Assessing zebra chip resistance of advanced potato clones under field conditions in the Toluca valley, Mexico

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Abstract

Zebra chip, also known as ‘potato purple-top’ and ‘internal tuber browning’ is threatening potato production in Mexico, Central America, the United States, and New Zealand. The disease is caused by the phloem-limited ‘Candidatus Liberibacter solanacearum’ (Lso), for which potato psyllid, Bactericera cockerelli is the vector. Currently, ZC management is mainly based on insecticide applications targeted against the potato psyllid, underscoring the need for development of potato varieties that are resistant to Lso and/or potato psyllid. A field study was carried out during three years in the Toluca Valley, Mexico, to assess the zebra chip resistance of six advanced potato clones. In addition, the commercial variety Fianna was included as a control. There were no significant differences in yield and number of potato psyllid nymphs per plant among the seven potato clones. However, significant differences were observed in the percentage of healthy tubers, area under disease progress curve in the foliage and in the severity of the internal tuber discoloration. The six potato clones showed higher tolerance to ZC symptoms than Fianna.

Additional Key words: Candidatus liberibacter solanacearum, Bactericera cockerelli, potato purple top.

Determinación de la resistencia contra el manchado interno del tubérculo de clones avanzados de papa bajo condiciones de campo en el Valle de Toluca, México

Resumen

“Zebra chip” (ZC), también conocida como “papa manchada” y como “punta morada de la papa”, es una enfermedad que afecta la producción de papa en México, América Central, Estados Unidos y Nueva Zelanda. La enfermedad es causada por la bacteria Candidatus liberibacter solanacearum, la cual es transmitida por el psílido de la papa Bactericera cockerelli. Actualmente, el control de la enfermedad se basa en la aplicación de insecticidas contra el insecto vector, por lo que es necesario generar variedades resistentes contra ZC. Con el objetivo de evaluar la resistencia de seis clones avanzados de papa, durante tres años se realizó un estudio de campo en el Valle de Toluca, México. La variedad Fianna fue utilizada como testigo. No hubo diferencias significativas en rendimiento y número de ninfas de B. cockerelli por planta entre los siete genotipos de papa. Sin embargo, hubo diferencias significativas en el porcentaje de tubérculos sanos, en el área bajo la curva de los síntomas de

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la enfermedad en el follaje y en la severidad del manchado interno de los tubérculos. Los seis clones de papa mostraron mayor tolerancia a la enfermedad que la variedad Fianna.

**Palabras clave adicionales:** *Candidatus* liberibacter solanacearum, *Bactericera cockerelli*, Punta morada de la papa.

**Introduction**

Zebra chip (ZC) disease is one of the main potato production constraints in Mexico, New Zealand, United States, and Central America (Munyaneza, 2012). The symptoms of ZC, also known as ‘potato purple-top’ and ‘internal tuber browning’ in Mexico, include plant stunting, bulging of the stem in areas of leaf insertions, formation of aerial tubers, and the tendency of the top leaves to turn yellow or purple, depending on varieties. These above-ground symptoms of ZC resemble those caused by infection of phytoplasma in potato (Munyaneza, 2012). The tubers from ZC-infected plants exhibit internal browning and generally do not sprout, if sprouting does occur, the sprouts are very thin or threadlike and result in weak or short-lived plants (Munyaneza, 2012). The internal browning of the tubers in a pattern of striations becomes more pronounced when tubers are fried. This is what has led the disease to become known as ‘zebra chip’.

