Aa	30.00 Aa	30.00 Aa	29.17
Balesta	CGL	20.00 Aa	26.67 Aa	30.00 Aa	43.33 Ab	30.00
Betânia	CPL	30.00 Aa	30.00 Aa	23.33 Aa	40.00 Ab	30.83
Branca de Paris	ROM	30.00 Aa	30.00 Aa	23.33 Aa	40.00 Ab	30.83
Joker	CPL	30.00 Aa	30.00 Aa	30.00 Aa	40.00 Ab	32.50
NUM DM 17	CGL	33.33 Aa	30.00 Aa	46.67 Ab	23.33 Aa	33.33
Vitoria de Santo Antônio	BHD	33.33 Aa	23.33 Aa	23.33 Aa	60.00 Ab	35.00
Crespa Repolhuda	CGL	33.33 Aa	26.67 Aa	23.33 Aa	66.67 Bc	37.50
Valmaine (PI 543959)	ROM	36.67 Aa	26.67 Aa	36.67 Ab	46.67 Ab	36.66
Flashy Trouts Back	ROM	36.67 Aa	46.67 Aa	26.67 Aa	43.33 Ab	38.33
Veneranda	CGL	40.00 Aa	30.00 Aa	46.67 Ab	33.33 Aa	37.50
Hanson	CGL	30.00 Aa	23.33 Aa	63.33 Bc	50.00 Bb	41.66
Rubi Crespa	CPL	53.33 Ab	43.33 Aa	33.33 Aa	33.33 Aa	40.83
BRS Mediterrânea	CGL	36.67 Aa	33.33 Aa	30.00 Aa	70.00 Bc	42.50
Salinas 88	ICE	30.00 Aa	33.33 Aa	66.67 Bc	40.00 Ab	42.50
Crespa Itapuã Super	CGL	56.67 Ab	40.00 Aa	43.33 Ab	46.67 Ab	46.67
PI 342444	BHD	40.00 Aa	30.00 Aa	70.00 Bc	50.00 Bb	47.50
Vanguard 75 (PI 536812)	ICE	70.00 Bc	43.33 Aa	60.00 Bc	26.67 Aa	50.00
Prado Mimosa	MIM	40.00 Aa	33.33 Aa	60.00 Bc	70.00 Bc	50.83
Salad Bowl	MIM	33.33 Aa	40.00 Aa	46.67 Ab	83.33 Bd	50.83
Crespa Verde	CGL	43.33 Aa	66.67 Bb	66.67 Bc	43.33 Ab	55.00
Simpson	CGL	40.00 Aa	50.00 Aa	53.33 Ac	100.00 Bd	60.83
BRS Lélia	CGL	43.33 Ba	23.33 Aa	93.33 Cd	90.00 Cd	62.50
Crespa Verão	CGL	40.00 Aa	33.33 Aa	80.00 Bd	100.00 Bd	63.33
UCO 7107	MIM	53.33 Bb	33.33 Aa	66.67 Bc	96.67 Cd	62.50
BRS Leila	CGL	63.33 Bb	70.00 Bb	83.33 Bd	46.67 Ab	65.83
Aurélia	BHD	76.66 Ac	56.67 Ab	70.00 Ac	83.33 Ad	71.67
Elisa	BHD	76.66 Ac	100.00 Ac	90.00 Ad	93.33 Ad	90.00
Regina de Verão	BHD	90.00 Ac	100.00 Ac	96.67 Ad	83.33 Ad	92.50
Mean	-----	42.12	38.89	49.29	54.04	
CV	-----			20.25		

¹Disease severity, calculated by the McKinney index (1923), using the frequencies of disease classes considering a scale of grades from 1 to 5. ²Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test (P<0.05). **To obtain the letters and CV, the disease index was transformed by the square root of X+1. *Lettuce morphotypes: ‘Crispy Green Loose-Leaf’ (CGL), ‘Crispy Purple Loose-Leaf’ (CPL), ‘Iceberg’ (ICE), ‘Butterhead’ (BHD), ‘Cos/Romaine’ (ROM) ‘Mimosa’ (MIM), and ‘Batavian’ (BAT).

A subgroup of accessions including ‘Hanson’, ‘BRS Lélia’, ‘Crespa Verão’, ‘PI 342444’, and ‘Prado Mimosa’

displayed a peculiar type of species-specific resistance to *B. basicola*. (**Table 2**). The accessions ‘Salinas 88’, ‘PI 342444’, and ‘BRS Mediterrânea’ showed a resistance reaction against isolates of *B. basicola* but displayed heterogeneous responses against the two isolates of *B. rouxiae*. The accession ‘Crespa Repolhuda’ exhibited an interesting pattern of heterogeneous response, showing superior levels of resistance to three isolates (two *B. basicola* and one *B. rouxiae*), but displayed a susceptible reaction to the *B. rouxiae* EH-2733 isolate.

