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Resistance to Bacterial Wilt in *Solanum commersonii* Dun.

Resistance to Bacterial Wilt in *Solanum commersonii* Dun.

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Abstract

The levels of resistance to bacterial wilt (BW) of potatoes caused by *Ralstonia solanacearum* (Rs) are limited. *Solanum commersonii* (cmm) is a wild tuber-bearing potato highlighted for its resistance to BW. This research aimed to (i) characterize cmm genotypes for resistance to Rs (race 3, biovar 2) using a soil inoculation method, (ii) determine to what extent resistance in cmm is transmissible through sexual reproduction to a susceptible genetic background, and (iii) determine the relationships between a set of Rs strains (race 3, biovar 2) and cmm genotypes in the expression of resistance. The screening was performed under controlled conditions of temperature and light. Accessions collected from different regions of Uruguay showed diversity for resistance to BW: some genotypes were asymptomatic in the response, while for others the symptoms were similar to the susceptible control. Two cmm genotypes with contrasting responses to BW were crossed, and an offspring of 121 genotypes was obtained. The distribution of BW resistance levels in the progeny suggested a polygenetic control for BW resistance; though this conclusion is preliminary regarding that one of the cmm parents was triploid. A factorial experiment using five *R. solanacearum* strains isolated in Uruguay and five cmm clones showed differences in the virulence between strains. There was no interaction between plant genotype and bacterial isolate, and therefore, under the conditions of this research, BW resistance in cmm was not dependent on the isolate of the pathogen.

Keywords: inoculation, bacterial wilt, Ralstonia solanacearum, Solanum tuberosum, disease resistance

Resumen

Los niveles de resistencia a la marchitez bacteriana (MB) de la papa causada por *Ralstonia solanacearum* (Rs) son limitados. *Solanum commersonii* (cmm) es una especie silvestre destacada por su resistencia a MB. Los objetivos de este trabajo fueron (i) caracterizar genotipos de cmm para resistencia a Rs (raza 3, biovar 2) utilizando un método de inoculación en suelo, (ii) determinar en qué medida la resistencia de cmm es transmisible por vía sexual en un trasfondo genético susceptible, y (iii) determinar la relación entre un grupo de cepas de Rs raza 3 biovar 2 y genotipos de cmm en la expresión de la resistencia. Se utilizó una metodología de inoculación en suelo bajo condiciones de temperatura y luz controladas. Para genotipos de cmm colectados en distintas regiones de Uruguay, se encontró variabilidad en la resistencia a MB, desde respuesta asintomática en algunos genotipos hasta síntomas en nivel similar al control susceptible. Mediante el cruzamiento de dos genotipos de cmm con respuestas contrastantes a MB se obtuvo una progenie de 121 genotipos. La distribución de los niveles de resistencia en la progenie indicaría un control poligénico, aunque esta conclusión es

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preliminar, ya que se encontró que uno de los parentales era triploide. Un ensayo factorial utilizando cinco cepas de Rs aisladas en Uruguay y cinco genotipos de cmm, reveló diferencias en la virulencia entre cepas. No se observó interacción entre genotipo y cepa, por lo que en las condiciones de este trabajo la resistencia no fue dependiente del aislamiento del patógeno.

Palabras clave: inoculación, marchitez bacteriana, Ralstonia solanacearum, Solanum commersonii, resistencia a enfermedades

Introduction

Genetic resistance is a key factor when integrating a control strategy for Bacterial Wilt (BW) in potatoes caused by the bacterium *Ralstonia solanacearum* (Rs). However, the generation and use of potato germplasm with stable levels of resistance to wilting, has been very limited (Priou and others, 2005; Lopes, 2009).

