

Plant Pathol. J. 38(3) : 239-247 (2022) https://doi.org/10.5423/PPJ.OA.01.2022.0008 eISSN 2093-9280

Research Article Open Access

Field Performance of Resistant Potato Genotypes Transformed with the EFR Receptor from *Arabidopsis thaliana* in the Absence of Bacterial Wilt (*Ralstonia solanacearum*)

Marco Dalla-Rizza ^(D)^{1†*}, Claudia Schvartzman^{1†}, Sara Murchio¹, Cecilia Berrueta², Federico Boschi³, Mariana Menoni³, Alberto Lenzi², and Gustavo Gimenez²

¹Unidad de Biotecnología, Instituto Nacional de Investigación Agropecuaria, Canelones, CP 90100, Uruguay

²Programa de Producción Hortícola, Instituto Nacional de Investigación Agropecuaria, Canelones, CP 90100, Uruguay

³Instituto Nacional de Semillas, Canelones, CP 90100, Uruguay

(Received on January 20, 2022; Revised on May 9, 2022; Accepted on May 10, 2022)

Bacterial wilt caused by the pathogen Ralstonia solanacearum is a devastating disease of potato crops. Harmonizing immunity to pathogens and crop yield is a balance between productive, economic, and environmental interests. In this work, the agronomic performance of two events of potato cultivar INIA Iporá expressing the Arabidopsis thaliana EFR gene (Iporá EFR 3 and Iporá EFR 12) previously selected for their high resistance to bacterial wilt was evaluated under pathogen-free conditions. During two cultivation cycles, the evaluated phenotypic characteristics were emergence, beginning of flowering, vigor, growth, leaf morphology, yield, number and size of tubers, analyzed under biosecurity standards. The phenotypic characteristics evaluated did not show differences, except in the morphology of the leaf with a more globose appearance and a shortening of the rachis in the transformation events with respect to untransformed Iporá. The Iporá EFR 3

[†]These authors contributed equally to this work. *Corresponding author. Phone) +598-2-3677641, FAX) +598-23677609 E-mail) mdallarizza@inia.org.uy ORCID Marco Dalla-Rizza https://orcid.org/0000-0003-4065-0940

Handling Editor : Cheol Seong Jang

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http:// creativecommons.org/licenses/by-nc/4.0) which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Articles can be freely viewed online at www.ppjonline.org.

genotype showed a ~40% yield decrease in reference to untransformed Iporá in the two trials, while Iporá EFR 12 did not differ statistically from untransformed Iporá. Iporá EFR 12 shows performance stability in the absence of the pathogen, compared to the untransformed control, positioning it as an interesting candidate for regions where the presence of the pathogen is endemic and bacterial wilt has a high economic impact.

Keywords : defense genes, genetic transformation, immunity

Social pressure on caring for the environment and the concept of One Health is increasingly prevalent at the level of consumers and society as a whole (Andrivon et al., 2022). In this sense, the genetic control of diseases together with the increase in yield has been prioritized by breeders (Brown, 2015). Durable resistance to bacterial and fungal diseases continues to be a challenge in breeding programs. Bacterial wilt is a widespread vascular disease that affects more than 450 plant species in tropical, subtropical, and temperate regions of the world (Elphinstone, 2005), some of great economic importance such as potato, tomato, bell pepper, eggplant and tobacco (Hayward, 1991). Ralstonia solanacearum responsible for this disease, is a soil-borne pathogen that enters the plant through wounds in the root tissues and progressively invades the vascular tissues, causing partial or total wilting and, finally, the death of the plant (Hayward, 1991). Vascular diseases have been shown to be the most destructive to annual crops and woody perennials. Because vascular pathogens live inside their host plants,

they make cultural, chemical and biological control measures inefficient, highlighting the need to design high-level strategies to combat them (Yadeta and Thomma, 2013). Given the complexity of its control under production conditions, host resistance is the most sustainable and environmentally effective strategy. However, there are currently no potato cultivars resistant to *R. solanacearum* available for production.