Inheritance studies conducted by Sala *et al.* (2003) proposed a dominant monogenic model (called *Tb* gene/locus) controlling resistance to *T. basicola* in the accession ‘PI 342444’. Herein, accessions of ‘Cos/Romaine’ and ‘Batavian’ morphotypes and a large majority of accessions of ‘Crispy loose-leaf’ were resistant to all isolates of both fungi, indicating that the *Tb* locus might also control resistance to isolates of the species *B. rouxiae*. However, the original source of the *Tb* locus (‘PI 342444’) displayed good levels of resistance to *B. basicola* isolates (EH-2733 and EH-2740) but not to *B. rouxiae* isolates (EH-2741 and EH-2743) in the bioassay #2, indicating a species-specific reaction of this accession. Therefore, alternative hypotheses were proposed suggesting either the presence of two distinct genes (one controlling resistance to *B. rouxiae* and other to *B. basicola*) in close linkage within a putative cluster of resistance genes, which is a common feature in the lettuce genome (McHale *et al.*, 2009; Christopoulou *et al.*, 2015) or the presence of distinct resistant gene(s) in the accessions of ‘Cos/Romaine’, ‘Batavian’, and ‘Crispy loose-leaf’ morphotypes. In this context, allelic tests involving crossings of ‘PI 342444’ and these novel sources of large-spectrum resistance should be carried out to assess the genetic control of this trait.

No clear-cut host specificity was observed across the four isolates of the two fungal species in relation to lettuce accessions. Only a slight variability in the aggressiveness/virulence profile of the isolates was observed within the subgroups of accessions. For example, the accessions ‘Hanson’, ‘BRS Lélia’, ‘Crespa Verão’, ‘PI 342444’, and ‘Prado Mimosa’ displayed resistance to *B. basicola* isolates EH-2733 and EH-2740, but they were susceptible to both *B. rouxiae* isolates. In fact, differential pathogenicity patterns among isolates from both *Berkeleyomyces* species have been reported, suggesting the presence of putative fungal pathotypes and/or races in lettuce (Nakane *et al.*, 2019; Souza *et al.*, 2025). Differential resistance patterns among morphotypes have been observed in other pathosystems involving lettuce. For example, the cultivars of the morphotypes ‘Cos/Romaine’ and ‘Mimosa’ behave as the most resistant to *Fusarium oxysporum* f. sp. *lactucae* race 1, while most cultivars from the ‘Iceberg’ morphotype were susceptible (Cabral *et al.*, 2019).

One of the few sustainable and durable methods for controlling pathogens is pyramiding multiple disease resistance genes into a single cultivar (Mundt, 2018). Effective resistance factors against other lettuce pathogens are also present in the five accessions that exhibited the highest levels of multi-resistance against isolates of both *Berkeleyomyces* species (*viz.* ‘Litte Gem’, ‘Maravilha 4 Estações’, ‘Salvius’, ‘Argeles’, and ‘La Brillante’). The cultivar ‘Little Gem’ is a source of resistance to *Xanthomonas campestris* pv. *vitians* (Bull *et al.*, 2007), whereas ‘Argeles’ was identified as the best source of resistance against all Brazilian isolates of *Bremia lactucae* (Franco *et al.*, 2021). The cultivar ‘La Brillante’ is another accession of interest from the breeding standpoint, showing high levels of resistance against all *Berkeleyomyces* isolates as well as to *B. lactucae*, *Verticillium dahliae*, *Orthotospovirus tomatomaculae*, *Orthotospovirus impatiens necromaculae*, and *X. campestris* pv. *vitians* (Sala *et al.*, 2008; Hayes *et al.*, 2011, 2014; Simko *et al.*, 2015; 2018; Fontes *et al.*, 2019). In turn, the cultivar ‘Salvius’ displayed high levels of tolerance for heat-associated physiological disorders, including tipburn and premature bolting (Holmes *et al.*, 2019). Thus, the use of this subgroup of accessions as potential sources of useful traits would be a judicious strategy for breeding programs aiming at the development of multi-resistant cultivars with adaptation to warm climates.

Dynamic plant-fungal interactions have been investigated in different pathosystems involving *Berkeleyomyces* species and dicotyledonous hosts (Mauk & Hine, 1988; Hood & Shew, 1997; Mims *et al.*, 2000). A subset of resistant tobacco and *Viola* accessions reacted to *Berkeleyomyces* isolates by exhibiting papillae and callose formation in sites of fungal invasion in epidermal cells (Hood & Shew, 1997; Mims *et al.*, 2000). This phenotypic response suggests the potential involvement of NB-LRR-like resistance genes to *Berkeleyomyces* (Wang *et al.*, 2021). However, in the lettuce *Berkeleyomyces* pathosystem, the genetic factors as well as the biochemical and cytological mechanisms have not yet been fully characterized. In the lettuce genome, numerous genes potentially involved in resistance responses have already been characterized, including factors encoding NB-LRR-like proteins (McHale *et al.*, 2009; Christopoulou *et al.*, 2015). The identification of sources with high levels of resistance for both *B. basicola* and *B. rouxiae* in accessions of different morphotypes opens the breeding

opportunity to incorporate these genetic factors in a wide range of commercial lettuce cultivars, being crucial for the development of lettuce cultivars with stable and durable resistance against *Berkeleyomyces* species.

AUTHORS' CONTRIBUTIONS

All authors contributed to the design and overall organization of the study. DNP, LSB, and AR developed the idea and wrote the manuscript in equal parts. DNP, CSC, TBT, MENF, RLS and CSC help with the lab assays, greenhouse works and inoculation procedures. All authors read, commented and approved the final version of the manuscript.

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Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

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DATA AVAILABILITY

Data will be made available upon request to the corresponding author.

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