v. 4, n. 7, 2024

International Journal of Biological and Natural Sciences

Acceptance date: 16/10/2024

SELECTION OF POTATO (*Solanum tuberosum* L.) GERMPLASM WITH POTATO PURPLE TOP SYNDROMES TOLERANCE CHARACTERISTICS

Isidro Humberto Almeyda León
INIFAP-CIRNE-Campo Experimental General Terán, Carretera Montemorelos-China, General Terán

Margarita Díaz Valasis
INIFAP-CIRNE Campo Experimental Valle de México, Texcoco, México

María Genoveva Álvarez Ojeda
INIFAP-CIRNE-Campo Experimental Río Bravo, Cd. Río Bravo, Tams

Abstract: Currently, the management and control of potato purple top syndrome is done by chemical control of the vector with massive applications of pesticides with a great adverse effect on the environment and human health. Therefore, it is essential to generate alternatives that allow a profitable and sustainable production for the producer, such as the use of varieties with characteristics of tolerance or resistance to the disease. The objective of this study was to select germplasm with tolerance characteristics to potato purple top syndrome, caused mainly by the bacterium *Candidatus* Liberibacter solanacearum. Ten potato genotypes (eight clones and the commercial varieties Gigant and Mondial) were evaluated. The variables evaluated for the selection of germplasm with tolerance characteristics to potato purple top syndrome were the percentage of tuber browning and sprouting. The damage values recorded based on tuber browning ranged from light (L) to very heavy (F+), especially when insecticides were not applied. Likewise, the percentage of tuber sprouting was variable among the different materials evaluated, highlighting clones 1-6-1, 1-13-1, 4-5-6, 4 and the commercial variety Gigant. The bacterium was detected in all materials from DNA extracted from tuber pulp. However, this pathogen was not detected when using DNA extracted from shoots from the same tubers that tested positive. With these results, the clones that showed the least tuber browning damage and the highest percentage of sprouting are an alternative for the selection of materials with tolerance to potato purple top syndrome.
Keywords: potato, syndrome ZC - PMP, tuber, browning

INTRODUCTION

In Mexico, potato (*Solanum tuberosum* L.) is planted in an area of approximately 60,000 ha under irrigated and rainfed conditions, with an annual production of 1'784,000 t (SIAP, 2020). Production is affected by various phytosanitary problems, the most important being the Zebra Chip (ZC) - Punta Morada de la Papa (PMP) complex, which is associated with *Candidatus* Liberibacter solanacearum *(*CaLso) and *Candidatus* Phytoplasma spp. (CaPhy) (Munyaneza, 2012). CaLso is a gram-negative αproteobacterium αproteobacterium, not culturable in vitro, limited to the phloem vascular system, is an obligate parasite of plants and insects, and exhibits horizontal and vertical transmission (Munyaneza *et al*., 2008; Secor *et al*., 2009; Munyaneza, 2012; Bertolini *et al*., 2015). This bacterium is a bacillus between 2 and 3 μm long and 0.2 to 0.3 μm wide. CaPhy has also failed to be cultured in the laboratory, lacks several biosynthetic functions, and generally have an average diameter between 200 and 800 nm (Marcone *et al*., 1999; Davis *et al*., 2005; Bai *et al*., 2006; Oshima *et al*., 2013). CaLso and CaPhy are transmitted mainly by the psyllid *Bactericera cockerelli* (Sulc) (Hemiptera: Psyllidae) (Almeyda-León *et al*., 2008; Butler and Trumble, 2012; Munyaneza, 2012; Ciubotaru *et al*. 2018), although some leafhopper species of the genera *Empoasca* and *Aceratagallia* are also implicated in CaPhy transmission (Almeyda-León *et al*, 2008). *Candidatus* Liberibacter solanacearum is distributed in Central America, Mexico, United States and New Zealand (Munyaneza *et al*., 2007, Munyaneza, 2012). In Mexico, losses caused by this pathogen, range between 30 and 95%; in most of the potato producing regions of Coahuila, Nuevo Leon and Guanajuato, there is up to 95% incidence of the bacterium, either as a simple infection and/or associated with CaPhy (Flores-Olivas, 2013; Gonzalez *et al*., 2014; Melgoza *et al*., 2018). In Mexico, an increase of 50% has been reported