The incorporation of resistance by genetic engineering has generated fast and safe alternatives to traditional breeding (Dong and Ronald, 2019, 2021). An interesting strategy is the expression of pattern recognition receptors (PRRs) to enhance the immune response, and thus generate resistance to pathogens (Boller and Felix, 2009; Gurr and Rushton, 2005; van Esse et al., 2019). Plants recognize the presence of pathogens through PRRs located on the cell surface and intracellular nucleotide-binding site leucine-rich repeat receptors (Lu and Tsuda, 2021). Plant PRRs recognize molecular patterns associated with pathogens or cell damage (PAMP or DAMP, respectively) leading to activation of immunity (PAMP-triggered immunity). In particular, the Arabidopsis thaliana immune EF-TU RECEPTOR (EFR) recognizes bacterial elongation factor Tu, triggering an immune response. The EFR gene transfer has been shown to increase resistance in Nicotiana benthamiana against Agrobacterium tumefaciens and Pseudomonas syringae pv. tabaci, in tomato against R. solanacearum (Lacombe et al., 2010), in rice against Xanthomonas oryzae pv. oryzae (Lu et al., 2015; Schwessinger et al., 2015), in wheat against Pseudomonas syringae pv. oryzae (Schoonbeek et al., 2015), in orange against Xanthomonas citri subsp. citri (Xcc) and Xylella fastidiosa subsp. pauca (Xfp) (Mitre et al., 2021), and in Medicago truncatula against R. solanacearum (Pfeilmeier et al., 2019; Piazza et al., 2021).

Recently, our group characterized the introgression of the EFR receptor in commercial potato (*Solanum tuberosum*) genotypes susceptible to *R. solanacearum*. In these studies, we demonstrate that EFR expression confers resistance to bacterial wilt in the potato cultivar INIA Iporá under controlled conditions in growth chambers, with a significant reduction in the progression of the disease (Boschi et al., 2017). Furthermore, it was confirmed that these genotypes show resistance under conditions similar to natural infection in the field. We evidenced a restricted colonization of the pathogen at the vascular level compared to non-transformed controls, which may explain the greater resistance to the disease (Fort et al., 2020).

Some reports indicate that the increase in immunity can be accompanied by performance penalties varying between 4% and 21% depending on the culture and the defense characteristic expressed (Heil, 2002; Ning et al., 2017).

The objective of this work was to evaluate the productive performance of two INIA Iporá potato transformation events with the *EFR* gene with high resistance to *R*. *solanacearum* (3 and 12) in the absence of the pathogen. Under biosafety standards and during two cultivation cycles, agronomic and yield characteristics were analyzed. This research contributes towards the generation of potato genotypes resistant to bacterial wilt of agronomic interest through the use of the *EFR* gene.

Materials and Methods

Genotypes evaluated and culture conditions. The commercial potato cultivar INIA Iporá, a high-yielding cultivar that does not possess genes for resistance to bacterial wilt, –like all commercially available potato cultivars—was used. It is characterized by producing a high number of stems per plant (5 to 8), with high productivity compared to other potato cultivars (ENC, INASE, 2011-2020) (ENC Instituto Nacional de Semilas, 2021). This genotype was transformed with the EFR receptor gene as previously described by Boschi et al. (2017). Two events that showed high resistance to *R. solanacearum*, INIA Iporá EFR 3 and INIA Iporá EFR 12 (Boschi et al., 2017), were selected for this evaluation.

The plants were micropropagated *in vitro* from nodes maintained in Murashige and Skoog medium with sucrose 30 g/l and maintained at 22°C with cycles of 16:8 h of light:dark. Two weeks later, the seedlings were transferred to nurseries containing commercial peat (TREF mix) and kept under greenhouse conditions with natural light until the minitubers were harvested. These minitubers were then used as seed under field conditions in a field destined for this purpose for the production of seed tubers to be used in agronomic evaluation trials.

Experimental design. The study was carried out at INIA Las Brujas experimental station (latitude 34"40' S, longitude 56"20' W, altitude 32 m.a.s.l.), under the current regulations for the evaluation of genetically modified material according to the commission for the risk management of the Ministry of Livestock, Agriculture and Fisheries (Uruguay). The test conditions were the same (both soil and environmental) for the three genotypes analyzed: INIA Iporá untransformedwt, INIA Iporá EFR 3, and INIA Iporá EFR 12 events.

Two field trials were carried out in the autumn 2020 and spring 2020/21 harvests. The autumn trial 2020 lasted 140 days (sowing 12/4/2019, harvest 4/22/2020). The spring



Fig. 1. Climatic conditions in the experimental period autumn (December 2019-April 2020) (A) and spring (September 2020-January 2021) (B). Average of 10 days is displayed. The bars represent precipitation in l/m^2 , the line is the average temperature in °C and the asterisks the meteorological frosts.

trial 2020/21 lasted 148 days (sowing 9/1/2020, harvest 01/27/2021). The agrometeorological conditions of these two periods are summarized in Fig. 1. The agronomic management of the trials was carried out according to the production practices for each of the seasons. A basic fertilization was applied at the time of planting with ammonium phosphate at a dose of 250 kg/ha and a refertilization with urea at a dose of 50 kg/ha. Weed management was carried out with mechanical hoes at the beginning of the crop. Later on, in specific situations, weed control was manually done. No insecticide or fungicide were applied to control pests or pathogens. Both tests were carried out with irrigation using the dripper system (one tape per bed with emitters spaced 30 cm apart and a flow rate per emitter of 1 l/h).