In Mexico, ZC is ubiquitous throughout the country, except in the Northwest (Sonora and Sinaloa States) and a small area in Tapalpa, Jalisco State, where very low incidence of the disease has been observed (Rubio-Covarrubias et al., 2006). Most of the potatoes in Mexico are grown in the central part of the country (Mexico, Tlaxcala, Puebla, Hidalgo, and Veracruz States) mainly on lands, with elevations between 2000 and 3500 m. The Toluca Valley, located in the central plateau of Mexico, used to be an important seed-potato producing region. Because of ZC, seed potato production no longer exists in this region (Rubio-Covarrubias et al., 2011). Prior to the discovery of the association of ZC with the newly described bacterium *‘Candidatus Liberibacter solanacearum’* (Lso) (Hansen et al., 2008; Liefting et al., 2008), the disease was believed to be caused by potato purple top phytoplasmas in Mexico, and vectored by the potato psyllid, *Bactericera (= Paratrioza) cockerelli* Sulc (Leyva-Lopez et al., 2002; Rubio-Covarrubias et al., 2006; Santos-Cevantes et al., 2010). Later studies showed that Lso was indeed widespread in Mexico and associated with the observed symptoms in potato crops (Munyaneza et al., 2009; Rubio-Covarrubias et al., 2011).

Currently, ZC management is mainly based on insecticide applications targeted against the potato psyllid. This control strategy is expensive and pesticide intensive (Butler and Trumble, 2012; Guenthner et al., 2012), underscoring the need for development of potato varieties that are resistant to Lso and/or potato psyllid. Plant resistance to *B. cockerelli*, with both antixenosis (decreased host selection by the insect) and antibiosis (decreases in survival of insects reared on
the resistant plant) has been reported in tomatoes (Casteel et al., 2006). Also, Liu and Trumble (2006) reported antixenosis (described as decreased feeding and oviposition) and antibiosis (increased developmental time and decreases in survival) in a wild-type accession of tomato. In addition, researchers have screened potato material for resistance to adult potato psyllids and identified putatively resistant/tolerant potato clones (Butler et al., 2011; Diaz-Montano et al., 2014).

The identification of ZC-resistant potato varieties or advanced breeding lines is needed for an efficient, sustainable, and integrated pest management strategy for the disease. A number of breeding programs for ZC resistance are underway in ZC-affected countries and are focused on the generation of varieties (Cadena-Hinojosa et al. 2003; Butler et al., 2011; Anderson et al., 2012; Butler and Trumble, 2012; Diaz-Montano et al., 2014; Rubio et al., 2013). While a few potato lines have been found to be tolerant to ZC, no tolerant or resistant varieties have been released so far. The present study reports results from ZC screening trials of six advanced potato breeding lines with good agronomic and commercial characteristics that have shown tolerance to ZC internal tuber discoloration.

**Materials and methods**

The six advanced potato clones used in the present study were selected from 800 lines previously screened under field conditions in the central part of Mexico. These advanced potato lines (8-65, 5-10, NAU, 99-38, 8-29 and 2-75) were selected based on their agronomic and marketable characteristics, in addition to ZC tolerance. Among the selected lines, the clones 8-65, 99-38, 8-29 and 02-75 are resistant to late blight (*Phytophthora infestans*), the clone NAU has good commercial characteristics for the fresh market, whereas the tubers of the clone 5-10 have shown good chipping quality. During three years (2010-2012), the six potato clones and Fianna, a commercial variety used as control, were field tested at the experimental station of INIFAP in Metepec, Mexico State, Mexico. This site is located in the Toluca Valley, which is well known for being the center of origin for late blight (Goodwin et al., 1992; Alarcón-Rodríguez et al., 2014), but also is a place with a high density of *B. cockerelli* and high ZC infection pressure (Rubio-Covarrubias et al., 2011, 2013). The potatoes of the seven genotypes were planted each year in the 2nd week of July and clipped three months after planting, when most of the tubers had reached commercial size. Tubers were harvested 3 weeks after vine killing to allow hardening of their skin. After the sprouts started emerging from the soil surface, which occurred approximately 2 weeks after planting, fungicides were sprayed each week to protect the plants against late blight infection. Applications of insecticides were made weekly during the first 5 weeks post-emergence to help plant establishment and promote tuber setting and production. The insecticides were applied weekly to the foliage in the following order: thiamethoxam, flonicamid, imidacloprid, abamectin and bifenthrin. No further insecticides were applied to allow natural infestations of *B. cockerelli* and *Lso* infection under normal field conditions.

