Genetic tolerance to *Ceratocystis* wilt in melina (*Gmelina arborea* Roxb.)

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Abstract

Gmelina arborea is the second most planted tree species in Costa Rica, and one of the most important in several Latin American countries. In recent years, a disease caused by *Ceratocystis fimbriata*, has spread throughout Latin America, leading to the complete root of the tree in a few months. This study was conducted to evaluate the incidence, severity and genetic tolerance control of the *Ceratocystis* wilt in *G. arborea*. Data is based on a 2.4-year-old clonal trial, with 34 elite genotypes, at Río Jiménez, Guácimo in the Costa Rican Caribbean region. The variables diameter at breast height (DBH), total height, disease incidence and disease severity were evaluated. A pathogenicity index was proposed as a new selection criterion. SELEGEN (EMBRAPA) software was used for the evaluation of the genetic control in all traits investigated. Overall incidence rate was 39 % and severity was 13 %. Mean clonal heritability for severity was $h^2_{mc} = 0.59$ and for incidence $h^2_{mc} = 0.47$, these values were high evidencing that both traits are under a strong genetic control. The pathogenicity index becomes a useful discrimination criterion for ranking genotypes by their disease tolerance. An infection pattern inversely proportional to the DBH was observed. Although none of the 34 genotypes evaluated recorded total resistance to *C. fimbriata*, eight of these genotypes show strong genetic tolerance to the disease, and high productivity. Their use can be encouraging and will contribute to the reduction of *Ceratocystis* wilt impact in the country, as well as in the tropical Latin America region.

Keywords: *breeding, clonal forestry, genetic resistance, pathology, tropical disease.*

Introduction

Gmelina arborea (melina) is a large to medium-sized deciduous tree, native to the tropical and subtropical regions of Asia and widely planted in the tropical regions of America, Asia, and Africa (Pathala et al., 2015). It is the second most widely planted tree species in Costa Rica, with a wide commercial demand in Ecuador and Colombia as well, and subjected to breeding programs in Costa Rica (Lokmal 1994; Hogde & Dvorak 2004; Kumar 2007; Murillo et al., 2016). Its importance lies in its rapid growth rate, high productivity and wide tolerance to site conditions (Ávila et al., 2016), as well as its wide use of wood in industry (Moya 2004).

Despite its growth potential and economic value, melina is susceptible to a number of diseases in plantations (Anuagasi et al., 2017). So far, diseases of most concern in America are vascular wilts caused by *Ceratocystis fimbriata* in Brazil and Ecuador, as well as the death of melina in Costa Rica (Oliveira et al., 2015; Arguedas et al., 2018), which was recently reported to be caused by *C. fimbriata* (Méndez et al., 2020). The incidence of this disease in Costa Rica is estimated at a rate of 40 % in commercial plantations (Arguedas et al., 2018).

Among the symptoms that can be observed are, wilt and leaf loss, sprouting on the stem at the base of the tree, trunk rot with cankers and exudations, necrotic spots inside the stem and finally, tree death (Salas et al., 2016b; Arguedas et al., 2018; Méndez et al., 2020). This disease has been observed to occur at seven months age in plantations, and severely reduces growth and development, causing serious economic losses (Murillo et al., 2016).

C. fimbriata is a saprophytic fungus present in soils and a primary pathogen in angiosperms and gymnosperms (Harrington et al. 2014). It is distributed in tropical, subtropical and temperate regions of the world (Holland et al. 2019). *C. fimbriata* is capable of infecting at least 31 species from 14 economically important families (Holland et al. 2019; Valdetaro et al. 2015). Species of the genus *Ceratocystis* have various means of transmission and dispersal, including insects, wind, water, root grafts, and pruning tools; infecting its host generally through wounds (Ferreira et al. 2010; Harrington et al. 2011). In Costa Rica, there is evidence of a relationship between disease incidence and site conditions; according to Arguedas et al (2018), there is higher incidence in plantations with high stand density and soils with higher clay content. However, the etiology of the diseases has not been established yet (Méndez et al., 2020).