In both trials the experimental design was a randomized complete block with three replications. In the autumn 2020 trial, the plot had two rows spaced 0.8 m apart and 5 m long, the target plant density was 40,000 plants/ha, consisting in 5 plants per plot. All plants in the plots were analyzed. In the spring trial 2020/21 the design and conformation of the plots were the same as the autumn trial 2020, with 30 plants per plot.

Phenotype characterization. In the study of the phenotypic characterization, the guidelines of the Union for the Protection of New Varieties of Plants (UPOV), the document prepared for potato cultivation TG/23/6 (International Union for The Protection of New Varieties of Plants. 2004), were followed. The characteristics studied were: percentage of emergence measured at 30 days of cultivation, vigor evaluated at 57 and 70 days in the spring harvest and at 47 and 54 days in autumn, plant height measured in spring at 75 days and in autumn 54 days after planting. In the autumn cycle, at 54 days the observations of leaf morphology, number of stems and plant architecture were made. The number of days to flower initiation was quantified, defined when 50% of the plants had the first open flower. The vigor of the plants was described according to a scale of 1 to 9, being 1 very little vigorous and 9 very vigorous.

Performance analysis. In the yield analysis, the procedure of the National Evaluation of Potato Cultivars was followed, a mandatory requirement for the National Registry of Cultivars (ENC Instituto Nacional de Semilas, 2021). To measure total yield, all the tubers in the plot were harvested, counted, and weighted. The time of harvest was defined according to the time of senescence. The harvested tubers were classified according to tuber weight considering the following categories: commercial (greater than 80 g), seed (between 40 and 80 g), and discard (less than 40 g).

Statistical analysis. Analysis of variance was performed to study the differences between genotypes and the Tukey test was performed to separate means (P < 0.05). The statistical study was carried out using Infostat and R platform.

Results

Phenotype characterization. The phenotypic characterization carried out included stem length, leaf length, leaf width, leaf opening, number of stems per plant and height of the three genotypes studied in the two trials. Phenotypic traits were only evaluated in autumn 2020, and plant height of the three studied genotypes in both seasons, presented in Table 1. The main differences observed between the genotypes were in the length of the second stem, resulting in INIA Iporá EFR 12 being shorter than INIA Iporá EFR 3 and Iporá wt. In days to flowering, a trend towards later flowering was observed in transformed plants, being consistently higher in EFR 12. Likewise, a certain difference was observed in the visual appreciation of the leaf opening in EFR 12 (Fig. 2). For the other phenotypic traits, no rel-

242

Dalla-Rizza et al.

Table 1. Phenotypic characterization	of the Iporá wt	INIA Iporá EFR 3	3 and INIA Iporá EFR	12 genotypes. autu	mn and spring trial data
~ 1	1		1	0 71	1 0

Champetanistic	A	Spring 2020/21				
Characteristic	wt	EFR 3	EFR 12	wt	EFR 3	EFR 12
Second stem length (cm)	57.58 a ^a	52.02 ab	48.59 b	-	-	-
Leaf length (cm)	18.25 a	17.33 a	18.83 a	-	-	-
Leaf width (cm)	10.50 a	8.67 a	10.08 a	-	-	-
Leaf opening	Intermediate open	Closed	Closed	-	-	-
No. of stems ^b	5	6	6	5	5	5
Plant height (cm)	60.54 a	59.80 ab	54.29 b	37.80 ab	34.40 b	42.70 a
Emergence ^c	94	98	96	98	96	96
Vigor ^d	9	8	7	8	7	9
Days to flowering ^e	30	35	37	59	64	68

Genotypeswt, untransformed; EFR 3, INIA Iporá EFR 3; EFR 12, INIA Iporá EFR 12.

^aANOVA, Tukey's test; means with a common letter are not significantly different (P > 0.05).

^bMeasured at 75 days.

°% Evaluated at 30 days from sowing.

^dRange from 1 to 9 (1: less vigorous, 9: more vigorous).

^eDays from sowing until first flower open.



Fig. 2. Leaf opening characteristic of each genotype. (A) Untransformed Iporá (wt). (B) Iporá EFR 3. (C) Iporá EFR 12.

evant clear trends were observed.

