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Assessment of resistance to *Phytophthora cinnamomi* Rands in Mexican race avocado genotypes by electrical conductivity

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1 ABSTRACT
Phytophthora root rot caused by *Phytophthora cinnamomi* Rands., is distributed worldwide and causing great economic losses to the avocado producers. The search for avocado rootstocks that present resistance against this rot has been done by screening in germplasm banks and wild materials of this species. Resistance of avocado to *P. cinnamomi* has been determined through of different methods, both direct (shoots, stem and roots inoculation with zoospore suspension) and indirect (mycelium attraction and electrolyte leakage). In this study, the objective was to evaluate the resistance of ten Mexican race avocado genotypes against isolates of *P. cinnamomi* (Pc1, Pc2) by changes in the electrical conductivity in inoculated roots fragments suspension. Electrical conductivity was measured and registered 24, 48, 72 and 96 h after inoculation. Variance analysis was carried out and no effect was found on genotypes x isolates interaction, yet significant differences were found within genotypes and within isolates. 'Duke 7' genotype was identified as resistant, showing the lowest electrical conductivity average value of 0.141 mS·cm^{-1} from all the evaluated genotypes. *P. cinnamomi* isolate Pc2 was found to be significantly ($P \leq 0.05$) more pathogenic by inducing an average change of 0.536 mS·cm^{-1} on the electrical conductivity of the avocado genotypes, while isolate Pc1 showed an average value of 0.470 mS·cm^{-1}. Most genotypes presented values greater than 1 on the ratio in electrical conductivity change. Therefore, even when this technique cannot be used as an absolute test for resistance, it is suggested to be considered as a complementary test in a rootstock selection process for resistance.

2 INTRODUCTION
Plant disease from avocado (*Persea americana* Mill.) known as root rot, is caused by *Phytophthora cinnamomi* Rands., and is distributed worldwide (Pegg *et al*., 2002), causing great economic losses to the producers. The search for avocado rootstocks that present resistance against this rot has been done by screening in germplasm banks and wild materials of this species. Among the direct and indirect methods that have been reported for resistance determination of avocado to *P. cinnamomi* are: mycelium attraction by callus in *in vitro* tissue cultures (Dizon *et al*., 2000), etiolated shoots inoculation with zoospore suspension (Dolan and Coffey, 1986), inoculation of plants at the base of the stem with zoospore suspension

(Andrade-Hoyos *et al.,* 2015; Ochoa-Fuentes *et al*., 2015), stem injury technique (Rodríguez *et al*., 2017), temperature controlled water tanks technique (Andrade, 2012), hydroponic systems (Neilsen, 2016), seeds or seedlings sowing in infected substrate (Ploetz *et al*., 2002), evaluations on infested fields (Gallo *et al*., 2003) and changes in electrical conductivity of inoculated roots fragments suspension (Zilberstein and Pinkas, 1987; Gabor and Coffey, 1991; van der Merwe *et al*., 1992; Salgado and Fucikovsky, 1996; Rodríguez *et al*., 2015). The electrolyte leakage from fine roots is a measurement of the capacity of the membranes in the root system to retain ions (Ritchie *et al*., 2010). Damaged membranes tend to liberate ions due to the penetration capacity of the pathogen, as well as the action of extracellular enzymes involved in the degradation of host cell wall. This is because, in infected cells, the membrane separates from the cell wall indicating a severe turgor loss together with a thickening of the cell wall and endodermis destruction causing a severe imbalance in osmoregulation (Oßwald *et al*., 2014). If electrolyte leakage is quantified by changes in electrical conductivity of the solution, an indicator of the viability of the root system can be obtained. Electrolyte leakage has been used to evaluate the effect of injuries caused by cold, rough plant handling, desiccation, cold or warm storage and other types of stress in root viability and plant vigor (Ritchie *et al*., 2010). Therefore, the objective of this study was to evaluate the resistance of ten Mexican race avocado genotypes against two isolates of *P. cinnamomi* by changes in the electrical conductivity in inoculated roots fragments.

