EVALUATION OF TOLERANCE TO VEGETATIVE ANTRACNOSIS OF NEW MANGO GERMPLASMS IN MEXICO[1]

[EVALUACIÓN DE LA TOLERANCIA A LA ANTRACNOSIS VEGETATIVA DE NUEVOS GERMOPLASMAS DE MANGO EN MÉXICO]

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SUMMARY

Anthracnose (*Colletotrichum gloeosporioides* Penz.) is one of the most important diseases of mango (*Mangifera indica* L.) due to its wide distribution in the world and to induce severe epidemics in the vegetative and reproductive stages, causing important production losses. The objective of this research was to evaluate the tolerance to *C. gloeosporioides* infection in the vegetative stage of eleven new mango cultivars in Mexico with potential in the export market. Leaves of 15 days old of development were inoculated with the *Gro* isolate highlighted in virulence. Based on the incubation, period, incidence and severity, "Alphonse", "Neelum", "Kesar and Ivory" cultivars were low susceptibility, "Nam Doc Mai", "Rosygold", "Cotaxtla" were highly susceptible, and "Fairchild", "Ataúlfo Diamante", "Ataúlfo Zafiro" and "Mallika" had medium susceptibility. The longer incubation periods determined the lowest severity (r^2= -0.89 and -0.90) and incidence (r^2= -0.77 and -0.85). The incidence correlated positively with severity (r^2= 0.86 and 0.92), both inoculation techniques (DLT and ALT) were useful to induce typical symptoms of anthracnose and to estimate the expression of virulence (incubation period, incidence and severity) of the pathogen.
Key words: *Mangifera indica*; *Colletotrichum gloeosporioides*; cultivars; severity; susceptibility.

RESÚMEN

La antracnosis (*Colletotrichum gloeosporioides* Penz.) es una de las enfermedades más importantes del mango (*Mangifera indica* L.) debido a su amplia distribución en el mundo y por inducir severas epidemias en etapas vegetativas y reproductiva que conllevan a importantes pérdidas de producción. El objetivo de esta investigación fue evaluar la tolerancia a la infección por *C. gloeosporioides* en etapa vegetativa de once nuevos cultivares de mango en México con potencial en el mercado de exportación. Se inocularon hojas de 15 días de desarrollo con el aislamiento virulento *Gro*. Basándose en el período de incubación, incidencia y severidad, los cultivares "Alphonse", "Neelum", "Kesar e "Ivory" presentaron baja susceptibilidad, "Nam Doc Mai", "Rosygold", y "Cotaxtla" fueron altamente susceptibles mientras que "Fairchild". "Ataúlfo Diamante", "Ataúlfo Zafiro" y "Mallika" exhibieron susceptibilidad media. Los períodos de incubación más largos determinaron la menor severidad (r^2 = -0.89 y -0.90) e incidencia (r^2 = -0.77 y -0.85). La incidencia se correlacionó positivamente con la severidad (r^2 = 0,86 y 0,92), ambas técnicas (DLT y ALT) de inoculación fueron útiles para inducir síntomas típicos de antracnosis y para estimar la expresión de virulencia (período de incubación, incidencia y gravedad) del patógeno.
Palabras clave: *Mangifera indica*; *Colletotrichum gloeosporioides*; cultivares; severidad; susceptibilidad.

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INTRODUCTION

The mango (*Mangifera indica* L.) is grown in most tropical and subtropical areas of the world and represents the third tropical fruit of economic importance (Galan, 2009). India is the leading producer, while Mexico is the leading exporter (FAOSTAT, 2016). In Mexico between 1997 and 2004, exports declined by 24 points due to the lack of new yellow polyembryonic competitive cultivars that demand the US market, the main export destination of mexican mango (Malc *et al*., 2011). In Mexico, "Ataúlfo" is the main cultivar and its production is limited due to problems by phytosanitary, fertilization and marked (Becerra, 1995; Malc *et al*., 2011).

Anthracnose (*Colletotrichum gloeosporioides*) is one of the most important diseases in pre and post-harvest fruits in all producing areas in the world. The pathogen also infects leaves, flowers and branches. The disease is more severe under high relative humidity and abundant rainfall; anthracnose may cause loss of production of 50 to 100% in orchards with poor agronomic management (Arauz, 2000; Monteon-Ojeda *et al*., 2012). Most studies on anthracnose epidemiology or anthracnose pathogenesis emphasize commercially critical phenological stages, such as flowering and fruiting, however mango trees are highly susceptible to anthracnose mango during non-critical stages such as vegetative stage, have higher epidemic rates due to this polycyclic pathogen. Secondary infection cycles usually determine the intensity, frequency and duration of epidemics and promote high severity during commercially critical phenological stages such as fruiting (Tivoli *et al*., 2006). This problem is potentiated in mango since this crop have several (more than three) vegetative flows, depending on the cultivar (Delgado *et al*., 2011; Litz, 2009). This phenomenon may contribute significantly to the increase the inoculum and disperse it. It has been observed that inoculation of leaves and detached fruits offers a good positive prediction of mango tolerance so that these techniques could be used for the screening of germplasm (Bigirimana 2001, Iwaro, 1997, Tu 1986). In all mango producing areas it is necessary to implement integrated phytosanitary management strategies that incorporate the evaluation of new cultivars showing tolerance to anthracnose. The objective of this research was to evaluate the tolerance to anthracnose in the vegetative stage of 11 cultivars of yellow skin in Mexico that include genotypes of extensive cultivation in Mexico ("Cotaxtla"), recent introduction ("Nam Doc Mai", "Rosigold", "Mallika", "Ivory", "Alphonse", "Neelum", "Fairchild" and "Kesar", from the Tropical Botanic Garden, Florida, USA) and recent registration ("Ataúlfo Diamante" and "Ataúlfo Zafiro") INIFAP (acronym of National Agricultural and Livestock Forestry Research).