During the three years of the study, potatoes of each of the seven genotypes were planted in a complete block design with 6, 10 and 4 replications in 2010, 2011 and 2012, respectively. The experimental unit was 1, 1, and 5 plants in each of the 3 years, respectively. The rows were 90 cm wide and plants were separated 30 cm inside the rows. The *B. cockerelli* population was monitored by using 3 yellow sticky traps placed 15 m apart in the middle and two edges of the experimental site and the number of adult insects caught on each trap was recorded.
weekly for the 3 years of field evaluations. The traps were placed in the same experimental plot even when there were no potato plants during the winter and spring. The average of the 3 traps per week was used to describe the fluctuation of the insect population in the experimental site. At the end of each potato growing season, prior to vine-cutting, approximately 60 *B. cockerelli* adults from each plot were collected and shipped to USDA-ARS Wapato, WA and tested for Lso by PCR as described by Munyaneza *et al.* (2010).

The variables used to measure ZC resistance in the plants and tubers were: number of *B. cockerelli* nymphs per plant, Area Under Disease Progress Curve (AUDPC), potato yield, percentage of healthy tubers per plant, and severity of internal tuber discoloration. The percentages of the foliage with ZC symptoms were recorded weekly for each plant and used to calculate the AUDPC according to Shaner and Finney (1977).

Three months after planting, when most of the tubers had a commercial size, each plant was clipped at the base and the number of *B. cockerelli* nymphs was scored. All plants were hand-harvested and the tubers of each plant were weighed and stored at room temperature for 5 months, after which the tubers developed sprouts. The number of tubers per plant with normal sprouts (healthy tubers), wire sprouts, and without sprouts was recorded.

The numbers of *B. cockerelli* adults caught in yellow sticky traps during the three years of this study (Figure 1) fluctuated among years. However, lower numbers and no large differences were observed during the potato-growing season from mid-June to September in all three years. This may be related to the application of insecticides during the early stages of

Then, each tuber was cut in cross section and the severity of internal tuber browning was visually scored in raw slices using a scale from 0 to 5, with 0 indicating no discoloration and 5 corresponding to severe discoloration. The severity of internal tuber discoloration was calculated by averaging the scores of the diseased tubers per plant.

Initially, a test of normality was performed for each variable (yield, number of nymphs, % of healthy tubers, AUDPC, and severity of tuber discoloration). The test indicated that only yield was normal, with most variables zero-inflated. Due to this distribution, data were analyzed with generalized mixed models (GMM) fit with appropriate probability distributions (negative-binomial or Poisson) and performed with the package glmmADMB in R 3.1.1. (R core team, 2014) For each variable, the model included a fixed term for clone and a random effect term for year. Data are presented as Analysis of deviance (type II tests). Significant models were further examined using contrasts comparing the mean of the control with the means of the 6 clones. Yield was examined using a generalized linear mixed model with a fixed term for clone and random effect term for year. The relationship among variables was measured by calculating the Spearman correlation coefficients.

**Results and discussion**

potato growth. Although it is important to consider that once the psyllid adults settle onto plants they are mostly sessile, and because they do not have to fly to look for food, the number of adults caught in the yellow sticky traps is lower. The rain that normally occurs during the potato growing season may also contribute to preventing psyllids from leaving the plant canopy.
The general average of the percentage of healthy tubers was 31.7%, which means that the 68.3% of tubers were ZC infected. This high disease incidence is probably related with the high populations of *B. cockerelli* in the experimental site, as determined by the population dynamic (Figure 1) and the presence of nymphs on the plants (general average of 41 nymphs per plant). Besides the high insect population density, the Lso infection rate of the adult insects may contribute to explain the high ZC incidence. The analysis of insects collected in the experimental plot showed that 22, 2 and 7% were positive for Lso in 2010, 2011 and 2012 respectively. Variation from 2.8 to 7.5% in Lso infection rates among 3 years (2009-2011) was observed in the Low Rio Grande Valley, Texas, where the percentage of ZC incidence in tubers was up to 57.5% (Goolsby *et al.*, 2012). Low Lso infection rates of the adult insects are enough to spread the disease in an entire potato field because it has been demonstrated that the adult potato psyllids are highly efficient vectors of Lso (Buchman *et al.*, 2011). The authors observed that a single adult potato psyllid can inoculate Lso to potato in a period of six hours.