Solanum commersonii Dun. (cmm) (Hawkes, 1994) is a diploid species noted for its resistance to BW (Laferriere and others, 1999; Kim-Lee and others, 2004; Galván and others, 2006; Carputo and others, 2009; Siri and others, 2009). The use of cmm in the genetic improvement of potatoes has been scarce, among other factors, due to barriers to post-scigotic hybridization with *Solanum tuberosum* that involve endosperm balance (Johnston and others, 1980). However, successful introgression experiences have been reported using different mechanisms to evade the barriers of incompatibility (Laferriere and others, 1999; Carputo and others, 1997; González and others, 2010) that allow the use of cmm in potato improvement.

Laferriere and others (1999) and Carputo and others (2009) found resistance in cmm for Rs race 3 biovar 2 (R3bv2). In each of these cases, a cmm genotype and a single Rs strain were used. Kim-Lee and others (2004) evaluated four different genotypes of the same cmm accession (PI320266) for different strains of races 1 and 3. For the first time, they concluded the variability within cmm for the resistance to Rs, and the need to have characterized germplasm for genetic improvement. The first comprehensive characterization studies of cmm germplasm for resistance to R3bv2 were reported by Galvan and others (2006) and Siri and others (2009), with an inoculation method based on injuries in the aerial part of the plant that does not represent the natural infection process. Indeed, with this inoculation, some active defense mechanisms of the plant could be evaded, since the bacterium is a soil pathogen that enters the plant through root injuries.

Using a method of Rs inoculation, under controlled conditions, representative of the natural infection process in roots would be very useful to characterize cmm germplasm in pre-improvement programs for resistance to BW.

The effective use of BW resistance is limited due to the lack of knowledge of its genetic basis. This knowledge would be useful to define efficient crossing strategies in the incorporation of resistance in susceptible germplasm. *Solanum phureja* is probably the most studied source of resistance. Research carried out with segregating populations inoculated with a strain of race 1, suggested the action of three dominant and independent genes with a possible effect of other modifying genes (Rowe and Sequeira, 1970). Subsequently, strain-specific resistance was found for race 1 and race 3 in *S. phureja*, and also, loss of resistance effectiveness associated with high temperatures (French and de Lindo, 1982; Tung and others, 1990a). These studies pointed to the strong variability in the resistant phenotype as a difficulty in the analysis of data, especially by temperature and light.

Subsequently, some studies incorporated resistance provided by the clone "Cruza 148", of Mexican origin and unknown pedigree, and by "AVRDC-1287.19", of Taiwanese origin and product of the crossing between *S. chacoense and S. raphanifolium* (Schmiediche, 1988). Partial resistance was determined for a strain of race 1, very closely related to environmental adaptation to elevated temperatures, which would determine a resistance of complex and polygenic heritability (Tung and others, 1990b). In further studies, the same authors detected significant plant-pathogen-environment interaction. Changes in the aggressiveness of the used strains, associated with temperature changes, were the main source of instability in these experiments (Tung and others, 1990a).

The R3bv2 of *R. solanacearum* has very little genetic variability (Fegan and Prior, 2005), a result confirmed by Siri and others (2011) for Uruguayan strains. However, Uruguayan strains were distinguished in their virulence on tomato, potato and three cmm genotypes (Siri and others, 2011). Determining differences in virulence between strains, or strain-specific resistance expression, would guide choosing the appropriate strain for the evaluation of cmm genotypes for BW resistance. The

analysis of the interaction strain x host, in addition, would generate clues on the genetic basis of resistance.

This study aimed to (i) characterize different cmm genotypes for pathogen R3bv2 resistance using an inoculation method in soil under controlled environmental conditions, (ii) determine to what extent cmm resistance is sexually transmissible in a susceptible genetic background, and (iii) determine the relationship between five strains of Rs R3bv2 and five cmm genotypes in the expression of BW resistance.

Material and methods

Plant material

Three independent trials were conducted: (i) evaluation of a collection of cmm genotypes, (ii) evaluation of an F1 progeny of cmm, and (iii) evaluation of the interaction between cmm genotypes and Rs strains.