MATERIALS AND METHODS

The study was developed during 2015-2016 at the Academic Unit of Agricultural and Environmental Sciences of the Universidad Autonoma de Guerrero, located in the municipality of Iguala, Guerrero, Mexico (18 ° 25' N, 99° 35´ W at 731 masl). Mango plants of 18 months-age of the cultivars "Cotaxtla", "Nam Doc Mai", "Rosigold", "Mallika", "Ivory", "Alphonse", "Neelum", "Fairchild", "Kesar" "Ataulfo Diamante" and "Ataulfo Zafiro" were planted in 5 L capacity plastic pots in a nursery (plastic caliber 600 and mesh with 50% of shade). The plants were fertilized weekly with Steiner nutrient solution applied to the soil (1 L^{-1} per plant), spray to foliage (1 mL L^{-1}), and watered every two days until field capacity.

Inoculum

Inoculations were carried out using a previously evaluated mono-conidial strain of *C. gloeosporioides* (*Gro*) obtained from disease fruits in the laboratory of the Phytopathology Institute of the Colegio de Postgraduados, Mexico; isolated and identified from mango commercial orchards with anthracnose in the state of Guerrero, Mexico. This isolate has high growth, germination and sporulation rates as well as virulence in mango leaves (data not show). Conidial suspension was prepared when a 2 cm of diameter PDA mycelia growth disc from a 7-day-old culture (incubated on PDA at 24 ± 3 °C) were placed in a blender (Oster® 500w) with 50 mL of sterile distilled water and blended four times during two-second intervals to promote conidia and acervuli detachment. The mix was pass through a 200 mesh / in^2 and the filtrate was collected into a glass beaker and vortex (Mixer®) for 10 seconds. Conidial concentration was adjusted to $1X10^5$ conidia mL^{-1} using a Neubauer chamber and suspended in Polyoxyethylene-20-sorbitan monolaurate (0.1%).

Inoculation on detached leaves

Detached leaves were inoculated using a modified technique that consisted in: vegetative buds were marked on each cultivar plants in order to observe foliage development; leaves were detached when they were 15 days-old. Leaves were disinfested with sodium hypochlorite (NaCl) at 0.5% for 30 s, rinsed three times with sterile distilled water and blotted in a laminar flow chamber. Sterile towels were placed inside a clean, disinfested plastic container (25 x 35 x 10 cm) and the towels were saturated with sterile distilled water. The disinfested and dried mango leaves were placed on the wet towels inside the plastic container. Half of each leaf (respecting to the central nerve) was inoculated by depositing three drops (50 µL) of the inoculum in the abaxial surface. Drops were placed separately and approximately 35 mm each

other. The plastic containers with the inoculated leaves were sealled and incubated at 24 ± 3ºC, under alternate 12:12 dark (initial condition): light conditions and 100% relative humidity. Five leaves were placed in each container (experimental unit) and five containers (replicates) per cultivar (treatments). The incubation period (time from inoculation to symptoms) and incidence (%) were evaluated one time and severity (%) was evaluated eight days after inoculation measuring each necrotic lesion diameter (cm) with a digital Vernier. Temperature, relative humidity and light intensity were controlled using a humidifier and PAR light lamps and measure with a datalogger HOBO® Model U12. A completely randomized statistical design was used in this experiment and the whole experiment was replicated three times.

Inoculation on attached leaves

Vegetative buds were marked and when leaves were 15-days-old the leaves were disinfested with 0.5% NaCl solution for 30 s, rinsed three times with sterile distilled water using a hand-held backpack sprayer (Swissmex®) and dried at environment temperature (29-31 ºC) for 10 min. Inoculum was spread on one half (considering the central foliar nerve as reference) of both abaxial and adaxial surface using a soft brush with approximately 500-600 µl (camel hair). Five leaves per plant (experimental unit) were inoculated and four plants (replicates) per cultivar (treatments) were evaluated. Inoculation was carried out before sunset (18:00 h ± 300-450 lx). Inoculated plants were covered with a dark plastic bag during the first 12 h and then kept in the nursery (temperature 29-31 ºC, relative humidity 85 to 90% and photoperiod 12±1 h light, recorded with a datalogger HOBO® Model U12) until symptoms appeared. Incubation period (days after inoculation) and incidence (%) were recorded. Severity (%) was evaluated 15 days after inoculation by digital images. Proportion of affected area was determined using GIMP 2.0 software for Windows®. A completely randomized block statistical design was used for this experiment and the whole experiment was replicated three times.

Re-isolating strains

Vegetative tissues with symptoms of anthracnose collected from the experimental units of both trials (detached and attached leaves) were fragmented into pieces of approximately 1 cm in length, isolating were made using a monosporic culture technique and the species was corroborated using the taxonomic keys of Ainsworth *et al*. (1973), Barnett and Hunter (1998) and Sutton (1992).

Statistical analysis. For each one of the trials was made an variance analysis (GLM) and mean tests (LSD, p = 0.05) performed with SAS v.9.1.3 statistical software (SAS Institute Inc, 2003). In order to detect correlations among variables, Pearson correlation tests and Linear and power (log-log) regression analysis were performed using Sigma-Plot® calculating *P* and r^2 values.

Cultivars categories

In detached leaf technique (DLT) it was considered as susceptibility "low" the cultivar that showed more than three days of incubation period, less than 86% and 2.5% of incidence and severity respectively, as "medium" those with more three days of incubation period, between 86.1 and 92% of incidence and from 2.7 to 3.6% of severity and as "high" to those with less than three days of incubation period, more than 93% and 4% of incidence and severity respectively; in the other hand in attached leaf technique (DLT) it was considered as susceptibility "low" the cultivar that showed more than seven days of incubation period, less than 50% and 2.1% of incidence and severity respectively, as "medium" those with more seven days of incubation period, between 51 and 64% of incidence and from 2.1 to 2.7% of severity and as "high" to those with less than seven days of incubation period, more than 62% of incidence and more than 3% of severity.