The analysis of correlation (Table 1) indicates negative numbers between nymphs and the percentage of healthy tubers, and positive ones with AUDPC and the severity of tuber discoloration. These results confirm the association between *B. cockerelli* and ZC, which has been very well documented and extensively reviewed (Munyaneza, 2012; Butler and Trumble, 2012; Lin and Gudmestad, 2013). This association was also previously demonstrated in the same location where the present field study was carried out.
which is considered as a place with high density of *B. cockerelli* and high ZC infection pressure (Rubio-Covarrubias *et al*., 2011, 2013). The clear expression of ZC symptoms in both, the above and below ground parts of the plants, suggests that the time period during which plants were not sprayed with insecticides, and consequently exposed to greater numbers of psyllids (5 weeks before clipping), was enough to result in the infection of the plants with Lso. Previously, it was reported that ZC infections initiated five weeks before harvest can cause ZC symptoms in field grown potatoes (Rashed, 2013; Wallis *et al*., 2014) and yield losses have been observed in the range from 49.9 to 87.2%, depending on the resistance to ZC of diverse potato varieties grown in the field (Munyaneza *et al*., 2011).

Table 1. Spearman correlation among yield, number of nymphs per plant, % of healthy tubers, AUDPC and severity of tuber discoloration. N=123 to 130.

<table>
<thead>
<tr>
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<th>YIELD</th>
<th>% HEALTHY TUBERS</th>
<th>AUDPC</th>
<th>TUBER DISC.</th>
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</thead>
<tbody>
<tr>
<td>NYMPHS</td>
<td>-0.149</td>
<td>-0.455 *</td>
<td>0.414*</td>
<td>0.433*</td>
</tr>
<tr>
<td>YIELD</td>
<td>0.209*</td>
<td></td>
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<tr>
<td>% HEALTHY TUBERS</td>
<td></td>
<td>-0.372*</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>AUDPC</td>
<td></td>
<td>-0.495*</td>
<td>-0.382*</td>
<td>0.385*</td>
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*P<0.05

The analysis of deviance indicated that there were statistical differences among the seven genotypes in the percentage of healthy tubers, AUDPC and tuber discoloration, nevertheless, no differences were detected in yield and number of nymphs. Based on these results, the means of the significant variables were further analyzed. However, it is important to make some considerations about yield and number of nymphs. The general mean was 41 nymphs per plant, which indicates that there was a high population density of insects at the end of the potato growing season, which may have induced the insects to colonize all the available plants, regardless of the individual plants relative attractiveness to the psyllids. At low densities, it is possible that insects can be selective, but as densities increase they are likely forced to move onto other, less suitable, plants. This may eventually result in infestation of the entire field. Regarding plant resistance to the potato psyllid, both antibiosis and antixenosis have been reported in potatoes (Butler *et al*., 2011; Diaz-Montano *et al*., 2014; Prager *et al*., 2014). However, the presence of these resistance mechanisms cannot be demonstrated in the present study.

Regarding the yield, the analysis of deviance indicated that the seven genotypes had similar yields with an average of 1.26 kg/plant, which may be considered a normal yield. To explain these results, it should be considered that all the six clones were previously selected because they had good performance in the Toluca Valley and Fianna had also shown high yield, which is one of the reasons it has been the main commercial variety in this region. Furthermore, it should be considered that the plants were protected with insecticides during the first five weeks after their emergence from the soil surface, a period that lasts until the tuber initiation stage, by which time the plants...
might have developed enough foliage to support their tuber growth.

The means of the three variables that presented statistical differences (% of healthy tubers, AUDPC, and severity of tuber discoloration), were analyzed by comparing Fianna with each clone (Fig. 2). This figure shows significant differences between Fianna with 8-65 and 99-38 in percentage of healthy tubers, with 8-65, 5-10, 8-29 and 02-75 in AUDPC and with all clones in tuber discoloration. Collectively, these results indicate that the 6 clones performed better than the commercial variety Fianna. The clone 8-65 presented the highest % of healthy tubers, the lowest AUDPC and the lowest severity of tuber discoloration. The response of all these variables suggests that, among the 6 clones, 8-65 possesses the highest tolerance to ZC.