Performance characterization. Yield analysis was performed with the two trials together (autumn and spring environment) to verify the existence of genotype-by-environment interaction. The ANOVA showed that there was significance in the model, between the genotypes, between the environments and also in the genotype (G) by environment (E) interaction. The GxE interaction was significant, which would indicate that in different environments the agronomic outcome may vary. Therefore, the analysis of each genotype in the two evaluated periods of autumn and spring was carried out separately (Table 2). In each productive cycle (autumn and spring) the total yield per area and the main components of yield, average number of tubers per plant and average weight of the tubers production were quantified for each genotype (Table 3, Fig. 2). All genotypes reached a higher total yield in the autumn trial (average yield, 34.8 t/ha) where there were favorable environmental conditions for the crop compared to the spring trial (average yield, 18.8 t/ha). In both cycles, the Iporá EFR 3 genotype exhibited significantly lower performance than the Iporá wt. Iporá EFR 12, on the other hand presented a lower yield than Iporá wt in autumn, but in spring it surpassed the untransformed control (Table 3, Fig. 3).

The difference between Iporá EFR 3, and Iporá wt in yield could be explained as a result of a lower number of tubers per plant produced in the transformed genotype in both cycles. No significant differences were observed in

Variance source	Sum of squares	Degrees of freedom	Mean square	F-statistic	P-value
Model	2,502,278,217	9	278,030,913	19.55	0.0002
Genotype	579,721,027	2	289,860,514	20.38	0.0007
Environment	1,151,776,011	1	1,151,776,011	80.99	< 0.0001
Repetition	134,852,236	4	33,713,059	2.37	0.139
Interaction GxE	635,928,943	2	317,964,472	22.36	0.0005
Error	113,769,597	8	14,221,200		
Total	2,616,047,814	17			

Table 2. Joint analysis of variance of the three genotypes in two environments (autumn and spring)

Table 3. Total yield (kg/ha), average number of tubers per plant and average weight of tubers per genotype and per trial

Line	Total yield (kg/ha)	Tubers	Average weight of tubers (g)	
Autumn trial				
Iporá wt	48.014 a ^a	17.73 a	67.60 a	
Iporá EFR 3	27.895 b	11.33 b	62.20 ab	
Iporá EFR 12	28.499 b	16.67 a	42.57 b	
Average	34.803	15.24	57.46	
DMS $P > 0.05$	6.618	3.67	19.91	
CV (%)	8.39	10.64	11.91	
Spring trial				
Iporá wt	18.075 ab	10.47 ab	43.83 a	
Iporá EFR 3	10.731 b	5.87 b	44.83 a	
Iporá EFR 12	27.608 a	13.60 a	50.70 a	
Average	18.804	9.98	46.45	
DMS $P > 0.05$	12.987	5.83	10.94	
CV (%)	23.73	20.09	8.09	

CV, coefficient of variation.

^aANOVA, Tukey's test; means with a common letter are not significantly different (P > 0.05).

the mean weight of the tubers between Iporá EFR 3 and wt. However, the difference in yield between Iporá EFR 12 and Iporá wt in the autumn cycle was explained by the lower average weight of the tubers compared to the Iporá wt control. In the spring cycle, on the other hand, Iporá



Fig. 3. Total average yield (kg/ha) of each genotype in the autumn 2020 and spring 2020/21 trials. Bars represent means \pm standard error. Bars with different letters indicate $P \le 0.05$ by Tuckey test.

EFR 12 did not present significant differences in yield or its components with respect to the untransformed control. Complementing the analysis, Table 4 shows the distribution of the number of tubers per plant and its representation by category in each trial. The average number of tubers per plant in Iporá EFR 3 was lower, which explains the reduction in yield in both trials. This effect was not observed for Iporá EFR 12, which has a slightly higher number of tubers than Iporá without transformation in the spring trial.

Table 4. Average tuber number per plant during autumn and spring trial

Line -				Tu	bers			
	Autumn trial					Spring trial		
	Average	Commercial (%)	Seed (%)	Discard (%)	Average	Commercial (%)	Seed (%)	Discard (%)
Iporá wt	17.7	22.2	38.5	39.7	10.5	12.1	27.4	60.5
Iporá 3	11.3	22.3	34.4	43.2	5.9	10.2	30.7	59.1
Iporá 12	16.7	11.4	31.0	57.4	13.6	22.6	24.0	53.5

Commercial, greater than 80 g; Seed, between 40 and 80 g; Discard, less than 40 g expressed as a percentage and total average per plant.