RESULTS AND DISCUSSION

Varietal tolerance

According to the results observed in the detached (DLT) and attached leaf techniques (ALT), cultivars “Alphonse” and “Neelum” show the lowest values of severity (1.65-2.11%) and incidence (49.41-60.83%) with the most extended period of incubation (7.43-8.87 *days*) that classified them as low susceptibility to anthracnose. In contrast, “Nam Doc Mai”, “Rosygold” and “Cotaxtla” cultivars show the highest incidence and severity, with the short period of incubation, being highly susceptible to anthracnose. “Fairchild”, “Ataúlfo Diamante”, “Ataúlfo Zafiro” and “Mallika” were in the medium; “Kesar” (DLT) and “Ivory” (ALT) were of low susceptibility to anthracnose (Figure 1, Tables 1 and 2). It has been implied documented that fruits of commercial cultivars are susceptible to anthracnose, however, no studies were found of tolerance mango screening to anthracnose in vegetative tissue (Nishijima, 1993; Paez, 1997; Pernezny and Ploetz, 2000). We observed that all cultivars evaluated were affected by anthracnose, and "Alphonse", "Neelum", "Kesar" and "Ivory" show the highest tolerance. These results were similar to those reported by Lei et al. (2006) and Chanana et al. (2005) who observed moderate resistance in “Mallika” cultivar, but contrasted with Nishijima (1993) and Haggag (2010) who reported that "Neelum" and Alphonse" were very susceptible and classified "Fairchild" as resistant. Severity of anthracnose in the

field may depend on the complex of species (*C. gloeosporioides* is the main species found in field) that cause the disease (Lima *et al*., 2013) and the virulence, population structure, interaction of the isolates that determine the seasonal pressure and fluctuation of inoculum, environmental inductivity and susceptibility of the infected tissue associated with its phenological development (Arauz, 2000; Monteon-Ojeda *et al*., 2012). Nishijima (1993) propose four susceptibility levels (resistant, moderately resistant, susceptible and very susceptible) while Pernezny and Ploetz (2000) suggest only three levels of susceptibility (highly susceptible, susceptible and moderately susceptible). In these experiments, levels or groups of susceptibility were determinate by the severity, incidence and incubation period, cultivars with similar levels were grouped obtaining three categories of severity (high, medium and low susceptibility). This experiments were performed providing optimal environmental and management conditions for the development of the pathogen, including the high susceptibility of leaves from 15 days of development to anthracnose determined in previous studies (unpublished data); It is expected that the expression of susceptibility for a cultivar under field conditions may vary among regions depending on environmental conditions (wet, sub-humid or dry tropics) and virulence of the pathogen (Afanador-Kafuri *et al*., 2003; Alahakoon *et al*., 1994; Gomes *et al*., 2010; Rojas-Martinez *et al*, 2008).