Effective disease management in forest plantations relies on multidisciplinary collaboration and dynamic approaches (He et al., 2016). In tree breeding programs, the selection of individuals with a clear disease resistance pattern or tolerance is usually sought. These should be properly determined based on parameters associated with effective genetic control, such as genotypic variation, heritability values, genetic ranking, among others (Telford et al., 2014; Salas et al., 2016a).

Therefore, clonal selection based on genetic tolerance against a specific disease, can not only increase timber production in the next plantations, but will also contribute to a better stand density management, with an important economic impact for producers (Salas et al., 2016a). One of the first steps to be taken is the determination of disease incidence and severity in the breeding population. Genetic collections should be screened in order to determine their degree of tolerance or resistance. Several approaches can be implemented, which could be classified as direct (based on pathogen inoculation) and indirect (based on field trials) methods.

The indirect method is based on field measurements in genetic tests established in different sites, which have previously been identified as highly contagious with this disease (Salas et al., 2016a; Murillo et al., 2016). Despite not being a completely safe method, it allows screening and showing a first selection criterion in the breeding population (Telford et al., 2014; He et al., 2016). Since polygenic resistance is often more durable than monogenic resistance, it has been suggested that breeding strategies should take these criteria into account (Palloix et al., 2009).

Therefore, an extensive melina breeding program has been launched through the Tree Breeding Cooperative GENFORES in several countries in Latin America, with the intention of searching for the genetic variation related to resistance to *C. fimbriata*. In addition to breeding efforts, other management approaches need to be incorporated in the global strategy against diseases. Since the greater the number of breakthrough resistance factors, the greater the number of virulence mutations required in the pathogen genome and, the less likely their occurrence (Palloix et al., 2009). The main objective of the present study was to determine whether there is a genetic control of tolerance to "Ceratocystis wilt", in a clonal trial established in the Costa Rican Caribbean region.

Materials and Methods

A 2.4 years-old melina clonal trial was evaluated, established in Río Jiménez of Guácimo, Caribbean region (10.251356 'N, -83.576124' W), in a semi-flooded area, on an inceptisol soil (Alvarado et al., 2014). The trial was established in April 2016, under a good weed control, but without land preparation, as it was previously planted with crops. About two tons_{ha}⁻¹ of calcium carbonate was applied on site along the tree planting lines. In the second year, a first commercial thinning was performed on 33 % of the population. The trial site belongs to the very humid premontane forest life zone of the Holdridge classification, with a precipitation ranging from 3500-4600 mm year⁻¹, no drought period and a mean annual temperature of 26 °C.

The clonal test was established following a randomized complete block experimental design developed by GENFORES (Murillo et al., 2019). Within the six blocks, 34 elite genotypes were tested, each represented by two pairs of ramets randomly distributed within each block, with the restriction of not planting two pairs of the same clone together. Thus, 24 plants in the entire trial represented each genotype. Commercial seed from the best local seed orchard was used as a control lot. Trees were planted at 4 x 4 m spacing. All trees were measured for DBH (1.3 m) and total height with a telescopic pole.

Disease severity and incidence determination

Disease incidence was assessed by binomial scoring of all individual trees, as healthy (without symptoms = 0) or sick (with visible symptoms = 1). Both general and clonal incidence, were determined by equation 1 (Cavallini, 1998):

$$Incidence = \frac{\text{Total number of affected trees}}{\text{Total number of trees}} * 100 \quad (1)$$

Based on the severity scale proposed by Salas et al. (2016a, 2016b) trees were scored in four categories from "1" to "4". Where the value of "1" was assigned to a completely healthy tree or with no visible symptoms. While a value of "4" was assigned to a completely dead tree, with no foliage, no bark and dark stem. With this record, the severity index (Equation 2) was determined for each of the 34 test clones, based on the evaluation of all its individual trees.