These data seem to infer that while tuber differentiation was affected in Iporá EFR 3, in Iporá EFR 12 the filling of the tuber was mainly affected in the autumn trial, resulting in the highest percentage of tubers in the lowest category observed for this genotype. while in the spring trial, EFR 12 did not show difficulty in filling, obtaining a result even higher than the wt.

During the autumn cycle, the incidence of skin disease in potato tuber, known as 'scabies' caused by *Streptomyces scabies* in transformed events, was observed. In Iporá EFR 3 it affected up to 25% of the tubers and in Iporá EFR 12 it was less than 5%, while it was not observed in the nontransformed one. The incidence of this disease is normally higher in the autumn cycle due to cool temperatures and rainfall (Fig. 1). During the spring cycle, the disease did not appear.

Discussion

Several authors reported in different plant species the positive effect of the use of the *EFR* gene on the control of bacterial pathogens in controlled conditions (Kunwar et al., 2018; Lacombe et al., 2010; Mitre et al., 2021; Pfeilmeier et al., 2019; Schoonbeek et al., 2015; Schwessinger et al., 2015). Previously, our research group reported the effect of the EFR gene in controlling the development of bacterial wilt in potato (Boschi et al., 2017; Fort et al., 2020). In this work, we evaluated the effect of the *efr* gene on agronomic aspects and performance in the absence of disease, advancing in the search for resistant germplasm of commercial interest.

Regarding phenotypic characteristics, differences were only observed in the transformed events with respect to the untransformed variety in the leaf opening and in the length of the second stem (Table 1). Leaf thickness and habit are very important elements in defining performance. The more open leaves, the higher the light extinction coefficient. The dry matter production of any crop is determined by the capture and utilization efficiency of solar energy. According to Boote and Loomis (1991), the capture depends on the incident radiation, the leaf area and the extinction coefficient of light (k, directly depends on the arrangement of the leaves, insertion angle).

The productive performance of both production cycles, autumn and spring, were different, being higher in autumn (Table 3). Although in the conditions of Uruguay there is a tendency to the autumn cycle being more productive, the differences are not so pronounced (on average 5% higher according to the Farmer Observatory [Observatorio granjero]) (Mercado Modelo, 2017). In the trial, these differences were on average greater than 50% and this could be due to the high temperatures of the spring cycle that affected during the tuberization period. But, in addition, the tuberization period considered from the beginning of flowering time to harvest in autumn was 106 days, and in spring 85 days. These differences are related to the different photoperiod, temperature, heliophany and rainfall conditions that occurred during the two growing cycles, where the cooler night temperatures in autumn favored tuberization (Fig. 1). During the harvest period for both trials there was abundant rainfall that made harvesting more complex.

Yield components in potato crops comprise the average number of tubers produced and the average size of the tubers, being strongly affected by the interaction of radiation intercepted by canopy and temperature (Struik, 2007). A relevant aspect is the delay in flowering time observed in the two transformed genotypes. The peak of stolon production and the beginning of tuberization occurs during the flowering period; therefore, flowering is considered an indicator of tuber formation. The crops start the tuberization at the moment of beginning of flowering (first flower open) or just before that moment (Celis-Gamboa et al., 2003). The observed delay in flowering (5 days in EFR 3 in both cycles and 7 and 9 days in EFR 12 in autumn and spring, respectively) implies a delay in the onset of tuber filling. The longest delay of EFR 12, could explain the greater differentiation of tubers, that could cause greater competition between them, affecting the final size. According to Struik (2007), the relative variability of the size of the tubers (the coefficient of variation of the size of the tuber) is rather constant once the tuberization is complete and depends on the number of tubers, although the relation can be complex.

Iporá EFR 12 showed a high productive potential associated with a good average number of differentiated tubers per plant in both cycles (Table 4). However, the productive result obtained by Iporá EFR 12 was very different between the cultivation cycles. In autumn, a lower development of the aerial part was observed in this genotype (see plant height, vigor and height of the second stem) (Table 1), reducing the source. In addition, the delay in flowering and filling of the tubers together with greater competition between a high number of differentiated tubers could negatively affect the final size and yield. This did not happen in spring, where the development of the plants was higher, the filling of the tubers occurred in a better way, achieving a good average size of tubers and yield compared to the rest of the genotypes. Iporá EFR 3 showed a lower yield in both production cycles, mainly explained by a lower number of differentiated tubers per plant. However, the lower number reduced the competition between them and allowed a better

filling of the differentiated tubers.