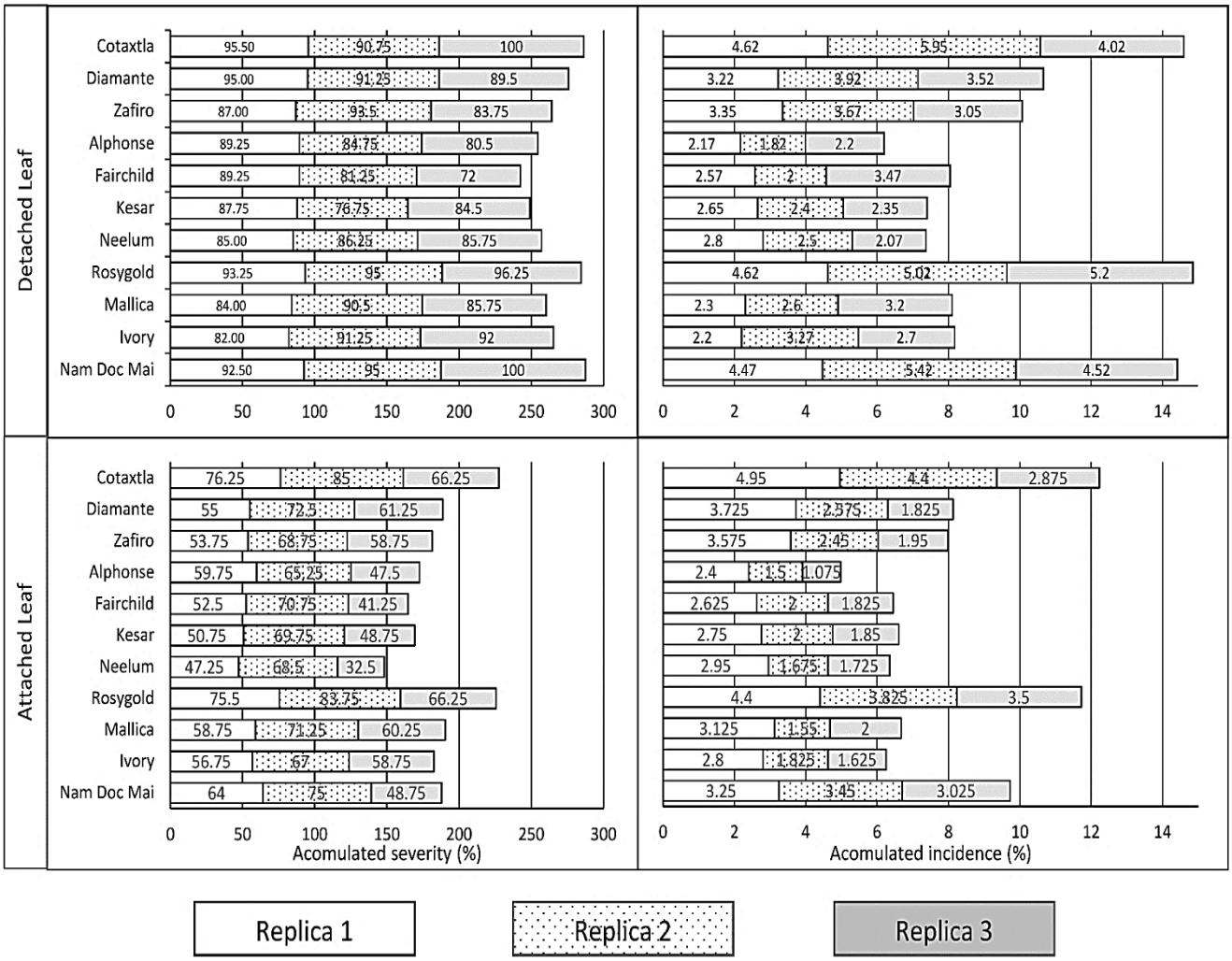

Figure 1. Incidence and accumulative severity of anthracnose of 15-days old leaves of eleven mango cultivars (*Mangifera indica L.*) of yellow skin inoculated with the high virulence isolation of *Colletotrichum gloeosporioides* (Gro) with the methods of detached and attached leaf in laboratory and nursery respectively. Each bar represents the absolute values observed during three experimental replicates (cycle 2015-2016).

Table 1. Susceptibility of 15 days old leaves of eleven mango cultivars (*Mangifera indica* L.) to anthracnose (*Colletotrichum gloeosporioides*) according to the incubation period, incidence and severity, inoculated in the laboratory with the detached leaf technique.

Cultivar	Incubation period (*dai*)[2]	Incidence (%)	Severity (%)	Susceptibility range
Alphonse	4.45 a	84.83 d	2.06 d	Low
Neelum	3.85 a	85.66 d	2.45 d	Low
Kesar	3.95 a	83.00 d	2.46 d	Low
Fairchild	3.79 a	80.83 d	2.68 c	Medium
Ataúlfo Zafiro	4.11 a	88.08 b	3.35 c	Medium
Ivory	4.34 a	88.41 b	2.72 c	Medium
Mallika	4.00 a	86.75 b	2.7 c	Medium
Ataúlfo Diamante	4.22 a	91.91 b	3.55 b	Medium
Nam Doc Mai	2.18 b	95.83 a	4.80 a	High
Cotaxtla	2.17 b	95.41 a	4.86 a	High
Rosygold	2.26 b	94.83 b	4.94 a	High
LSD	1.15	8.28	0.85	
CV (%)	18.95	5.48	15.07	

(1)Means followed by equal letters in the columns are not significantly different, LSD test (p>0.05), probability. (2) *dai* = Days after inoculations.

Table 2. Susceptibility of 15-days old leaves of eleven mango cultivars (*Mangifera indica* L.) to anthracnose (*Colletotrichum gloeosporioides*) according to the incubation period, incidence and severity, inoculated in the nursery with the attached leaf technique.

Cultivar	Incubation period (*dai*)[2]	Incidence (%)	Severity (%)	Susceptibility range
Alphonse	8.87 b	57.50 c	1.65 e	Low
Neelum	8.01 b	49.41 c	2.11 d	Low
Ivory	7.43 d	60.83 b	2.08 d	Low
Fairchild	9.15 a	54.83 c	2.14 c	Medium
Kesar	8.80 b	56.41 c	2.20 c	Medium
Zafiro	7.60 c	60.41 b	2.65 c	Medium
Diamante	7.49 d	62.91 b	2.70 c	Medium
Mallika	7.48 d	63.41 b	2.22 c	Medium
Nam Doc Mai	6.54 e	62.56 b	3.24 b	High
Cotaxtla	5.95 e	75.83 a	4.07 a	High
Rosygold	5.58 e	75.16 a	3.90 a	High
LSD	1.07	9.07	0.59	
CV (%)	8.9	8.7	9.1	

(1)Means followed by equal letters in the columns are not significantly different, LSD test (p>0.05), probability. (2) *dai* = Days after inoculations.

Comparative evaluation of the detached and attached leaf techniques

Methods tested in this research were effective to induce symptoms of anthracnose, to estimate virulence parameters and to classify the susceptibility of mango cultivars. These results were consistent with Rojas-Martinez et al. (2008) and Leandro (2001). Although the two techniques (Detached Leaf Technique and Attached Leaf Technique) reflected differences in susceptibility of cultivars, it was observed that severity and incidence were always higher (Tables 1 and 2) and the incubation periods were up to 50% shorter when the DLT, a cause of the could be the regularity of the condition in the lab are more stable compared to the nursery. Technique was used (Figure 2). The re-isolating of strains confirmed to *Colletotrichum gloeosporioides* like the causing pathogen of vegetative mango anthracnose and permitted the observation of differential kind of symptoms in respect

to the technique utilized. In terms of detached leaf technique, it was observed big circular to epileptic dark spots with acervuli, in contrast with the attached leaf technique, where presented small angular depressed dark spots limited by nerves like in those origin with natural infections (Figure 3). According to Liu et al. (2007), isolates of *C. linicola* inoculated on plants of *Arabidopsis thaliana* by attached technique caused infections and typical symptoms but no on detached leaves due to physiological and molecular changes associated with leaf separation such as initiation of ethylene-dependent senescence and inactivation of defense pathways that contribute to colonization of the fungus. These authors demonstrated that salicylic, jasmonic acids and ethylene that are less active in separate leaves than in intact leaves mediate the common defense system of plants. The possibility of using either of the two techniques (DLT or ALT) to classify the susceptibility of mango cultivars was showed by Iwaro (1997) who demonstrated that a reliable prediction of cocoa resistance to *P. palmivora* can be determine using leaf and fruit detached or attached to the plant. King et al. (1997) reported that incubation and sporulation periods of detached strawberry fruits inoculated with *Colletotrichum spp*. were positively correlated (R=95) with the results obtained in fruits adhered to plants.