$$Severity\ index = \frac{N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4}{N_1 + N_2 + N_3 + N_4} \quad (2)$$

Where,

N_1 = number of trees (ramets) of a given clone "i" with a score of 1 or healthy, with no visible disease symptoms;

N_2 = number of trees (ramets) of a given clone "i" scored by 2 or with some visible disease symptoms like obvious leaf wilt; the stem may have small necrotic wounds with black exudation in locations other than pruning sites; sprouts break may initiate. Not all symptoms are expressed;

N_3 = number of trees (ramets) of a given clone "i" scored by 3 with more than 2 visible disease symptoms such as canker lesions on the bark with signs of rot, bark exposure and bulging,

prominent exudation; loss of more than 50 % of the leaf area in a progressive pattern; sprouts developed;
 N_4 = number of trees (ramets) of a given clone "i" scored by 4 or completely dead.

A pathogenicity index was determined on a scale of 1 to 100 (Equation 3), for the entire assay, as well as for each clone in the trial, where the value of 100 corresponds to a completely healthy population.

$$\text{Pathogenicity index} = 100 - \left(1 + \left(\frac{\text{Sev1} + 1 + \text{Sev2} + 2 + \text{Sev3} + 3 + \text{Sev4} + 4}{3} \right) \right) * 100 \quad (3)$$

Where,

Sev1 corresponds to the number of trees (ramets) of a given clone "i" rated with severity "1" or completely healthy;

Sev2 corresponds to the number of trees (ramets) of a given clone "i" rated with severity "2" or with some visible disease symptoms, such as obvious leaf wilt; the stem may have small necrotic wounds with black exudation;

Sev3 corresponds to the number of trees (ramets) of a given clone "i" rated with severity "3" or visibly diseased, with more than 50 % loss of the leaf area and signs of rot;

Sev4 corresponds to the number of trees (ramets) of a given clone "i" qualified as "4" or completely dead.

A genetic ranking of this pathogenicity index was obtained using SELEGEN software (Resende, 2016), which provided a list of all 34 clones, from the highest (more tolerant) to the lowest (least tolerant). The range of values was subdivided into three thirds, to facilitate their interpretation as high, medium and low tolerant.

Genetic control analysis

Genetic test was planted following GENFORES's experimental design, which is a randomized complete block (RCB) design, with six blocks per trial site. Each block is composed of four ramets (plants) per clone, arranged in two non-contiguous pairs, randomly distributed within the block. The clones within blocks were considered as the experimental unit (plot). Therefore, 24 plants (ramets) represented each clone. After 30 days, the test was replanted to reduce mortality and imbalance.

Genetic analysis of DBH, total height, incidence, severity and pathogenicity index, were performed with SELEGEN software (Resende, 2016). Data were analyzed with model 4 (equation 4), which is specific for genetic tests under a completely random block design, in a single location, with unrelated clones and several ramets per clone in the experimental unit.

$$y = Xr + Zg + Wp + e \quad (4)$$

Where:

"y" is a data vector, "r" is a vector of repetition effects (assumed as fixed) and added to the general mean; "a" is a vector of individual additive genetic effects (assumed as random), "p" is a vector of plot effects assumed as random, and "e" is a vector of residuals or the error term (random). Capital letters represent incidence matrices for the mentioned effects. From the analysis, it was determined the following variance components and parameters for each of the traits investigated: genetic variance

among clones (σ_g^2), plot variance (σ_{plot}^2), environmental variance (σ_e^2), total phenotypic variance (σ_p^2), individual (H_g^2) and mean ($H_{m(clonal)}^2$) clonal broad sense heritabilities, coefficient of determination for the plot effect (block x family) (C_{plot}^2); It accounts for variances among plots within blocks, selective accuracy (A), assuming complete survival, individual additive genetic variation coefficient ($CV_{gi}(\%)$), genotypic variation coefficient among clones ($CV_g(\%)$), environmental variation coefficient variation ($CV_e(\%)$), relative variation coefficient (CV_r), standard deviation and the variance of the error term by,