For the characterization of resistance to BW, 32 clones from different regions of Uruguay were evaluated. Two clones of *S. phureja* (phu) (192 and 195) were also included, from the potato genetic improvement project of INIA Uruguay (not previously evaluated) and the cultivar *S. tuberosum* (tbr) 'Chieftain' (United States) as a susceptible control. After the first characterization of the resistance, the clones of cmm 05.02.6 and 04.204.3 were selected as parents of the progeny given the resistance and susceptibility they showed, respectively, within the same pattern of morphological characteristics. The clones of cmm F97, F102, F118, 05.02.6 and 04.204.3 and the 'Chieftain' cultivar were selected for their different levels of resistance in the characterization of the progeny to perform the interaction test between genotype and strain. All the material for the evaluation was obtained from the *in vitro* active genebank of the National Institute of Agricultural Research (INIA by its Spanish acronym).

Crossing

The genotypes of cmm 05.02.6 and 04.203.4 selected for crossbreeding were grown in greenhouse with controlled temperature (25 ºC) and photoperiod (14 hours of light) conditions. Pollen of genotype 04.204.3 used as the male parent was collected to pollinate emasculated flowers of plants used as females (genotype 05.02.6). Berries were harvested 50 days after pollination. The extracted seeds were treated by immersion in a solution of 1500 ppm of gibberellic acid for 24 hours in order to lift the dormancy. Then, they were sown *in vitro* in MS medium (Murashige and Skoog, 1962) in order to introduce and preserve the progeny for evaluation and use in breeding. One hundred forty-five individuals of the progeny were obtained (designated with the letter F).

Evaluation of resistance to bacterial wilt

The strain used in the characterization of Cmm accessions was UY036. Due to the availability at the time of the experiment, the strain used for the progeny characterization of the genotypes cmm 05.02.6 and 04.203.4 was UY043. In the interaction experiment between host genotypes and strains, the strains UY031, UY035, UY036, UY041 and UY043 were used. All strains were collected from infected commercial potato crops in Uruguay and belong to the R3bv2A of the pathogen (Phylotype II, Sequevar 1- 2) (Siri and others, 2011). The strains were preserved at 70 ºC in the Chemistry College, Universidad de la República.

A soil inoculation methodology was used to differentiate degrees of resistance to BW in seedlings from *in vitro* multiplication, based on Montanelli and others (1995). The inoculum was prepared as a suspension of bacteria in physiological serum (Thurston and Lozano, 1968), at a concentration of 1 x 108 cfu·mL-1. It started from colonies incubated in petri dishes for 48 hours at 28 °C in Kelman growth medium containing 2,3,5-triphenyl tetrazolium chlorate (TTC) (Kelman, 1954). The inoculum concentration was estimated by 0.1 absorbance in spectrophotometer (OD 550 nm), and verified by the sowing of dilutions at 1 x 105 and 1 x 106 in TTC medium, which obtained counts of 100 to 300 cfu for the dilution of 1 x 105, and 0 to 100 cfu for the dilution of 1 x 106.

For the resistance evaluations, seedlings with five to eight expanded leaves, derived from the *in vitro* micropropagation, were used in order to ensure uniformity and to obtain replicas (clones) of each genotype. For this, mononodal cuttings were grown in MS medium for three weeks, followed by two weeks of greenhouse acclimatization in the inoculation container. The container consisted of individual cells of 17 cm3 for each seedling, with 4 g of commercial horticultural substrate free of pathogens. In the characterization trial of a collection of cmm, 12 repetitions (seedlings) were used for each genotype, while in the other two trials, 26 repetitions per genotype were used, except for some individuals of the progeny who had lower *in vitro* multiplication rate. In all experiments, seedlings were inoculated with physiological serum without bacteria as a negative control.