Evaluation of severity

Visual estimation of the number of lesions influenced the accuracy and precision with respect to the actual proportion of the infected area, the greater the number of lesions caused that the overestimation was also greater. The use of visible light photographs and digital image analysis is an increasingly used tool to solve these problems, being the software easy to use (Do Vale, 2001). In this study, the use GIMP 2.0 software for Windows® that resulted effective to evaluate the severity of artificially induced foliar anthracnose in mango cultivars. The same method was used by Sauceda-Acosta et al. (2015) to evaluate the severity of wheat rust by *Puccinia triticina* with Image J 1.0 software for Windows® and Wijekoon et al. (2008) to accurately quantify the severity of leaf lesions caused in different hosts by *C. destructivum*, *C. dematianum* and *Oidium sp*., with Scion Image® software. In fruits, Corkidi et al. (2006) quantified the severity of anthracnose (*C. gloeosporioides*) in the all surface area of the mango fruits using a three-dimensional image analysis system with high correlation with respect to the real severity (R^2= 0.99). In our research, the visual and digital estimation methods of lesions obtained with DLT and ALT had a high correlation (r= 0.97) (Figure 2), thus they can be used interchangeably to evaluate the anthracnose of the mango, depending on the conditions of the experimental studies.

Correlation analysis

Regardless of the technique of inoculation (DLT or ALT), longer incubation periods correlated to lowest severity (r= -0.90 DLT, -0.89 ALT) and incidence (r= -0.77 DLT, -0.85 ALT). In contrast, incidence positively correlated to severity (r= 0.92 DLT, 0.86 ALT) (Figures 1 and 2). Relationship between these variables was previously reported by Chala et al. (2010), who reported a significant correlation (R^2= 0.86-0.92) between incidence and severity of anthracnose in leaves of *Sorghum vulgare* inoculated with *C. sublineolum*. In addition, Lamsupasit (1993) observed that sporulation and incubation period of *C. gloeosporioides* in six accessions of the *Stylosanthes hamata* legume had positive correlation (P <0.05) to resistance (AUDPC) of adult plants in the field, and documented a close relationship between the incubation period, daily sporulation and AUDPC (R^2= 0.90). In peach and nectarine cultivars was observed that greater resistance to *Monilinia fructicola* is correlated to thicker and denser epidermis than in susceptible cultivars, factors that delay the penetration of the fungus and promote longer incubation periods (Adaskaveg *et al*., 1989, 1991). According to Niks (1986), in non-hypersensitive resistance, such as pre-haustorial resistance, longer incubation periods and less sporulation may be observed, possibly due to the frequent failure of haustorial development, as in *Puccinia hordei* in barley Partially resistant, or also to the slow growth of the fungus colonies, as it happens in powdery mildew (*Erysiphe graminis* f. Avenae) of oats (Carver and Carr, 1978). The correlation between the two tested techniques (DLT or ALT) tested in this study shows that a quick laboratory inoculation offers important advantages in detecting tolerance saving time and resources. Both techniques are useful to evaluate the disease with accuracy and precision and could be implemented in breeding programs that consider early screening of genotypes for anthracnose in vegetative tissue when it is not possible to realize it in inflorescences and fruits. This approach is particularly useful when introducing new cultivars of commercial interest in the export market and prioritizing the sanitary stage of the materials to estimate the potential epidemic impact and the demand for health management technology for their cultivation. The advantages of DLT have been documented by Tu (1986) who concluded that the DLT technique was efficient to evaluate the pathogenicity, virulence and resistance of bean varieties to *C. lindemuthianum* and multiple pathogens using a single plant and avoiding undesirable situations such as inoculum limitations and adverse environment conditions. Similarly, Bigirimana (2001) showed that the inoculation of detached leaves exceeded the inoculation in attached leaves of seedlings due to its higher speed and sensitivity, even though the three methods allowed characterizing similarly resistance of

bean varieties to *C. lindemuthianum*. Moral and Trapero (2009) found high correlation between the incidence and severity of olive anthracnose in the field and detached fruits. Miles *et al*., 2012 demonstrated that different techniques of inoculation of *C. acutatum* on detached fruits (spray, drip and injection) on blueberries were useful to evaluate resistance since they operate with the same efficiency and provides a rapid assessment of resistance.