3 METHODOLOGY

The present study was carried out during the summer of 2017 at the Agronomy School of the Universidad Autónoma de Nuevo Leon' in Mexico. Ten Mexican race avocado genotypes, 'Todo el Año', 'Plátano Delgado', 'Bola', 'Leonor', 'Plátano', 'Plátano Temprano', 'Silvestre', 'María Elena', 'Criollo 6', 'Criollo 3', from the municipalities of Aramberri and General Zaragoza in Nuevo León, Mexico, were evaluated for resistance to two isolates of *P. cinnamomi* (Pc1 and Pc2). 'Hass' genotype (Mexican race X Guatemalan race, from seed) was used as susceptible control (Ramírez *et al*., 2014; Rodríguez *et al*., 2017) and 'Duke 7' genotype (Mexican race, from clonal origin) as moderate resistant control (Coffey, 1987). Five 6-months old seedlings per avocado genotype were used, where from each seedling, the substrate was removed by water immersion. Six root fragments of 40 mm of length were cut and rinsed with distilled water (dH_2O), five were placed in the same container with 20 ml of dH_2O and 0.5 ml of inoculum; the last remaining was placed on a separate container with 20 ml of dH_2O without inoculum that was used as a blank. A double blank was established, consisting in a container with 20 ml of dH_2O inoculated with mycelium suspension and without root fragments. Four erlenmeyer flasks of 250 ml were prepared with 100 ml of V8 Juice® clarified liquid medium (200 $ml \cdot L^{-1}$ V8 Juice®, 2 $g \cdot L^{-1}$ $CaCO_3$, and 800 $ml \cdot L^{-1}$ of dH_2O, clarified by centrifugation) prepared as suggested by Zentmyer *et al.* (1976). The flasks were sterilized by autoclaving at 121 ºC and a pressure of 1.05 $kg \cdot cm^{-2}$ for 15 min, allowed to cool down at room temperature and later inoculated with eight 0.5 cm of diameter cylinders with mycelium taken from the periphery of a growing fungal colony on an agar plate. Mycelium was allowed to grow for 7 days at dark and 25 ºC on a rotatory shaker at 200 rpm. The content of 2 flasks of the same isolate was homogenized, from which 0.5 ml of mycelium suspension (85 $mg \cdot ml^{-1}$) of *P. cinnamomi* served as inoculum for the containers with the roots fragments. In order to determine the concentration of mycelium in dry weight per volume, two volumes of 50 ml of the homogenized mycelium were taken and filtered with Whatman® No. 1 filters and allowed to dry at 60 °C in a stove until constant weight. Electrical conductivity was measured and registered after 24, 48, 72 and 96 h with a portable conductivity meter (HI-9814, Hanna Instruments, USA) for all the treatments, rinsing the measuring probe with dH_2O between measurements to avoid cross-contamination in the samples. The present experiment followed a completely randomized design with factorial arrangement A x B, where factor A corresponded to the 12 avocado genotypes and factor B corresponded to the two *P. cinnamomi* isolates, giving a total of 24 treatments with 5 replicates each. Variance analysis and Tukey´s means comparison ($P \leq 0.05$) were done using the Statistic Software Diseños Experimentales FAUANL v. 1.6 (Olivares-Sáenz, 2015). The ratio in the electrical conductivity change caused by the isolates Pc1 and Pc2 on avocado genotypes was also calculated, this was done dividing the value of electrical conductivity from the inoculated genotype by the electrical conductivity of the same genotype without inoculum.

4 RESULTS AND DISCUSSION

The twelve avocado genotypes showed an increase in electrical conductivity through time since the inoculation with the *P. cinnamomi* isolates was done (figure 1). The controls without inoculum tended to be stable, this was not the case for 'Duke 7' and 'Hass', which presented an increase in electrical conductivity; however, after 72 h there was a reduction in the changes of electrical conductivity for 'Duke 7' and 'Hass', tended to stabilize after been inoculated with Pc1 and diminished in the treatment with Pc2.