Kunwar et al. (2018) reported the performance of EFR transformed tomato plants in the field in the presence of R solanaceaurm. In this work, there was no performance penalty in controlled field conditions in presence of the pathogen, the yields reported reached up to a 170% higher in the transformed genotypes when compared with nontransformed plants. Analyzing the performance of each genotype, Iporá EFR 3 presented a decrease in yield close to 40% in reference to Iporá wt in both production cycles. Instead, INIA Iporá 12, while showing a reduction ~40% in autumn when compared with non-transformed genotypes, showed a yield of 150% vs. Iporá wt in the spring assay. Considering the two crop cycles, the average yield of EFR 12 maintains a higher average yield than the other genotypes evaluated. This is a preliminary observation that will have to be corroborated in successive trials. The potential of EFR-12 genotype as a resistant genotype can be highlighted, it performed better in a growth-limiting environment, under stress conditions. High temperatures in tropical climates favor the spread of the pathogen, and certainly is prevalent in tropical regions. Another favorable point is the longer vegetative period to flowering onset, as this represents higher leaf area index (LAI). The fraction of light passing through the canopy without interception by the leaves depends on the LAI which in turn determines the proportion of ground covered by green leaves. Intercepted radiation has been found to be linearly correlated with the quantity of dry matter produced which, in the case of potatoes, is distributed over the tubers and the other parts of the plant (Haverkort, 2007). Besides, EFR-12 has also longer tuberization period under environmental favorable conditions. Taking these factors into consideration (better performance under stress conditions, longer vegetative period until the onset of flowering and thus longer tuberization period) could explain a regular performance in different environments.

The performance differences observed between the two transformation events could be partially explained by the transformation methodology used (Boschi et al., 2017). *Agrobacterium tumefaciens*-mediated genetic transformation does not control gene copy number, and insertion site is random. It is well documented that this transformation methodology could influence the level of gene expression, as well as performance (Dong and Ronald, 2021; Kamthan et al., 2016). Precision enhancement, where the selective insertion of transgenes occurs in predetermined places in the plant genome, called genomic safe ports, is a desirable alternative with a relatively simple methodology. None of the previous evaluations of the *EFR* gene in different plant genetic backgrounds were carried out using precision breeding techniques that can control the copy number and the safe genomic insertion (Dong and Ronald, 2019; Dong et al., 2020), being an interesting strategy for advance in this field

Considering the contrasting yield behavior of EFR 12 during the two cycles, loss of yield was not observed and this rule out a negative impact of the EFR gene on development and productivity. According to Mitre et al. (2021) the EFR gene was evaluated in different genetic backgrounds, including proof of concept works such as commercial varieties of tomato, potato, rice, wheat, sweet orange, and resulting in a contribution in terms of control of bacterial wilt. This indicates that the EFR gene represents a contribution to resistance regardless of the genetic environment of the construct. The performance results found in Iporá-EFR highlight the importance of using transformation methods that allow precise insertion in genomic safe ports, which avoid expression in the host plant beyond that expected. The value of Iporá EFR 12 as a parental genotype is highlighted as it presents resistance in a commercial variety with a tetraploid genetic background. Additionally, the INIA Iporá cultivar presents immunity to PVY (Dalla Rizza et al., 2007), presents pollen with a low percentage of viability, and in Uruguay it is one of the cultivars most recognized by farmers for its high adaptability and production potential. The results shown both in resistance to R. solanacearum (Boschi et al., 2017), and in productivity in the two seasons make INIA Iporá EFR 12 a very interesting alternative for production sites where the presence of R. solanacearum affects crops severely or makes potato production unviable due to its serious impact. The results presented here encourage further field studies to assess the yield production of Iporá EFR genotypes in the presence of the pathogen. Even INIA Iporá 3, that showed lower production percentages in both trials could comprise an important germoplasm in endemic regions were no resistance variants are available.

Conclusion. The Iporá EFR 12 genotype in the absence of *R. solanacearum* presented an annual productive yield similar to INIA Iporá wt. Iporá EFR 12 is expected to maintain productive stability when bacterial wilt is present in the planting plots. This genotype could be an interesting variety for production where bacterial wilt disease is endemically present. Likewise, Iporá EFR 12 can be an alternative for the genetic base of future cultivars.

Currently, the EFR potato with bacterial wilt resistance highlights the importance of a regulatory system that can perform environmental risk assessment in countries where the disease is endemic and the potato is a staple food. The breeders of this germplasm share this advance for its use in regions that suffer from this cultural problem with prior agreement of use.