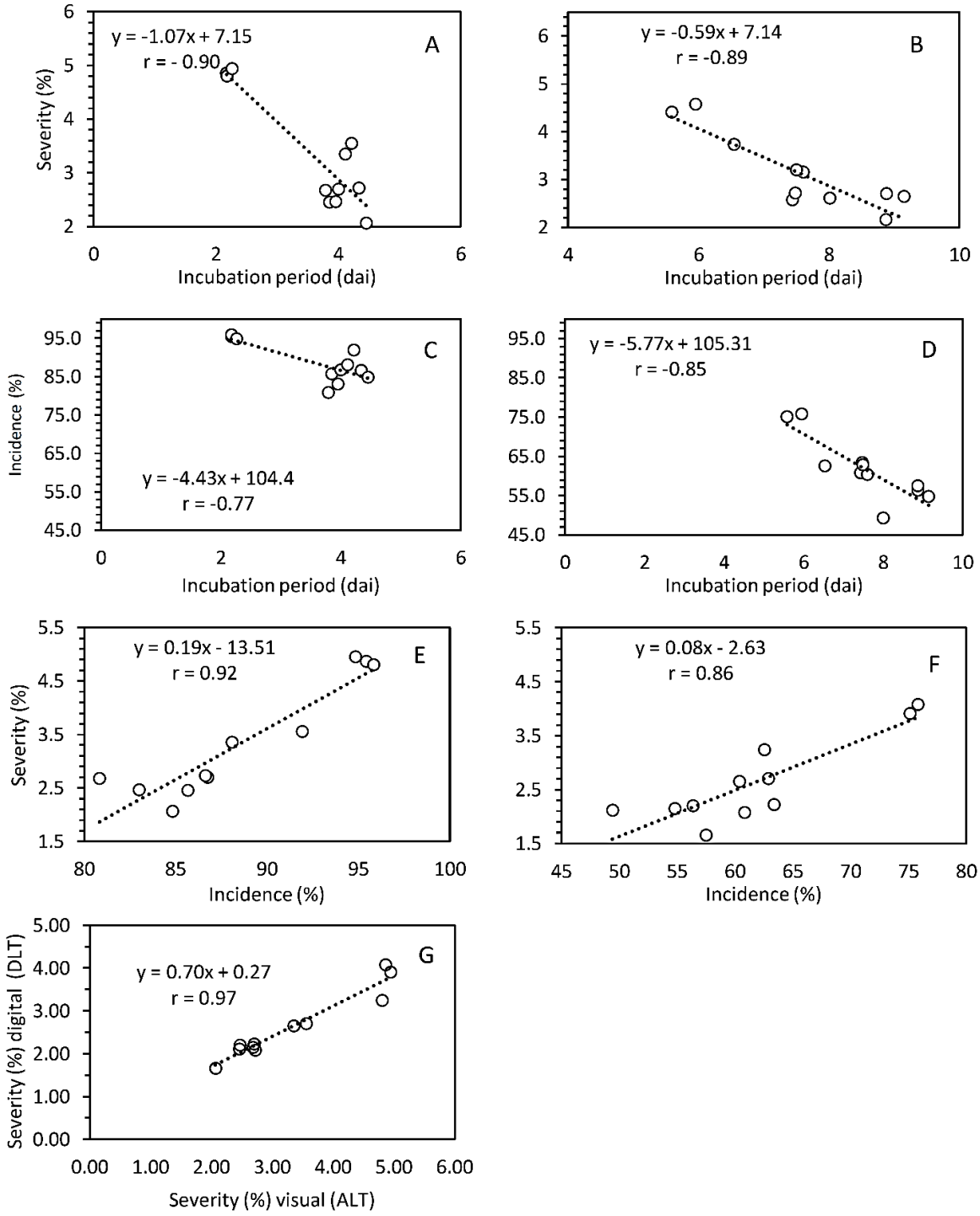

Figure 2. Linear regression analysis for severity, incidence and incubation period, of 15-days old leaves of eleven mango cultivars (*Mangifera indica L.*) of yellow skin inoculated with the high virulence isolation of *Colletotrichum gloeosporioides* (Gro). Detached method (A, C, E) and attached method (B, D, F). Correlation between visual and digital estimation of severity in DLT and ALT (G). r= Pearson's coefficient.