in the use of insecticides for the control of the psyllid *B. cockerelli* Sulc, making up to 70 applications per cycle, which affects physicochemical and bromatological characteristics of the product, edaphological characteristics, the environment, insect resistance and the considerable reduction of natural enemies, which causes an increase in production costs (Hernández *et al*., 2018). Therefore, it is essential to generate alternatives that allow obtaining a profitable and sustainable production for the producer, such as the use of varieties with disease tolerance or resistance characteristics. Based on the above, the objective of this work was to select potato germplasm with tolerance characteristics to potato purple top syndrome.

MATERIALS AND METHODS

Tubers of 8 potato clones and two commercial varieties were collected in the State of Mexico and showed normal sprouts, without symptoms of infection by the bacterium *Candidatus* Liberibacter solanacearum (Figure 1).

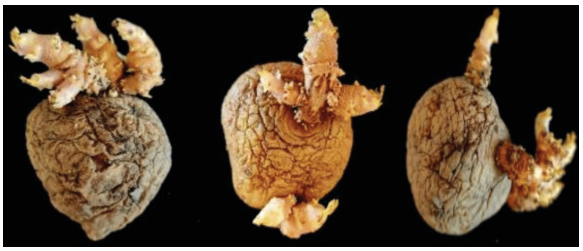

Figure 1. Potato tubers without apparent symptoms of PMP infection.

These materials were grown under field conditions with and without insecticide application and the degree of browning and percentage of sprouting were recorded. Genomic DNA extraction was performed by the CTAB method reported by Almeyda *et al* (2001), using ribs from potato leaves with and without symptoms of infection by PMP syndrome. Detection of the bacterium was performed using the Polymerase Chain Reaction-Endpoint technique and the primers Lp16S-2F/Lp16S-2R, designed on the sequence of the gene coding for the 16S ribosomal RNA of the bacterium and amplifying a fragment of approximately 872 bp (Hansen *et al*., 2008), were used. PCR reactions were performed in a final volume of 25 µL containing: 5.0 µL of PCR Buffer (1X) including dNTP's and $NaCl_2$, 2.0 µL of each indicator (25 pMoles), 100 ng of DNA and 0.3 Units of the DNA Taq Polymesara and 13.7 µL of sterile H2O. The amplification program was: an initial denaturation cycle at 95 °C for 5 min, followed by 35 cycles at 95 °C for 1 min, 60 °C for 30 s and 72 °C for 1 min, and a final extension cycle at 72 °C for 10 min. The amplified fragments were fractionated on 1.5 % agarose gels, stained with Gel-Red dye and visualized in an ultraviolet light transilluminator.

RESULTS AND DISCUSSION

The degree of browning recorded ranged from light (L) to very heavy (F+), in the clones and varieties evaluated, the highest browning values were observed when insecticides were not applied. Clone 1-13-1 and the variety Gigant recorded moderate (M) to moderate-strong (M-F) tuber browning values without insecticide application and even the percentage of sprouting was higher under this management condition (Table 1).

However, it is important to note that the sprouting of the tubers of all clones and of the two varieties was of the type known as thread stalk, which is not desirable if this material is to be used as seed in a subsequent crop cycle. The bacterium *Candidatus* Liberibacter solanacearum was detected from DNA extracted from tubers collected from all materials evaluated. However, the bacterium was not detected when using DNA extracted from sprouts from the same tubers that tested positive (Table 2 shows the results obtained from 10 tubers and their sprouts).

Clone	Treatment		Degree of browning	Percentage of sprouting	Type of sprouting
	With application	No application			
1-6-1	X		M, F	73.8	Thin sprout
1-6-1		X	F	56.2	Thin sprout
8-29	X		M-F	30.0	Thin sprout
8-29		X	F	0.0	-------
1-13-1	X		L, M-F, F	51.5	Thin sprout
1-13-1		X	M, M-F	71.4	Thin sprout
3-7-8	X		M, M-F	71.8	Thin sprout
3-7-8		X	F	27.5	Thin sprout
4-5-6	X		M, M-F	72.7	Thin sprout
4-5-6		X	F	42.8	Thin sprout
01-8	X		M	40	Thin sprout
01-8		X	F	6.8	Thin sprout
4-6-7	X		M-F	44.1	Thin sprout
4-6-7		X	M	10.0	Thin sprout
4	X		F+	79.1	Thin sprout
4		X	M-F	38.7	Thin sprout
Gigant	X		M-F	92.3	Thin sprout
Gigant		X	M	100.0	Thin sprout
Mondial	X		F+	77.5	Thin sprout
Mondial		X	F+	50.0	Thin sprout