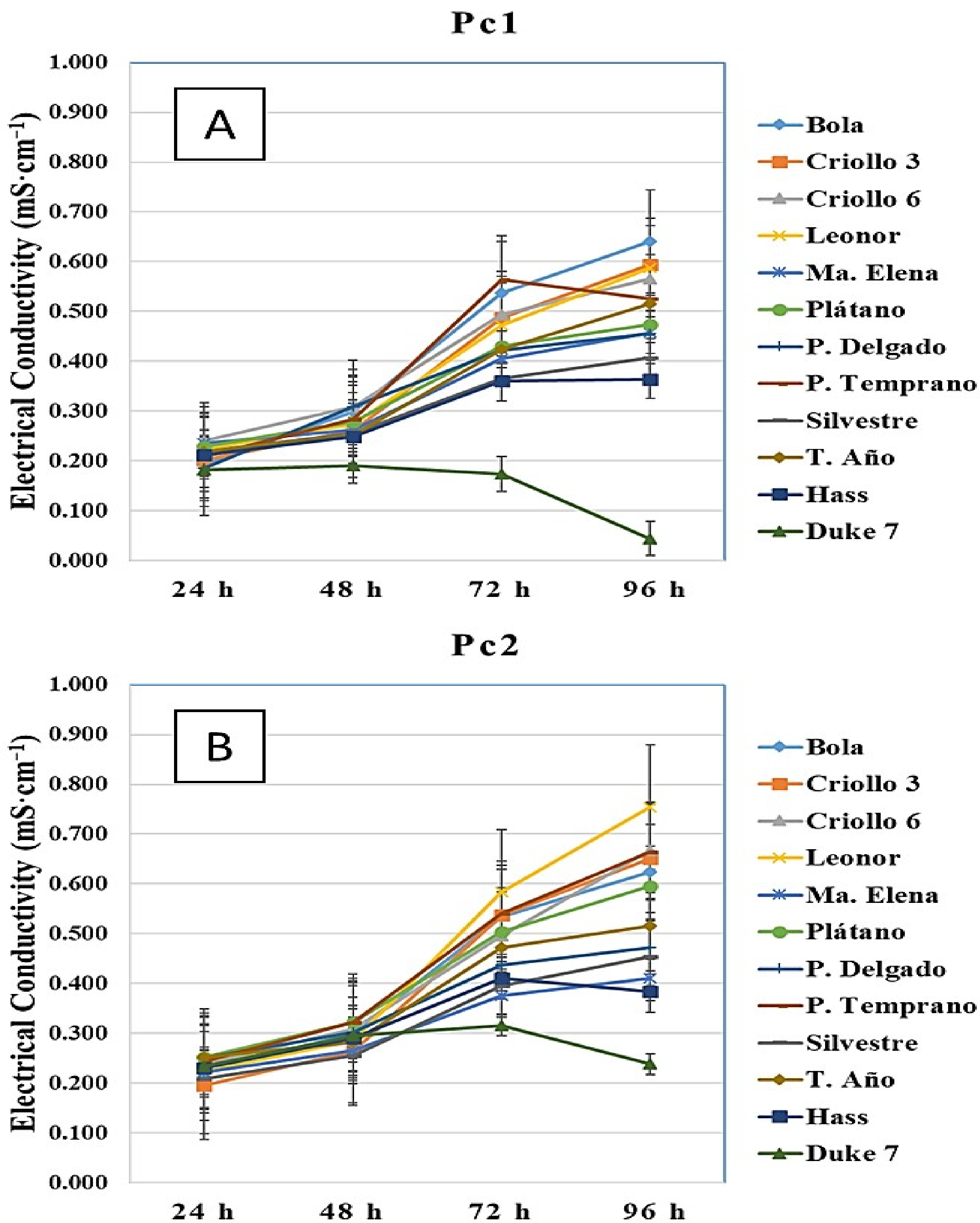

Figure 1: Changes in electrical conductivity for the avocado genotypes through time. A) Twelve avocado genotypes evaluated for resistance against Pc1 isolate of *Phytophthora cinnamomi*. B) The same twelve avocado genotypes evaluated for resistance against Pc2 isolate of *P. cinnamomi*.

From treatments with Pc1, 'Bola' was the most susceptible genotype showing the highest increase value in electrical conductivity of 0.640 $mS \cdot cm^{-1}$, followed by 'Criollo 3' (0.594 $mS \cdot cm^{-1}$) and 'Leonor' (0.566 $mS \cdot cm^{-1}$). In the case of treatments inoculated with Pc2, the most susceptible genotypes were 'Leonor' with 0.754 $mS \cdot cm^{-1}$, 'Criollo 6' with 0.668 $mS \cdot cm^{-1}$ and 'Plátano Temprano' with 0.664 $mS \cdot cm^{-1}$. 'Duke 7' behaved as the most resistant genotype, presenting the lowest electrical conductivity increase value for both isolates Pc1 (0.044 mS·cm^{-1}) and Pc2 (0.238 mS·cm^{-1}). Although 'Hass' has been reported as a susceptible genotype (Ramírez *et al.*, 2014; Rodríguez *et al.*, 2017), according to the results in the present study, it might be possible that this genotype presents some resistance to *P. cinnamomi*, since for both isolates was the second genotype with the lowest electrical conductivity increase values after 'Duke 7', showing electrical conductivity increments of 0.364 mS·cm^{-1} for treatments with Pc1 and 0.384 mS·cm^{-1} for Pc2. At the variance analysis, no significant differences were found ($P \leq 0.177$) at the genotypes x isolates interaction, significant differences were founded only within avocado genotypes ($P \leq 0.000$) and within *P. cinnamomi* isolates ($P \leq 0.001$). In the Tukey's means comparison ($P \leq 0.05$), 'Duke 7' genotype was identified as resistant, presenting the lowest electrical conductivity average value from all evaluated genotypes (figure 2). The differences in susceptibility or resistance showed by the genotypes might be due to variability at genetic-, physiological-, and biochemical level presented by the avocado genotypes and *P. cinnamomi* isolates, as well as its interaction with the environment.