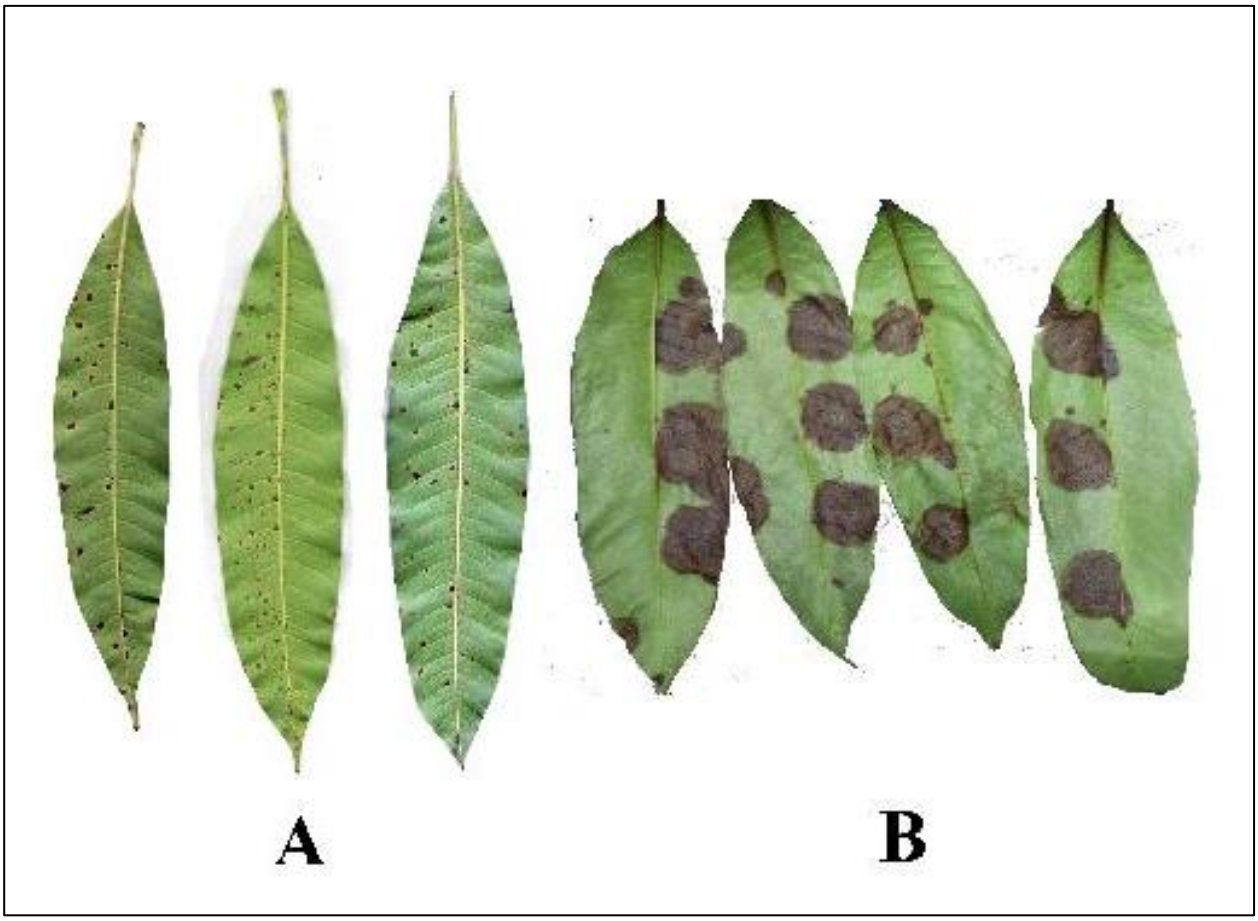

Figure 3. Anthracnose symptoms induced by *C. gloeosporioides* using ALT (A) and DLT (B).

CONCLUSIONS

The mango cultivars "Alphonse", "Neelum", "Kesar" e "Ivory" are the most tolerant to anthracnose. There was a negative correlation between the incubation periods with the incidence and severity, and positive between the incidence and the severity. The detached and attached leaf techniques are useful to induce anthracnose and estimate the expression of virulence of *C. gloeosporioides*, however, the detached leaf technique promote the highest values of the disease and increases the experimental work efficiency. Digital image processing is a useful tool for the precise evaluation of the anthracnose in vegetative tissue of mango and can be incorporated in the evaluation of different parasitic interactions.

REFERENCES

Adaskaveg, J.E., Feliciano, A.J., Ogawa, J.M. Comparative studies of resistance in peach genotypes to *Monilinia fructicola*. Phytopathology, v.79, p.1183-1184, 1989.

Adaskaveg, J.E., Feliciano, A.J., Ogawa, J.M. Evaluation of the cuticle as a barrier to penetration by *Monilinia fructicola*. Phytopathology, v.81, n.10, p.1150, 1991.

Afanador-Kafuri, L., Minz, D., Maymon, M., Freeman, S. Characterization of Colletotrichum isolates from tamarillo, passiflora, and mango in Colombia and identification of a unique species from the genus. Phytopathology, v.93, n.5, p.579-587, 2003.

Alahakoon, P.W., Brown, A.E., Sreenivasaprasad, S. Genetic characterization of *Colletotrichum gloeosporioides* isolates obtained from mango. International Journal of Pest Management, v.40, n.2, p.225-229, 1994.

Arauz, L.F. Mango anthracnose: economic impact and current options for integrated management. Plant Disease, v.84 n.6, p.600-611, 2000.

Becerra-Leor, E.N. Enfermedades del cultivo del mango. In: Mata-Beltrán, I.; Mosqueda-Vázquez, R. (Ed.). La Producción de mango

en México. Noriega Editores, México, 1995. p.83–101.

Bigirimana, J., Höfte, M. Bean anthracnose: inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. Journal of Phytopathology, 149 v.7-8, p.403-408, 2001.

Carver, T.L.W., Carr, A.J.H. Effects of host resistance on the development of haustoria and colonies of oat mildew. Annals of Applied Biology, v.88, n.1, p.171-178, 1978.

Chala, A., Brurberg, M.B., Tronsmo, A.M. Incidence and severity of sorghum anthracnose in Ethiopia. Plant Pathology Journal (Faisalabad), v.9, n.1, p.23-30, 2010.

Chanana, Y.R., Josan, J.S., Arora, P.K. Evaluation of some mango cultivars under North India Conditions. In: Proceedings of International Conference on Mango and Date Palm: Culture and Export, June 2005. Proceedings. University of Agriculture, Faisalabad, Pakistan. 2005. p.36-40.

Corkidi, G., Balderas-Ruiz, K.A., Taboada, B., Serrano-Carreon, L., Galindo, E. Assessing mango anthracnose using a new three-dimensional image analysis technique to quantify lesions on fruit. Plant Pathology, v.55, p.250–257, 2006.

Delgado, P.H., Aranguren, M., Reig, C., Galván, D.F., Mesejo, C., Fuentes, A.M., Agusti, M. Phenological growth stages of mango (Mangifera indica L.) according to the BBCH scale. Scientia Horticulture, v.130, n.3, p.536-540, 2011.

Do Vale, F.X.R., Filho, E.I.F., Liberato, J.R., Zambolim, L. Quant–a software to quantify plant disease severity, 2001. Available at: <http://scholar.google.com.mx/scholar?hl=es&q=.+Quant%E2%80%93a+software+to+quantify+plant+disease+severity&btnG=&lr=> Accessed on: 28 January 2017.

Faostat. Organización de las Naciones Unidas para la Alimentación y la Agricultura, 2016. Available at: http://faostat3.fao.org/browse/Q/*/E Accessed on: 28 January 2017.

Galán, S. El cultivo del mango. 2nd ed. Editorial Mundi-Prensa, S.A. de C.V. México, 2009. 341 p.

Gupta, V.K., Pandey, A., Kumar, P., Pandey, B.K., Gaur, R.K., Bajpai, V., Sharma, S. Genetic characterization of mango anthracnose pathogen *Colletotrichum gloeosporioides* Penz. by random amplified polymorphic DNA analysis. African Journal of Biotechnology, v.9, n.26, p.4009-4013, 2010.

Haggag, W.M. Mango diseases in Egypt. Agriculture and Biology Journal of North America, v.1, n.3, p.285-289, 2010.