$$\sigma_p^2 = \sigma_g^2 + \sigma_{plot}^2 + \sigma_e^2,$$

$$H_g^2 = \sigma_g^2 / \sigma_p^2,$$

$$H_{m(clonal)}^2 = \sigma_g^2 / \{ \sigma_p^2 + (\sigma_{plot}^2 / 6) + [(\sigma_{aw}^2 + \sigma_e^2) / 24] \},$$

assuming complete survival, "6" is the number of replications, "4" is the number of trees within each plot (n=4), then 24 is the total number of effective ramets of each other in test.

$C_{plot}^2 = \sigma_{plot}^2 / \sigma_p^2$, it assumes complete survival, "6" is the number of replications (r=6), "4" is the number of trees within each plot (n=4), then 24 is the total number of effective trees in test.

$A = \sqrt{h_{m(clone)}^2}$, accuracy of clonal selection assuming complete survival.

To proceed with selection of genotypes scenarios, the genetic ranking of Pathogenicity Index and DBH growth were graphically compared, as suggested by Murillo et al. (2019). Each of the two ranking was divided into three thirds: high, medium and low performance. Subdivisions were obtained by (the difference between highest-lowest value)/3, as described by Resende et al. (2018). Finally, a deviance analysis was performed, as suggested by Murillo et al. (2019), to determine the existence of significant differences among genotypes.

Results

The mean DBH was 18.89 cm (9.5 cm/year) and the mean total height was 14.95 m (7.5 m/year) at 2.4 years-old, with a predominant diameter distribution between 15 to 25 cm. Only eight individuals were recorded between 5 to 10 cm in DBH in the trial (Figure 1).

Figure 1

Diameter distribution and degree of severity associated to *Ceratocystis* wilt in a 2.4-year-old clonal trial of *G. arborea* in Guácimo, Caribbean region of Costa Rica.

When severity is combined with diameter class, it is observed that the greatest number of trees affected with severity grade 2, 3 and 4 belong to the 15 to 20 cm diameter class. While healthy trees are mostly found in the 20 to 25 cm diameter class, which is above the mean diameter in the trial (Figure 1). It was also observed that trees with severity 4 predominate in the diameter classes below 15 cm.

At 2.4 years-old, an incidence of 39 %, a severity index of 1.65 and a mean pathogenicity index of 21.7 % were recorded. All clones recorded ramets with disease symptoms (Table 1). Incidence values per clone ranged from 15.8 to 68.4 %, while pathogenicity index ranged from 6.3 to 42.1 %. A group of 12 clones presented a low pathogenicity index (< 18), but with an incidence value reaching to 31 %. At the other extreme, nine highly susceptible genotypes recorded a pathogenicity index higher than 31 % and with an incidence value reaching 44 %.

Based solely on the pathogenicity criteria, a possible selection scenario would be to take genotypes with a maximum pathogenicity index of 10.5 %. Thus, the resulting subpopulation would consist of the eight most tolerant genotypes, whose maximum incidence value was 30 % (Table 1). However, the selection of the best materials must also consider productivity. Figure 2 shows the genetic rankings of the Pathogenicity Index and commercial volume (productivity). Here, a group of six genotypes (57, 51, 1, 31, 19, and 3) that meet both selection criteria can be distinguished. There is another group of genotypes (1N, 19CA, 8CA and 15N), that rank in the first positions of productivity, but are highly intolerant to *C. fimbriata*.

The commercial volume genetic ranking shows little or no difference between genotypes. Therefore, some clones further down the pathogenicity index ranking, may also be selected, as shown in Figure 2.