**Figure 2.** Means of percentage of healthy tubers, AUDPC and the severity of tuber discoloration in the seven potato genotypes. * Significant difference compared with Fianna, contrast test P<0.05.

In this study, the healthy tubers were those that had normal sprouts and no internal discoloration. It is well known that infection with Lso may result in tubers
with abnormal sprouts (Henne and Workneh, 2010; Munyaneza, 2012). The negative correlation between psyllid nymph numbers with healthy tubers and the presence of Lso-positive insects, indicate that psyllids transmitted the bacterium to the plants causing abnormal tuber sprouting. The clones 8-65 and 99-38 showed higher percentages of healthy tubers than the control (Fianna), which open the possibility that those two clones have some resistance mechanism that decreases the translocation of the bacterium from the foliage to the tubers. Further studies are needed to clarify this issue.

The AUDPC represents the physiological alterations caused by psyllid feeding and Lso infection, which probably result from blockage of the phloem (Munyaneza, 2012; Butler and Trumble, 2012; Lin and Gudmestad, 2013). Three clones (8-65, 5-10 and 8-29) presented lower ZC symptoms than Fianna; however, there were no differences in yield between these genotypes and Fianna. Under the environmental conditions in the Toluca Valley, Fianna is a variety with vigorous foliage, which may have contributed to support its tuber growth regardless of its ZC foliage damage. It is also important to consider that the plants were protected with insecticides until the tuber initiation stage and then the plants developed enough foliage to support their tuber growth.

Comparison of mean severity of tuber discoloration shows a clear difference between Fianna with the other six clones. Tuber discoloration may be regarded as the final and most important of the ZC symptoms. Since the severity of tuber discoloration was measured in freshly-cut tubers, it is assumed that the dark color was conferred by the enzymatic oxidation of phenolic compounds, which may be produced as a defense mechanism in ZC tubers (Navarre et al., 2009; Wallis et al., 2012; Wallis et al., 2014). Furthermore, the dark color after frying slices of diseased tubers has been associated with increasing amino acids and reducing sugars. However, the content of reducing sugars in the tubers may also be influenced by potato variety and the climatic conditions. The present field study was performed in a location with low temperature and humid conditions during the potato growing season, and it is well known that these climatic conditions may induce high tuber sugar content (Hamouz et al., 2004; Meulenaer et al., 2008). Based on these considerations, the color of fried slices could not be indicative of the effect of the ZC infection and effect of environmental conditions. Thus, in this study, the determination of the internal discoloration in raw tubers that presented other ZC symptoms, like wire sprouts or no sprouts, is regarded as more reliable.

Interestingly, the clone 02-75 did not show ZC symptoms in the foliage but presented internal tuber discoloration, which suggests separated resistance mechanisms in the foliage and in the tubers. Additional observations to this study have shown foliage ZC symptoms in the clone 02-75 when the plants were infected since the initial development stage (Rubio, unpublished). These findings support the work of Levy et al. (2011), who observed that the movement of Lso inside the plant may occur according with a source-to-sink metabolite stream and consequently depends on the developmental stage of the plant. The clone 02-75 initially exhibits vigorous foliage growth and if the plants are infected with Lso after this stage, when the carbohydrates from leaves are mobilized to the tubers, then the foliar symptoms may not be evident in the foliage but symptoms may appear in the tubers.

In conclusion, the high numbers of nymphs in the seven genotypes, the capture of adult insects during the entire year, and the presence of Lso in the insects confirm the highly ZC infective conditions in the experimental site. Compared with the
commercial variety Fianna, the six clones presented higher tolerance to ZC symptoms in the tubers and they possess commercial characteristics that make them candidates to be released as varieties. Among these clones, the most outstanding for its characteristics of ZC tolerance was the 8-65. In the present study it was not possible to clarify the exact mechanism of the tolerance exhibited by the tested clones and further studies are needed to elucidate this issue.

Conflicts of interest
This publication has no conflicts of interest.

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