Iwaro, A.D., Sreenivasan, T. N., Umaharan, P. Foliar resistance to *Phytophthora palmivora* as an indicator of pod resistance in *Theobroma cacao*. Plant Disease, v.81, n.6, p.619-624, 1997.

King, W.T., Madden, L.V., Ellis, M.A., Wilson, L.L. Effects of temperature on sporulation and latent period of *Colletotrichum* spp. infecting strawberry fruit. Plant Disease, v.81, n.1, p.77-84, 1997.

Leandro, L.F.S., Gleason, M.L., Nutter J.R.F.W., Wegulo, S.N., Dixon, P.M. Germination and sporulation of *Colletotrichum acutatum* on symptomless strawberry leaves. Phytopathology, v.91, n.7, p.659-664, 2001.

Lei, X.T., Zhao, Y.L., Yao, Q.S., He, Y.B., Sun, G.M., Ma, W.H., Zhan, R.L. Identification and analysis of the resistance of different mango cultivars to anthracnose (*Colletotrichum gloeosporioides*). Journal of Fruit Science, v.6, p.11, 2006.

Lima, N.B., De, A., Batista, M.V., De Morais, M.A., Barbosa, M.A.G., Michereff, S.J., Hyde, K.D., Camara, M.P.S. Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil. Fungal Diversity, v.61, p.75, 2013.

Litz, R.E. The mango: botany, production and uses. 2nd ed. Oxfordshire, UK: CABI, 2006. 680 p.

Liu, G., Kennedy, R., Greenshields, D.L., Peng, G., Forseille, L., Selvaraj, G., Wei, Y. Detached and attached Arabidopsis leaf assays reveal distinctive defense responses against hemibiotrophic *Colletotrichum* spp. Molecular Plant-Microbe Interactions, v.20, n.10, p.1308-1319, 2007.

Malc, R., Gehrke-Vélez., Castillo-Vera., Ruiz-Bello, C., Moreno-Martínez, L. Viabilidad y germinación del polen en mango (*Mangifera indica* L.) cv. Ataúlfo. Interciencia, v.36, n.5, p.378-385, 2011.

Iles, T.D., Hancock, J.F., Callow, P., Schilder, A.M.C. Evaluation of screening methods and fruit composition in relation to anthracnose fruit rot resistance in blueberries. Plant Pathology, v.61, n.3, p.555-566, 2012.

Monteon-Ojeda, A., Mora-Aguilera, J.A., Villegas-Monter, A., Nava-Diaz, C., Hernández-Castro, E., Hernández-Morales, J. Temporal analysis and fungicide management strategies to control mango anthracnose epidemics in Guerrero, Mexico. Tropical Plant Pathology, v.37, n.6, p.375-385, 2012.

Moral, J., Trapero, A. Assessing the susceptibility of olive cultivars to anthracnose caused by *Colletotrichum acutatum*. Plant Disease, v.93, n.10, p.1028-1036, 2009.

Niks, R. E. Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. Physiological and Molecular Plant Pathology, v.28, n.3, p.309-322, 1986.

Nishijima, W. Mango diseases and their control. In: CONFERENCE ON MANGO IN HAWAII, 3., 1993, Hawaii. Proceedings. University of Hawaii at Mānoa, College of Tropical Agriculture and Human Resources, 1993. p.20–24.

Paéz, A.R. Respuesta de cultivares de mango (*Mangifera indica*) a la antracnosis en la Costa Atlántica colombiana. Revista Corpoica, v.2, n.1, p.43-53, 1997.

Pernezny, K., Ploetz, R. Some common diseases of mango in Florida. FL, USA: University of Florida/Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, 2000. Available at:<https://3774ceeb-a-62cb3a1a-s-sites.googlegroups.com/site/dileepiiithyderabad/protection-of-fruits-crops/VH04800.pdf?attachauth=ANoY7cporvMKUM2BwXTttnymIdekYAoanA2YVrOqnFx4UUIY-AQ3O-fVZd9kcs_El8UOwoyBEyEsi4AKv0ibwQCNnmIN3rGbx0Di4y9VWmizCRqsa_3pZFTTuSHkd9n-aU0DdbW1-SYm42p2WyJRPtF-JtpvvKQ8GDSEmWU9hSCcv7EnDglfakBYAQxFdfVreAks6MBfhSUpX-kdX33r021z8EGx7PLc3PTKBvmciA7pRKIofT1rglNuenihcgwguFRlPyGEWTMC&attredirects=0> Accessed on: 28 January 2017.

Rojas-Martinez, R.I., Zavaleta-Mejia, E., Nieto-Angel, D. Virulence and Genetic Variation of Isolates of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. on Mango (*Mangifera indica* L.) cv. Haden. Revista Mexicana de Fitopatología, v.26, n.1, p.21–26, 2008.

Sauceda-acosta, C.P., Lugo-García, G.A., Villaseñor-Mir, H.E., Partida-Ruvalcaba, L., Reyes-Olivas, Á. Un método preciso para medir severidad de roya de la hoja (*Puccinia triticina* Eriksson) en trigo. Revista Fitotecnia Mexicana, v.38, n.4, p.427-434, 2015

Tivoli, B., Baranger, A., Avila, C.M., Banniza, S., Barbetti, M., Chen, W., Sadiki, M. Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. Euphytica, v.147, n.1-2, p.223-253, 2006.

Tu, J.C. A detached leaf technique for screening beans (*Phaseolus vulgaris* L.) in vitro against anthracnose (*Colletotrichum lindemuthianum*). Canadian Journal of Plant Science, v.66, n.3, p.805-809, 1986.

Wijekoon, C.P., Goodwin, P.H., Hsiang, T. Quantifying fungal infection of plant leaves by digital image analysis using Scion Image software. Journal of Microbiological Methods, v.74, n.2, p.94-101, 2008

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