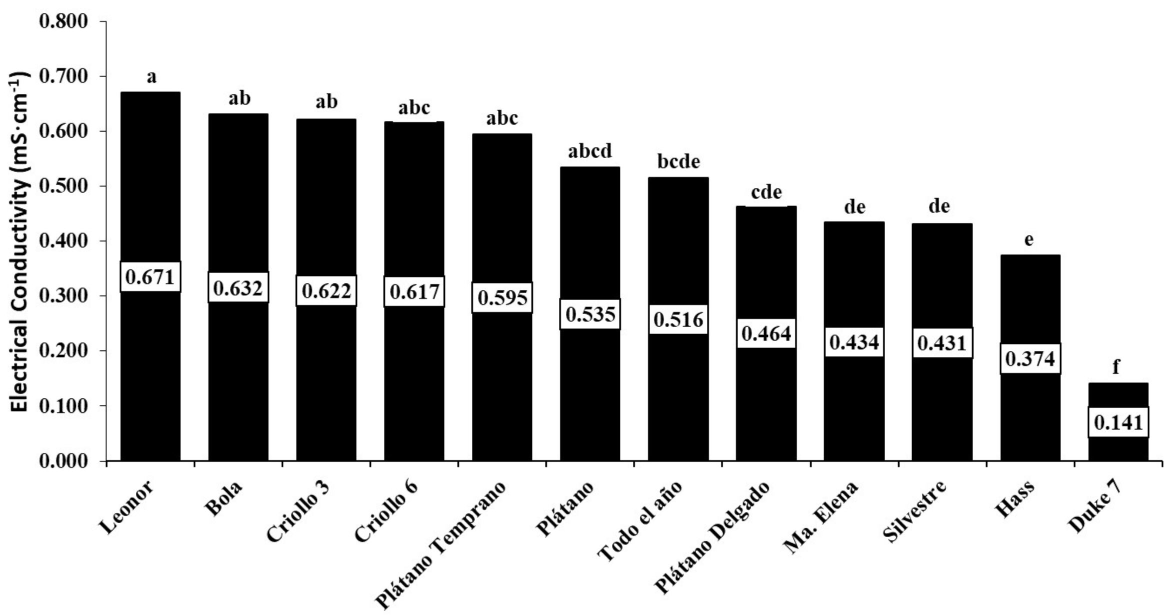

Figure 2: Electrical conductivity of roots from different avocado genotypes inoculated with *Phytophthora cinnamomi*. Different letters show significant differences (Tukey $P \leq 0.05$).

The results obtained for 'Duke 7' agree with the results published by Zilberstein and Pinkas (1987) and, Salgado and Fucikovsky (1996), by corroborating this genotype as resistant due to the low electrical conductivity value that it presented with respect to the genotypes that turned out to be susceptible. Furthermore, Hass showed a moderate level of resistance, coinciding with that reported by Neilsen (2016). On the other hand, Smith *et al.* (2011) indicated that clonally propagated Hass plants remain healthy in soils with high pressure from *P. cinnamomi*. Pc2 isolate was significantly ($P \leq 0.05$) more pathogenic, inducing an average of 0.536 mS·cm^{-1} in the change of EC on the different avocado genotypes, while Pc1 presented an average of 0.470 mS·cm^{-1}. Pathogenicity differences among *P. cinnamomi* isolates have been reported previously and may be the result of genetic variability within the species (Ochoa-Fuentes *et al*., 2009, 2015). The ratio in the electrical conductivity change indicates the effect of *P. cinnamomi* on each genotype (figure 3), this is done because a direct comparison between genotypes cannot be carried out due to the differences that can be observed in electrical conductivity caused by their own physiological and biochemical characteristics as genotype (Salgado and Fucikovsky, 1996).