For inoculation, a hole was made in the substrate with a plastic tip of micropipette to generate wounds at the root level. The hole about 1 cm deep, was made at 0.5 cm from the seedling. Next, 1 ml of the bacterial suspension was applied to each hole using a pipette. After inoculation, plants were kept in a chamber with artificial light (12 hours of light, 78 ìmol·m-2·s-1) and 28 °C at the root level. The location of the plants/clones of each genotype was not randomized within the chamber under the assumption of environmental homogeneity conditions. They were irrigated daily to keep the substrate moist, without causing excess water. Symptom assessment was done 28 days after inoculation (dai). Each of the plants of each genotype was assigned a value of "0" corresponding to the absence of symptoms, or "1" to the presence of wilt.

Data analysis

To determine the degree of resistance of each genotype, the proportion of wilted plants was calculated over the total number of inoculated plants. Then a category of reaction to the disease was assigned, according to the following arbitrary criterion: R=resistant (0.00-0.25), MR=moderately resistant (0.26-0.50), MS=moderately susceptible (0.51-0.75) and S=susceptible (0.76-1.00).

The resistance values of the progeny genotypes were compared using a Generalized Linear Model, assuming binomial distribution of the variable P (proportion of diseased plants of each genotype) (McCullagh and Nelder, 1989).

Only treatments (genotypes) with 20 to 26 inoculated plants entered the analysis, totaling 124 genotypes (121 genotypes of the progeny, the two parents and the susceptible control 'Chieftain'). Thirteen asymptomatic genotypes for all their replications (completely resistant) were analyzed outside this method since the variance estimation for the variable P was equal to 0. The confidence interval (95% confidence) was calculated for comparisons, based on the estimated proportion of diseased plants. Additionally, to visualize the differences between genotypes of the progeny, a cluster analysis was performed. The distance matrix was elaborated using the differences between the estimated proportions of diseased plants of each genotype. Ward's grouping method was used and the pseudo F and pseudo t^2 indexes were used as a cut point to determine the number of groups.

The interaction between host genotypes and Rs strains was evaluated by a completely randomized experimental design with a factorial arrangement of treatments (6 x 5), inoculating 26 plants for each of the 30 combinations between genotypes and strains. Each treatment was inoculated and analyzed in the same way as in the experiment to evaluate resistance in the progeny. The effects of the sources of variation (genotype, strain, genotype x strain) were analyzed using a Generalized Linear Model and assuming binomial distribution of the variable P (proportion of diseased plants of each genotype). The significance of the effects was tested by an F-test that originates from the quotient of two Chi-squares that come from model deviance and residual deviance.

Results

Evaluation of *S. commersonii* genotypes collected in Uruguay

The evaluation of BW resistance levels in 32 cmm genotypes using the UY036 strain of Rs showed wide variability (Table 1). Of the genotypes evaluated, 25% were R (P between 0.00-0.25), 19% MR (P between 0.26-0.50), 50% MS (P between 0.51-0.75) and 6% S (P between 0.75-1.00). The susceptible control cv. 'Chieftain' showed a proportion of diseased plants of 0.83. Genotypes 192 and 195 of *S. phureja*, presented intermediate and susceptible behavior, respectively. Cmm genotypes 05.02.6 (R) and 04.203.4 (MS) were selected for crossbreeding, for their contrasting resistance levels.

Obtaining a segregating cmm progeny for resistance to BW

Genotype 05.02.6 was used as a female given its low percentage of fertile pollen (11%) estimated by a staining technique. In addition, a low number of seeds was obtained per berry (5.64) compared to other crossbreeding between genotypes of this species. This indicates low fertility as a female of genotype 05.02.6 or some incompatibility condition between both parents used.

Due to the low efficiency of the crossing, the ploidy of the progenitors was studied by flow cytometry. The C content of genomic DNA was compared with a known diploid of cmm (genotype 04.02.3).

It confirmed that genotype 05.02.6 has 50% more genomes than the diploid control, and genotype 04.203.4 has the same as the diploid control. Therefore, based on flow cytometry, it was postulated that the female parent 05.02.6 would be triploid and the male parent 04.203.4 would be diploid (F. Santiñaque, pers. comm.).