It is emphasized the convenience of making precision improvements with the EFR receptor that avoid other expressions in the genome of the host plant beyond that expected, thus facilitating regulatory aspects.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

To Laura Orrego, for her constant bibliographic support; Adriana Reggio and Armando de Paz, for helping in field work. The authors are grateful for Cyril Zipfel's critical reading, suggestions and corrections to the manuscript.

Research project founded by the National Agency for Research and Innovation (ANII_FMV_136506).

References

- Andrivon, D., Montarry, J. and Fournet, S. 2022. Plant health in a one health world: missing links and hidden treasures. *Plant Pathol.* 71:23-29.
- Boller, T. and Felix, G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60:379-406.
- Boote, K. J. and Loomis, R. S. 1991. Modeling crop photosynthesis: from biochemistry to canopy. Crop Science Society of America, Madison, WI, USA. 140 pp.
- Boschi, F., Schvartzman, C., Murchio, S., Ferreira, V., Siri, M. I., Galván, G. A., Smoker, M., Stransfeld, L., Zipfel C., Vilaró, F. L. and Dalla-Rizza, M. 2017. Enhanced bacterial wilt resistance in potato through expression of *Arabidopsis* EFR and introgression of quantitative resistance from *Solanum commersonii*. Front. Plant Sci. 8:1642.
- Brown, J. K. M. 2015. Durable resistance of crops to disease: a Darwinian perspective. Annu. Rev. Phytopathol. 53:513-539.
- Celis-Gamboa, C., Struik, P. C., Jacobsen, E. and Visser, R. G. F. 2003. Temporal dynamics of tuber formation and related processes in a crossing population of potato (*S. tuberosum*). *Ann. Appl. Biol.* 143:175-186.
- Dalla Rizza, M., Vilaró, F. L., Torres, D. G. and Maeso, D. 2007. Detection of PVY extreme resistance genes in potato germplasm from the Uruguayan breeding program. *Am. J. Potato Res.* 83:297-304.
- Dong, O. X., Yu, S., Jain, R., Zhang, N., Duong, P. Q., Butler,

C., Li, Y., Lipzen, A., Martin, J. A., Barry, K. W., Schmutz, J., Tian, L. and Ronald, P. C. 2020. Marker-free carotenoidenriched rice generated through targeted gene insertion using CRISPR-Cas9. *Nat. Commun.* 11:1178.

- Dong, O. X. and Ronald, P. C. 2019. Genetic engineering for disease resistance in plants: recent progress and future perspectives. *Plant Physiol.* 180:26-38.
- Dong, O. X. and Ronald, P. C. 2021. Targeted DNA insertion in plants. Proc. Natl. Acad. Sci. U. S. A. 118:e2004834117.
- Elphinstone, J. G. 2005. The current bacterial wilt situation: a global overview. In: *Bacterial wilt: the disease and the Ralstonia solanacearum species complex*, eds. by C. Allen, P. Prior and A. C. Hayward, pp. 9-28. American Phytopathological Society, St. Paul, MN, USA.
- ENC Instituto Nacional de Semilas. 2021. Historical evaluation results, National potato cultivars, 2021. URL https://www.in-ase.uy/Publicaciones/Publicaciones.aspx?s=dt-enc [20 January 2022].
- Fort, S., Ferreira, V., Murchio, S., Schvartzman, C., Galván, G. A., Vilaró, F., Siri, M. I. and Dalla-Rizza, M. 2020. Potato plants transformed with the *Arabidopsis* EF-Tu receptor (EFR) show restricted pathogen colonization and enhanced bacterial wilt resistance under conditions resembling natural field infections. *Agrocienc. Urug.* 24:e413.
- Gurr, S. J. and Rushton, P. J. 2005. Engineering plants with increased disease resistance: how are we going to express it? *Trends Biotechnol.* 23:283-290.
- Haverkort, A. J. 2007. Potato crop response to radiation and daylength. In: *Potato biology and biotechnology: advances and perspective*, eds. by D. Vreugdenhil, J. Bradshaw, C. Gebhardt, F. Govers, M. Taylor, D. Mackerron and H. Ross, pp. 353-363. Elsevier, Amsterdam, The Netherlands.
- Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 29:65-87.
- Heil, M. 2002. Ecological costs of induced resistance. *Curr. Opin. Plant Biol.* 5:343-350.
- International Union for The Protection of New Varieties of Plants. 2004. Guidelines for the conduct of tests for distinctness, uniformity and stability - Potato (*Solanum tuberosum* L.). URL http://www.upov.int/edocs/tgdocs/en/tg023.pdf [20 January 2022].
- Kamthan, A., Chaudhuri, A., Kamthan, M. and Datta, A. 2016. Genetically modified (GM) crops: milestones and new advances in crop improvement. *Theor. Appl. Genet.* 129:1639-1655.
- Kunwar, S., Iriarte, F., Fan, Q., da Silva, E. E., Ritchie, L., Nguyen, N. S., Freeman, J. H., Stall, R. E., Jones, J. B., Minsavage, G. V., Colee, J., Scott, J. W., Vallad, G. E., Zipfel, C., Horvath, D., Wetwood, J., Hutton, S. F. and Paret, M. L. 2018. Transgenic expression of EFR and Bs2 genes for field management of bacterial wilt and bacterial spot of tomato. *Phytopathology* 108:1402-1411.
- Lacombe, S., Rougon-Cardoso, A., Sherwood, E., Peeters, N.,