Genetic control analysis

Individual heritability (H_g^2) was very low (< 0.06) for all the traits evaluated (Table 2). While the mean clonal heritability ($H_{m(clonal)}^2$) showed moderate values in all traits, with the exception of severity, which registered a value of almost 60 %. It was also observed that the genetic variance component (σ_g^2) exhibited a very low value in trait incidence, in contrast to the values exhibited by traits DBH, total height and severity. A higher genetic control was also determined in the severity trait than in the disease incidence, also corresponding to a higher coefficient of genetic variation ($r = -0.346$, Table 3). The accuracy (A) or reliability of the estimated parameters was high (> 68 %) for the severity and incidence traits, but moderate for the growth variables.

The DBH and height showed a moderate but significant genetic correlation with each other (Table 3). Both incidence and severity recorded a negative and moderate correlation with respect to tree height. Therefore, the data suggests a pattern of inverse relationship, where the smaller trees tend to show a higher rate of disease severity and incidence. While DBH shows a positive trend, although weak (not significant) in relation to both incidence and severity variables.

That is, the bigger the DBH, the lower the incidence and severity rate. However, in Figure 1, a dominant pattern is more clearly observed, the bigger the DBH, the greater the number of healthy trees.

Table 4 shows the results of the deviance analysis (ANO-DEV) for both, severity and diameter among the genotypes evaluated. Here it is observed that there are highly significant differences between genotypes for disease severity, but non-significant differences with respect to diameter growth.

Table 1

Incidence, severity and pathogenicity index of *Ceratocystis* wilt in *G. arborea*, in a clonal test at 2.4 years-old, Guácimo, Caribbean region of Costa Rica.

Clone	Incidence (%)		Severity grade (%)				Severity	Pathogenicity
	Healthy	Sick	1	2	3	4	Index	Index
57	81.3	18.8	81.3	18.8	0	0	1.19	6.3
MC0814	81.8	18.2	81.8	13.6	4.5	0	1.23	7.6
MC0902	84.2	15.8	84.2	5.3	10.5	0	1.26	8.8
51	71.4	28.6	71.4	28.6	0	0	1.29	9.5
1	81.0	19.0	81.0	9.5	9.5	0	1.29	9.5
53	70.0	30.0	70.0	30.0	0	0	1.30	10
58	78.3	21.7	78.3	13.0	8.7	0	1.30	10.1
MC2402	84.2	15.8	84.2	0	15.8	0	1.32	10.5
31	68.2	31.8	68.2	18.2	13.6	0	1.45	15.2
32PC	68.2	31.8	68.2	18.2	13.6	0	1.45	15.2
19	68.8	31.3	68.8	12.5	18.8	0	1.50	16.7
3	70.0	30.0	70.0	10.0	20.0	0	1.50	16.7
35PC	59.1	40.9	59.1	27.3	13.6	0	1.55	18.2
T	66.7	33.3	66.7	11.1	22.2	0	1.56	18.5
59	63.2	36.8	63.2	15.8	21.1	0	1.58	19.3
MC1313	63.2	36.8	63.2	15.8	21.1	0	1.58	19.3
26PC	55.0	45.0	55.0	30.0	15.0	0	1.60	20
5N	61.9	38.1	61.9	14.3	23.8	0	1.62	20.6
24	60.0	40.0	60.0	15.0	25.0	0	1.65	21.7
18PC	60.0	40.0	60.0	15.0	25.0	0	1.65	21.7
MC3204	52.6	47.4	52.6	26.3	21.1	0	1.69	22.8
12PC	47.6	52.4	47.6	33.3	19	0	1.71	23.8
33PC	60.0	40.0	60.0	15.0	15	10	1.75	25.0
20CA	57.9	42.1	57.9	15.8	15.8	10.5	1.79	26.3
4	61.1	38.9	61.1	11.1	11.1	16.7	1.83	27.8
27	63.2	36.8	63.2	0	26.3	10.5	1.84	28.1
19CA	53.3	46.7	53.3	6.7	33.3	6.7	1.93	31.1
4CA	38.9	61.1	38.9	27.8	33.3	0	1.94	31.5
26	55.6	44.4	55.6	5.6	27.8	11.1	1.95	31.5
33	47.4	52.6	47.4	10.5	42.1	0	1.95	31.6
8CA	43.8	56.3	43.8	25.0	18.8	12.5	2.00	33.3
1N	40.0	60.0	40.0	15.0	45.0	0	2.05	35.0
24CA	40.0	60.0	40.0	20.0	30.0	10	2.10	36.7
15N	40.0	60.0	40.0	6.7	53.3	0	2.13	37.8
20	31.6	68.4	31.6	21.1	36.8	10.5	2.26	42.1
Mean	60.8	39.2	60.8	16.0	20.3	2.8	1.65	21.7