Table 1. Proportion of plants with wilt at 28 days after inoculation with the UY036 strain of R3bv2 of *Ralstonia solaneacerum* in 32 accessions of *Solanum commersonii* collected in Uruguay. Two genotypes of *S. phureja* (192 and 195) are included. A genotype of *S. tuberosum* ssp. *tuberosum cv.* 'Chieftain' was included as a susceptible control.

Individual	Origin	p 28 dai [1]	RD [2]	Individual	Origin	p 28 dai [1]	RD [2]
04.09.T	Río Negro	0	R	6012	Canelones	0.58	MS
05.02.6	Canelones	0	R	04.203.4	INIA [3]	0.63	MS
04204.3	INIA [3]	0.11	R	10093	Salto	0.67	MS
09.02.3	Paysandú	0.11	R	10167	Paysandú	0.67	MS
20176	Unknown	0.17	R	50524	Canelones	0.67	MS
09.02.11	Paysandú	0.25	R	505212	Canelones	0.67	MS
505218	Canelones	0.25	R	5016	Canelones	0.75	MS
01.02.TA	Soriano	0.25	R	50526	Canelones	0.75	MS
10.01.18	Paysandú	0.33	MR	10177	Salto	0.75	MS
10085	Artigas	0.33	MR	10176	Salto	0.75	MS
50525	Canelones	0.42	MR	9028	Paysandú	0.75	MS
9027	Paysandú	0.42	MR	0701A10	Maldonado	0.75	MS
3037	Canelones	0.42	MR	4023	Colonia	0.75	MS
505216	Canelones	0.5	MR	Chieftain	USA	0.83	S
192	INIA [3]	0.5	MR	10073	Artigas	0.83	S
50527	Canelones	0.58	MS	10086	Artigas	0.83	S
11013	Canelones	0.58	MS	195	INIA [3]	0.85	S
9024	Paysandú	0.58	MS				

1Proportion of diseased plants at 28 days after inoculation. 2Response to disease: R=resistant (0.00-0.25), MR=moderately resistant (0.26-0.50), MS=moderately susceptible (0.51-0.75) and S=susceptible (0.76-1). 3Clones from the genetic improvement program of INIA, Uruguay.

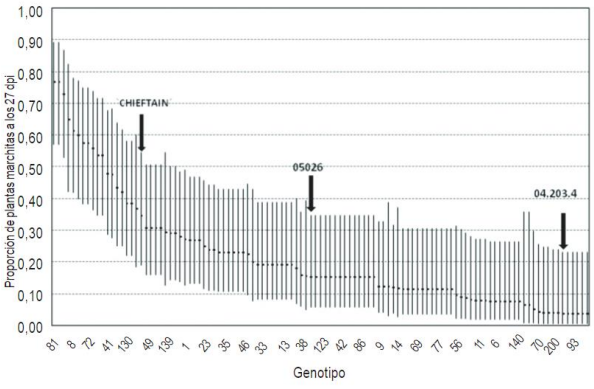
Distribution of resistance levels in progeny

Resistance to BW (proportion of plants with wilting) showed a continuous behavior in the progeny F1 (05.02.6 x 04.203.4) with an average P value of 0.19 and a range from P = 0.00 (asymptomatic plants) to P = 0.77 (high susceptibility).

Comparing confidence intervals assigned to the estimated means of each genotype (excluding 13 asymptomatic genotypes that therefore had zero variance) showed that only the most resistant and the most susceptible genotypes differed significantly (Figure 1).