Dahlbeck, D., van Esse, H. P., Smoker, M., Rallapalli, G., Thomma, B. P. H. J., Staskawica, B., Jones, J. D. G. and Zipfel, C. 2010. Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. *Nat. Biotechnol.* 28:365-369.

- Lu, F., Wang, H., Wang, S., Jiang, W., Shan, C., Li, B., Yang, J., Zhang, S. and Sun, W. 2015. Enhancement of innate immune system in monocot rice by transferring the dicotyledonous elongation factor Tu receptor EFR. *J. Integr. Plant Biol.* 57:641-652.
- Lu, Y. and Tsuda, K. 2021. Intimate association of PRR- and NLR-mediated signaling in plant immunity. *Mol. Plant-Microbe Interact.* 34:3-14.
- Mercado Modelo. 2017. Farmer Observatory. Potato crop, 2017. URL http://www.mercadomodelo.net [20 January 2022].
- Mitre, L. K., Teixeira-Silva, N. S., Rybak, K., Magalhães, D. M., de Souza-Neto, R. R., Robatzek, S., Zipfel, C. and de Souza, A. A. 2021. The Arabidopsis immune receptor EFR increases resistance to the bacterial pathogens *Xanthomonas* and *Xylella* in transgenic sweet orange. *Plant Bioetchnol. J.* 19:1294-1296.
- Ning, Y., Liu, W. and Wang, G.-L. 2017. Balancing immunity and yield in crop plants. *Trends Plant Sci.* 22:1069-1079.
- Pfeilmeier, S., George, J., Morel, A., Roy, S., Smoker, M., Stransfeld, L., Downie, J. A., Peeters, N., Malone, J. G. and Zipfel, C. 2019. Expression of the *Arabidopsis thaliana* immune receptor EFR in Medicago truncatula reduces infection by a root pathogenic bacterium, but not nitrogen-fixing rhizobial

symbiosis. Plant Biotechnol. J. 17:569-579.

- Piazza, S., Campa, M., Pompili, V., Dalla Costa, L., Salvagnin, U., Nekrasov, V., Zipfel, C. and Malnoy, M. 2021. The Arabidopsis pattern recognition receptor EFR enhances fire blight resistance in apple. *Hortic. Res.* 8:204.
- Schoonbeek, H.-J., Wang, H.-H., Stefanato, F. L., Craze, M., Bowden, S., Wallington, E., Zipfel, C. and Ridout, C. J. 2015. Arabidopsis EF-Tu receptor enhances bacterial disease resistance in transgenic wheat. *New Phytol.* 206:606-613.
- Schwessinger, B., Bahar, O., Thomas, N., Holton, N., Nekrasov, V., Ruan, D., Canlas, P. E., Daudi, A., Petzold, C. J., Singan, V. R., Kuo, R., Chovatia, M., Daum, C., Heazlewood, J. L., Zipfel, C. and Ronald, P. C. 2015. Transgenic expression of the dicotyledonous pattern recognition receptor EFR in rice leads to ligand-dependent activation of defense responses. *PLoS Pathog.* 11:e1004809.
- Struik, P. C. 2007. Above-ground and below-ground plant development. In: *Potato biology and biotechnology: advances and perspective*, eds. by D. Vreugdenhil, J. Bradshaw, C. Gebhardt, F. Govers, M. Taylor, D. Mackerron and H. Ross, pp. 219-233. Elsevier, Amsterdam, The Netherlands.
- van Esse, H. P., Reuber, T. L. and van der Does, D. 2019. Genetic modification to improve disease resistance in crops. *New Phytol.* 225:70-86.
- Yadeta, K. A. and Thomma, B. P. H. J. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. *Front. Plant Sci.* 4:97.