Figure 2

Pathogenicity index and commercial volume genetic ranking comparison, associated to *Ceratocystis* wilt in a 2.4-year-old clonal trial of *G. arborea* in Guácimo, Caribbean region of Costa Rica.

Table 2

Variance components and parameters in the evaluated traits associated to "Ceratocystis wilt", in a clonal test of *G. arborea* at 2.4 years-old, Guácimo, Caribbean region of Costa Rica.

Parameter	DBH	Total height	Severity	Incidence
σ_g^2	0.3726	0.0824	0.0456	0.0081
σ_{plot}^2	0.2524	0.3046	0.0066	0.0018
σ_e^2	15.4389	4.3886	0.7229	0.2130
σ_p^2	16.0639	4.7756	0.775	0.2230
H_g^2	0.0232	0.0172	0.0588	0.0364
$H_{m(clonal)}^2$	0.3522	0.2607	0.5935	0.4694
A	0.5934	0.5106	0.7704	0.6852
$CV_{gi}(\%)$	3.2301	1.9216	12.969	23.16
PEV	0.2414	0.0609	0.0185	0.0043
SEP	0.4913	0.2468	0.136	0.0656
Mean	18.897	14.938	1.646	0.389

σ_g^2 : genetic variance. σ_{plot}^2 : plot variance. σ_e^2 : error variance. σ_p^2 : total phenotypic variance. H_g^2 : individual heritability. $H_{m(clonal)}^2$: mean clonal heritability. A : accuracy. $CV_{gi}(\%)$: coefficient of individual genotypic variation. SEP: standard deviation. PEV: variance of the error.

Table 3

Genetic correlation matrix for variables total height, diameter at 1.3 m, severity and incidence in a 2.4-year-old clonal trial of *G. arborea* in Guácimo, Caribbean region of Costa Rica

Variable	Height	Severity	Incidence
DBH	0.446*	0.221	0.236
Height		-0.346*	-0.235
Severity			0.933**

* indicate the threshold for significance at 5 % and ** 1 %, respectively

Table 4

Analysis of deviance for diameter (DBH, above) and for severity (below) in a 2.4-year- clonal trial of *G. arborea* in Guácimo, Caribbean region of Costa Rica.

Effect	Deviance	χ^2	Variance Component	Determination coefficient
Clone	2557.00	2.25	Vg = 0.3726	$H_g^2 = 0.0232$
Plot	2554.91	0.16	Vplot = 0.2524	c2plot = 0.0157
Residual			Ve = 15.4389	
Full model	2554.75			
Clone	524.26	11.29 **	Vg = 0.0456 **	$H_g^2 = 0.0588 **$
Plot	513.01	0.04	Vplot = 0.0066	c2plot = 0.0085
Residual			Ve = 0.7229	
Full model	512.97			

χ^2 is the chi-square test, where values of 3.84 and 6.63 indicate the threshold for significance at 5% and 1%, respectively, ** indicates significant results.