No significant differences were found between the majority of the genotypes of the F1 progeny, the progeny and the susceptible control 'Chieftain'. Consequently, this experiment, that used the UY043 strain, did not confirm the contrast originally observed between the parents of the progeny when they were inoculated with the UY036 strain. 76% of the genotypes (92 out of 121) had an estimated proportion of diseased plants of less than 0.25. Only 9% of the progeny (11 genotypes out of 121) had a proportion of diseased plants of more than 0.50. The susceptible control 'Chieftain' presented a low number of diseased plants (P=0.35) compared to the previous trial (P=0.83) when the UY036 strain had been used.

Figure 1. Proportion of wilted plants (P) and confidence interval for each genotype of the F1 progeny of *Solanum commersonii* (05.02.6 x 04.203.4) 28 days after inoculation with *Ralstonia solanacearum,* R3bv2, strain UY043. The arrows mark the position of the parents and the susceptible control *S. tuberosum* ssp. *tuberosum* cv. 'Chieftain'. The horizontal axis has only a few identification numbers.



To obtain groupings of the genotypes according to the similarity of resistance levels, a cluster analysis was carried out using, as distance measurement, the differences between the estimated proportions

of diseased plants of each genotype that participated in the analysis (Figure 2). The cluster analysis coincided with the generalized linear model in the formation of three groups of genotypes (Table 2).

"Group 1" consists of the 11 individuals with the highest level of susceptibility. The susceptible control 'Chieftain' was part of the "group 2" that includes 30 of the 111 genotypes analyzed. Finally, the two parental genotypes of the family integrated the "group 3" together with the 70 F genotypes with the highest level of resistance within the included in the analysis.

The cmm genotypes chosen for this experiment represented different resistance levels within the inoculation of progeny with the UY043 strain: 05.02.6 (P=0.154), 04.203.4 (P=0.040), F97 (P=0.769), F102 (P=0.080) and F118 (P=0.000). The genotype and strain effects were significant, while the genotype x strain interaction was not significant (Table 3). Therefore, the resistance or susceptibility response was not affected by the strain used. The 'Chieftain' cultivar was the most susceptible for all strains (Table 4). The F102 genotype and the parental genotypes 05.02.6 and 04.203.4 had an intermediate response, not discriminating between them by any of the strains. The UY043 strain was the only one that obtained fewer plant deaths (lower virulence) on average for all genotypes (Table 4). This strain, which was used to characterize the progeny between genotypes 05.02.6 and 04.203.4, is the only one that could not discriminate between the most resistant genotypes and those with intermediate resistance (for example, genotypes F118 and F102).

Figure 2. Analysis of clusters by Ward method, using, as a distance measure, the differences between the estimated proportions of diseased plants of each of the genotypes of *Solanum commersonii* that participated in the statistical analysis. The groups formed by susceptible genotypes (S), moderately susceptible (MS), moderately resistant and resistant (MR/R) are indicated. The two parents of the progeny (05.02.6 and 04.203.4) and the susceptible control cv. 'Chieftain' (*S. tuberosum* ssp. *tuberosum*), are highlighted in bold. The genotypes F97 and F102 selected for the interaction test with strains of *Ralstonia solanacearum* are indicated by an asterisk

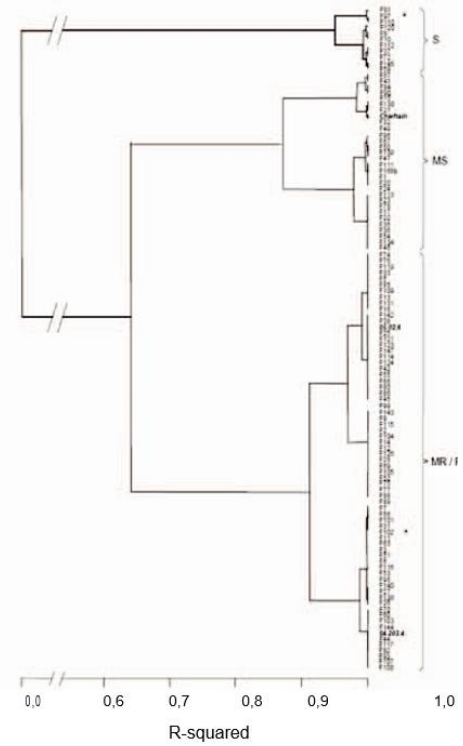


Table 2. Characterization of each group formed in the cluster analysis from inoculation with *Ralstonia solanacearum* of a family F1 of *S. commersonii* from the crossing between the parents 05.02.6 and 04203.4.