Degree of browning and percentage of sprouting of tubers of eight potato clones and two potato varieties with and without insecticide application.

L= Mild M= Moderate M-F= Moderate to Strong F= Strong F+= Very Strong

Sample	Result	Sample	Result
Tuber 1	+	Tuber 6	+
Outbreak 1	-	Sprout 6	-
Tuber 2	+	Tuber 7	+
Outbreak 2	-	Sprout 7	-
Tuber 3	+	Tuber 8	+
Outbreak 3	-	Outbreak 8	-
Tuber 4	+	Tuber 9	+
Outbreak 4	-	Sprout 9	-
Tuber 5	+	Tuber 10	+
Sprout 5	-	Sprout 10	-

Table 2. Results of the analysis for the detection of *Ca*. Liberibacter solanacearum in tubers and their shoots.

Based on the detection of CaLso by PCR, it can be inferred that the degree of browning is related to the concentration of the bacterium, since this pathogen was detected with greater sensitivity in tubers from plants without insecticide application, i.e. where the pressure of the inoculum was greater due to the presence of the vector. It is important to note that the infection and appearance of symptoms of PMP syndrome, may be associated with the crop cycle, since late-cycle materials are subject for a longer period of time to the presence of the vector and to the pressure of a greater amount of inoculum (Hernández *et al*., 2018). On the other hand, when no symptoms of infection of the bacterium are observed in tubers and there are healthy sprouts, this does not mean that such material should be used as seed because, as Pitman *et al*. (2011) point out, the transmission of CaLso from tuber seed to plant is feasible and constitutes the initial inoculum for a following crop cycle, having a greater impact on the presence and spread of the disease than the vectors themselves as mentioned by Hernández *et al*. (2018).

CONCLUSIONS

All the potato materials evaluated in this work show, to a greater or lesser degree, tuber browning damage, especially when chemical control of the bacterial vector, the psyllid *Paratrioza cockerelli*, is not carried out. However, Mexican materials such as clones 1-6-1, 1-13-1, 4-5-6, 4, are an option in the selection of materials tolerant to PMP syndrome, since they presented the lowest values of browning and the highest percentage of tuber sprouting.

REFERENCES

Almeyda, L. I.H., Rocha, P. M.A., Piña, R. J. and Martínez, S. J.P. 2001. The use of polymerase chain reaction and molecular hybridization for detection of Phytoplasma sp. in different plant species in México. 2001. Revista Mexicana de Fitopatología 19: 1-9.

Almeyda-León I.H., Sánchez-Salas, J.A. and Garzón-Tiznado, J.A. 2008. Vectores causantes de punta morada de la papa en Coahuila y Nuevo León, México. Agricultura Técnica en México 34: 141-150.

Bai, X., Zhang, J., Ewing, A., Miller, S.A., Jancso, R.A. and Shevchenko, D.V. 2006. Living with genome instability: The adaptation of phytoplasmas to diverse environments of their insect and plant hosts. Journal of Bacteriology 188: 3682–3696.

Bertolini, E., Teresani, G.R., Loiseau, M., Tanaka, FAO, Barbé, S. and Martínez, C. 2015. Transmission of 'Candidatus Liberibacter solanacearum' in carrot seeds. Plant Pathology 64: 276-285.

Butler, C.D. and Trumble, J.T. 2012. The potato psyllid, Bactericera cockerelli (Sulc) (Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. Terrestrial Arthropod Reviews 5: 87–111.

Ciubotaru, R.M., Cortiñas, A.J., Oyedele, J., Parnell, S., Schrader, G. and Zancanaro, G. 2018. Workplan and methodology for EFSA to develop plant pest survey guidelines for EU Member States. EFSA Support Publ. 15(3): 1399E.