Discussion

The DBH values and total height of the trees in the clonal trial show strong growth rates, similar to those reported by Murillo et al. (2016) in a melina trial in the southern Pacific of the country. In both cases, trees above 20 cm diameter class recorded the highest number of healthy trees. Thus, an infection pattern inversely proportional to the DBH was observed, that is, the lower the diametric class, the higher the incidence and severity. Furthermore, the genetic correlation matrix shows a trend in this direction, with a positive relationship between both growth traits with incidence and tolerance.

This growth behavior could be associated with the effect of the disease progression, which produces a blockage of water and photosynthesis products that cannot flow through the vascular system (Arguedas et al., 2018). Finally, it manages to affect trees growth as recorded in this study. Growth rate of DBH in *G. arborea* at this age was extremely accelerated in the site conditions, where it registers values from eight to 10 cm/year.

Once the disease clogs the vascular system, trees will rapidly experience a decrease in growth rate, which will continue until it comes to a complete growth stop and death process occurs. The incidence (39 %) recorded in this research was higher than the value reported by Murillo et al. (2016), with 21.6 % in a 2.7-year-old clonal trial in the South Pacific without thinning. A higher incidence in the Caribbean can be explained by several criteria, such as a different clonal composition used between both genetic tests and, a bigger proportion of clones highly susceptible to the pathogen.

Besides, the presence of a more aggressive (virulent) isolate of the pathogen, or the higher humidity regime in the Caribbean, may favor the spread of the pathogen. Undoubtedly, site conditions favor the appearance of this disease. The site historically registers flooding episodes that keep the water level high for prolonged periods (Arguedas et al., 2018). Salas et al. (2016a, 2016b) and Murillo et al. (2016) also reported a differential incidence rate among genotypes, in several clonal melina trials along the South Pacific of the country. While Arguedas et al. (2018) found incidence values up to 40 %.

Disease severity was reported from zero to 28 % in commercial plantations in Costa Rica at different ages (Arguedas et al., 2018). Differences in the rate of disease severity progress was found to be dependent on the genotype (Murillo et al., 2016). Thus, severity rates of 22 % found in this research are within the range reported in the country for young melina plantations. Few available studies show that site selection for plantation and genetic material, both have a significant influence on disease incidence and severity (He et al., 2016; Méndez-Báez 2011; Murillo et al., 2016; Hernández et al., 2021). In those investigations, a significant correlation between soil acidity and disease incidence was reported. Evidence suggest that a reduction of disease occurrence and severity can be achieved through better control of environmental factors, such as, avoiding water presence in the soil and controlling soil acidity by liming (Hernández et al., 2021). Disease prevention and

its rate of progress can also be reduced by strict genetic selection of commercial materials, as determined in this research.

Genetic control analysis

A high genetic control was found in both traits of disease severity and incidence. High mean clonal heritability values ($H^2_{m(clonal)}$) and a significant coefficient of genetic variation were determined. Similar values were reported by Salas et al. (2016a) and Murillo et al. (2016), oscillating between 0.58 and 0.63 respectively. Individual heritability (H^2_g) values were low for DBH and total height, but similar to those reported in other studies with melina. Lokmal (1994) in Malaysia, with H^2_g values for DBH of 0.12 at ages 5 to 6 years, as well as in commercial height with values of 0.068, 0.179, and 0.166 at ages 1, 5, and 6 years, respectively. Hogde & Dvorak (2004), reported heritability values for DBH ranging from 0.10 to 0.09 at ages 1 and 3 years, as well as in total height with values of 0.18 and 0.17 at ages 5 and 6 years. In another study in India, with 70 clones it was reported broad sense heritability for total height in 0.31 at five years-old (Kumar 2007). Finally, Hernández et al. (2021a, 2021b) reported in Costa Rica values for DBH ranging from 0.068 to 0.113 at 2 to 4 years-old. Therefore, these heritability results are consistent with other studies reported with melina elsewhere.