Groups	Number of genotypes	Proportion of diseased plants		
		Minimum	Maximum	Mean
1	11	0.54	0.77	0.63
2	30	0.23	0.48	0.30
3	70	0.04	0.20	0.11

Table 3. Effect of different sources of variation for the genotype interaction experiment by strain.

Source of variation	Degrees of Freedom Freedom	Value F [1]	Pr > F [2]
Genotype	5	27.08	<0.0001
Strain	4	9.09	<0.0001
Genotype*strain	20	1.03	0.4586

[1]Value F obtained from the quotient of two chi-squares that come from the deviances. [2]Probability of type 1 error for the value of F found.

Table 4. Proportion of diseased plants at 28 days after inoculation with *Ralstonia solanacearum* for the different genotypes chosen from *Solanum commersonii*, the susceptible control 'Chieftain' of *S. tuberosum* and the different R3bv2 strains used.

	Strain					Mean effect of
Genotype	UY031	UY035	UY036	UY041	UY043	genotype*
'Chieftain'	0.96	0.92	0.85	0.92	0.56	0.88 a
F97	0.88	0.80	0.54	0.58	0.58	0.70 b
F102	0.46	0.31	0.46	0.38	0.08	0.31 c
04203.4	0.42	0.42	0.31	0.38	0.12	0,31 c
05.02.6	0.50	0.38	0.35	0.35	0.04	0,27 cd
F118	0.21	0.08	0.13	0.04	0.08	0.09 d
Mean effect of the strain*	0.64 a	0.50 a	0.43 a	0.42 a	0.17 b	

* The means followed by the same letter do not differ significantly (α= 0.05).

In this study, the cultivar 'Cruza 148' and *S. phureja* 'BR-63.65' (cv. 'Molinera'), known for their good field resistance, presented the best behavior but still presented plants with synonyms.

Our results confirm the high resistance levels found for the species by Laferriere and others (1999), Kim-Lee and others (2004), Galván and others (2006), Carputo and others (2009) and Siri and others (2009). It is unknown whether the most resistant materials could have latent bacteria infections in asymptomatic tubers, an aspect that should be the subject of further studies. On the other hand, the variability found confirms the need to carry out sieving within the species before its use in breeding.

The presence of triploid cytotypes of cmm was described by Correll (1962) and by Tam and Hawkes (1986). The use of a triploid as a parent (05.06.2) hinders the analysis of the distribution of resistance levels in the progeny since the production of gametes is different from the expected for a diploid (Ramsey and Schemske, 1998). In turn, we do not know which of these gametes will provide the maternal complement necessary to generate a viable endosperm when combined with male gametes (Johnston and others, 1980). The production of 2n gametes by the male parent is another additional complexity. All these conditions limit the analysis of the distribution of resistance levels in the progeny since it is impossible to predict how many copies of alleles each individual received from the male or female parent.

On the other hand, the strain used to analyze the resistance levels of the F1 progeny was barely aggressive, which is evidenced in the low proportion of diseased plants of the susceptible control 'Chieftain' (0.35). The low aggressiveness of the UY043 strain, confirmed in the experiment of interaction between genotype and strain (Table 4), resulted in a low capacity to discriminate between individuals with high and intermediate levels of resistance. This low capacity to discriminate occurred due to both the confidence intervals from the generalized linear model, which could only differentiate the most resistant and most susceptible ends of the distribution, as well as the cluster analysis that grouped 70 of 122 genotypes as resistant. The use of a more virulent strain could determine significant differences between genotypes of narrower resistance strata.