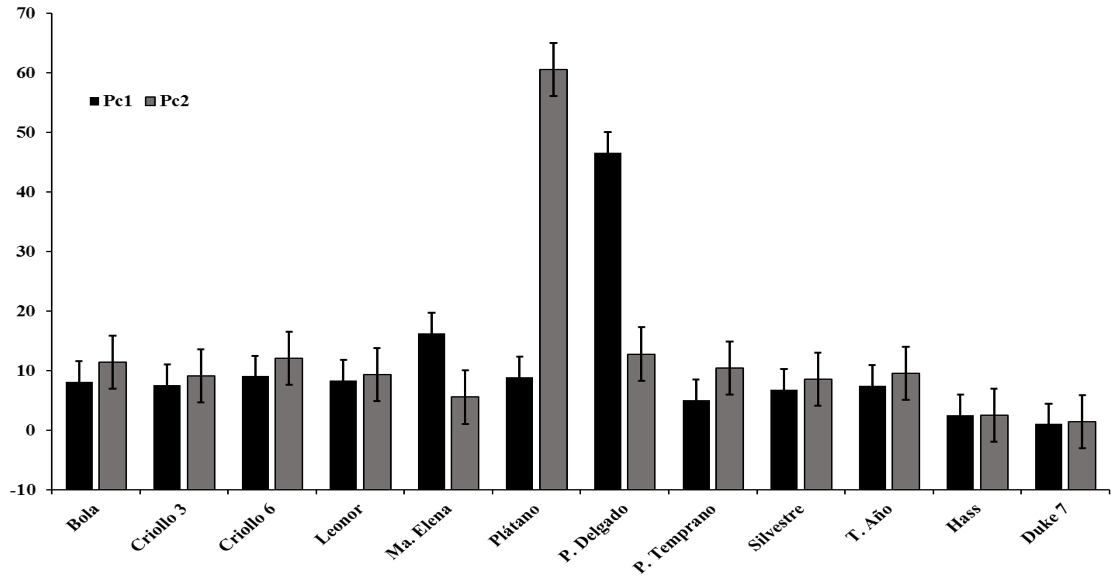

Figure 3: Ratio in the electrical conductivity change (Inoculated/Non-inoculated) of the avocado genotypes for the two isolates of *Phytophthora cinnamomi,* Pc1 and Pc2.

Most genotypes presented values higher than 1, indicating an effect of the inoculum over the membrane permeability of the roots fragments. 'Duke 7' presented the lowest values of 0.96 for Pc1 and 1.40 for Pc2, showing its resistance against the pathogen (Zilberstein and Pinkas, 1987). Additionally, the most susceptible genotypes to Pc1 were 'Plátano Delgado' with an electrical conductivity value of 46.60 and 'María Elena' with electrical conductivity of 16.27. For Pc2, the most susceptible was 'Plátano' showing an electrical conductivity value of 60.60. The differences detected in the level of susceptibility or resistance presented by the genotypes for each isolate of the pathogen may be due to the specific interaction, since one genotype may be resistant to one isolate and susceptible to another; likewise, an isolate can be virulent in one genotype and avirulent in another (Roelfs *et al*., 1992). It is important to consider that by means of this technique it is only possible to indirectly evaluate some of the different defense mechanisms that may occur, such as the accumulation of polyphenolic compounds around the cells that reinforce the cell wall and inhibit mycelial growth (Andrade, 2012), as well as the constitutive production of compounds with inhibitory activity against *P. cinnamomi* (Sánchez-Pérez *et al*., 2009), which would lead to less tissue damage due to the inhibition of the pathogen growth and therefore a minor change in the electrical conductivity increase. However, it is not possible to evaluate other mechanisms such as the capacity to regenerate new roots as a response of genetic resistance based on survival (Andrade, 2012), which is considered as another escape mechanism to the disease and that has been previously reported in 'Duke 7' (Kellam and Coffey, 1985).

6 CONCLUSION

The results obtained through this technique did not allow detecting any genotype with resistance equal to or better than 'Duke 7' but, allowed to infer the possible behavior of the tested genotypes against multiples isolates of the pathogen. Therefore, it is useful when the evaluation of a large number of avocado genotypes against one or more isolates of *P. cinnamomi* is desired, thus reducing the evaluation time and test surface needed. Subsequently, the genotypes selected as resistant must be further evaluated in an infected substrate or in infected fields in multiple locations. Finally, even when this technique cannot be used as an absolute test of resistance, it is suggested to be considered as a complementary test in a rootstock selection process for resistance.

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