Davis, R.E., Jomantiene, R. and Zhao, Y. 2005. Lineagespecific decay of folate biosynthesis genes suggests ongoing host adaptation in phytoplasmas. ADN Cell Biology 24: 832–840.

Flores-Olivas, A. 2013. Etiología, detección molecular y manejo integrado del síndrome de la punta morada de la papa en México. Revista Mexicana de Fitopatologia 31: 8-9.

González, C.A., Villavicencio, G.E.E., Torres, T.M.A., Zamora, V.V.M. and Almeyda, L.I.H. 2014. Efecto de antioxidantes y señalizadores en plantas de papa (*Solanum tuberosum* L.) infectadas con *Candidatus* Liberibacter solanacearum bajo condiciones de invernadero. Revista Colombiana de Biotecnología 16: 114-121.

Hansen, A.K., Trumble, J.T., Stouthamer, R., and Paine, T.D. 2008. New Huanglongbing (HLB) *Candidatus* species, "C. Liberibacter psyllaurous", found to infect tomato and potato is vectored by the psyllid *Bactericerca cockerelli* (Sulc). Applied and environmental microbiology 74(18): 5862–5865.

Hernández, G.V., Salas, M.M.A., Frías T.G.A., Aguirre, U.L.A., Flores, O.A. and Almeyda, L.I.H. 2018. Importance of the Seed-tuber and the Overgrowth *Lycium berlandieri* (Dunal) for the Potato Purple Top/Zebra Chip Epidemic Rev. Bio-Cien. 5(nesp), e442. doi: https://doi.org/10.15741/revbio.05.nesp.e442.

Marcone, C., Neimark, H., Ragozzino, A., Lauer, U. and Seemüller, E. 1999. Chromosome sizes of phytoplasmas comparing major phylogenetic groups and subgroups. Phytopathology 89: 805–810.

Melgoza, V.C.M., León, S.C.R., López, V.J.A., Hernández, E.L.A., Velarde, F.S. and Garzón, T.J.A. 2018. Presencia de *Candidatus* Liberibacter solanacearum en *Bactericera cockerelli* Sulc asociada con enfermedades en tomate, chile y papa. Revista Mexicana de Ciencias Agrícolas 9(3): 499-509.

Munyaneza, J.E., Crosslin, J.M. and Upton, J.E. 2007. Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "Zebra Chip," a New Potato Disease in Southwestern United States and Mexico. Journal Economic Entomology 100(3): 656-663.

Munyaneza, J.E., Buchman, J.L., Upton, J.E., Goolsby, J.A., Crosslin, J.M., Bester, G., Miles, G.P. and Sengoda, V.G. 2008. Impact of Different Potato Psyllid Populations on Zebra Chip Disease Incidence, Severity, and Potato Yield. Subtropical Plant Science 60: 27-37.

Munyaneza, J.E. 2012. Zebra Chip Disease of Potato: Biology, Epidemiology, and management. American Journal Potato Research 89: 329–350.

Oshima, K., Maejima, K. and Namba, S. 2013. Genomic and evolutionary aspects of phytoplasmas. Frontiers in Microbiology 4: 230. doi: 103389/fmicb.2013.00230.

Pitman, A.R., Drayton, G.M., Kraberger, S.J., Gent, R.A. and Scott, A. 2011. Tuber transmission of *Candidatus* Liberibacter solanacearum and its association with zebra chip on potatoe in New Zealand. European Journal of Plant Pathology 129: 389.

Secor, G.A., Rivera, V.V., Abad, J.A., Lee, M., Clover, G. R.G., Liefting, L.W., Li, X. and De Boer, S.H. 2009. Association of '*Candidatus* Liberibacter solanacearum' with Zebra Chip Disease of Potato Established by Graft and Psyllid Transmission, Electron Microscopy, and PCR. Plant Disease (93)6: 574-586.

SIAP. 2020. Panorama Agroalimentario. Servicio de Información Agroalimentaria y Pesquera. Ciudad de México, México. 200 p. https://www.inforural.com.mx/wp-content/uploads/2020/11/Atlas-Agroalimentario-2020.pdf.