The fact that, under the conditions of this study, individuals from the crossing between two individuals with partial resistance emerged completely susceptible, could have some of the following explanations: (i) that these individuals received fewer or more copies of some *locus/loci,* given the unbalanced segregation of the triploid and/or the production of male gametes 2n; (ii) some *locus/loci* are recessive from parents in heterozygosis (additive effects, dominance or overdominance); and (iii) that there are complex genetic effects resulting from specific interactions between the crossing of the selected genotypes. These three points would also explain the appearance of individuals more resistant than the two countries (not statistically proven in this study), and consequently, the tendency to the observed transgressive segregation.

A continuous distribution of resistance phenotype frequencies, such as the one obtained, is usually explained by several genes. A polygenic control for BW resistance in different wild *Solanum* diploids was found by Tung and others (1990b) using strains of races 1 and 3. The absence of co-evolution between the pathogen and the host would explain the

polygenic control, and the consequent absence of selection pressure for genes with specific resistance effects (Tung and others, 1990a). This could also be the situation for cmm and Rs. In other species such as tomatoes, polygenic control of BW resistance is also the most common form of inheritance (Yang and Francis, 2007). For these cases and from the point of view of genetic improvement, the accumulation of genes with a positive effect on the same individual is a key strategy. This process of accumulation or pyramidation can be used to accumulate resistance genes from the same source (for example, cmm), and to combine resistance from different species. The combination of various sources of resistance would contribute to the effectiveness and stability of the characteristic, for example in the face of changes in the environment.

This study did not detect any strain-specific resistance between the used strains of R3bv2 and the genotypes of cmm. Analysis of mean resistance levels by strain detected significant differences in virulence. A group of the four most aggressive strains distinguished itself from the less aggressive UY043 strain, in accordance with Siri and others (2011). Different levels of virulence in R3bv2 had been previously reported (French and de Lindo, 1982; Watanabe and others, 1992). The conformation of only two groups could be due to the limited number of strains analyzed in this study, as well as to the scarce genetic diversity found in general in the R3bv2 of Rs (Fegan and Prior, 2005) and in particular in the Uruguayan strains (Siri and others, 2011).

Genetic differences between the Rs strains evaluated would explain the differences in their pathogenic ability and virulence, but these differences did not determine interactions with the cmm genotypes. Except for the limited number of host genotypes evaluated, which prevents generalization for the species, the results indicate that the type of resistance to BW in cmm would not be strain-specific. This result differs from the interaction between strains of R3bv2 and genotypes of *S. phureja* found by French and de Lindo (1982). The strains used in that case, coming from distant geographical origins, probably had greater variability than those that participated in our trial.

In turn, the strain-specific resistance found in *S. phureja* corresponds to the opportunity for long-term co-evolution between the pathogen and the host, which may also explain the small number of genes postulated for that resistance (Rowe and Sequeira, 1970).

The first trial with the UY036 strain differentiated the level of resistance of the parents from the progeny, which was not confirmed by the following experiments. By ruling out changes in the order of resistance due to strain-host interaction effects, the differences in results would be explained by environmental variations that modify genotype responses with certain strains. Several studies have marked this difficulty when it comes to sieving under controlled conditions (Rowe and Sequeira, 1970; Tung and others, 1990a, 1990b.) Despite working under controlled experimental conditions, small temperature changes could cause changes in the aggressiveness of the strains or the level of resistance expression. This effect may have become important due to the lack of genotype randomization in the experiment. Therefore, the experiment replications and the block designs, even if variations in the uniformity of the environment are minimal, should be incorporated into the methodology. The use of virulent strains that manage to discriminate the different degrees of resistance better, and the use of the proportion of plants with symptoms of susceptible control cv. 'Chieftain' to evaluate the virulence of the strain and/or the adjustment of other conditions of the experiment are other elements to incorporate in the breeding methodology for BW resistance.

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