For disease tolerance, clonal mean heritability value ($H^2_{m(clonal)}$) was higher than 0.47 and the coefficient of genetic variation over than 13 %. Both parameters suggest breeding potential. Through controlled crosses between the most tolerant genotypes, an improvement in the degree of tolerance would be expected (Resende et al., 2018). However, further studies are needed to address the inheritance type and an understanding of the mechanisms of *Ceratocystis* wilt resistance (Palloix et al., 2009).

Figure 2 supports the selection of genotypes that meet both disease tolerance and productivity criteria. None of the 34 clones registered total absence of the pathogen. However, genotypes 57, 51, MC8014, 1, MC2402, 31, 19 and 3 (eight in total), reported an incidence under 32 %, a pathogenicity index lower than 17 % and, the best position in the genetic ranking of commercial volume (productivity). Therefore, these eight genotypes can be determined as the best option for establishing commercial plantations in the Caribbean region of the country.

The analysis of deviance shows that there are significant differences between clones with respect to disease severity, but not significant in relation to productivity. Therefore, the selection of clones for planting could be based mainly on the pathogenicity index, and more loosely on productivity. Thus, clones MC2402, MC0814, 31, 19 and 3, could be also considered for this selection, since all of them rank in the first positions with respect to the pathogenicity index (lower than 16.7). Meanwhile, their commercial volume is not significantly different with respect to the group of elite clones, positioned in the first third of the genetic ranking (Figure 2).

The exclusive use of these materials would possibly reduce not only the incidence rate and severity of the disease, but also its rate of productivity progress in commercial plantations (Salas et al., 2016a). In addition, a well-structured thinning program could be followed to prevent the progress of the disease in plantations. In this way, melina plantations could be maintained until 5 to 6 years age, with no less than 150 standing trees per hectare, as proposed by Murillo et al. (2016).

The pathogenicity index becomes a better criterion to rank genotypes according to their degree of disease tolerance. This index not only considers the relationship presence/absence of the disease (incidence), but also takes into account the frequency of individuals in each of the four severity categories. In this way, a weight is added that produces a better ranking of genotypes in terms of tolerance to this disease. Likewise, the pathogenicity index is based on severity values which, as a trait, recorded the highest clonal mean heritability values ($H^2_{m(clonal)}$) and a high coefficient of genetic variation (Table 2).

Therefore, it allows the construction of a better genetic ranking based on this index. The graphical approach of subdividing the genetic ranking into thirds, allows a quick visualization of the genetic structure of breeding population. A first third defines a significantly pathogen tolerant subpopulation; however, their incidence values are still relatively high, some of them are poorly productive. A better decision would be to choose a group of genotypes, whose pathogenicity index does not exceed the value of 10, as can be seen in the first third of the ranking. This elite group would be the commercial melina subpopulation selected to establish the plantations.

It is expected, that with this reduced number of individuals, a progressive decrease in incidence will be achieved. Especially to decrease, not only the onset of the disease, but also the rate of progression through the severity categories (Murillo et al., 2016). This genetic material is expected to give us more time until the disease manifests itself. Therefore, it will allow us to follow a more relaxed plantation management, via successive thinning from the second or third year of age.

Conclusions

The pathogenicity index becomes a useful discrimination criterion for ranking genotypes for disease tolerance. Mean clonal heritability values were high for incidence and severity, evidencing that both traits are under a high genetic control. An infection pattern inversely proportional to the DBH was observed, that is, the lower the diametric class, the higher the incidence and severity. There are eight out of 34 elite clones, which show a strong genetic tolerance to the disease, and a high productivity as well. Their use can contribute to the reduction of the impact of *Ceratocystis* wilt in the region.

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References

- Alvarado A, Mata R, Chinchilla M (2014) Arcillas identificadas en suelos de Costa Rica a nivel generalizado durante el periodo 1931-2014: II. mineralogía de arcillas en suelos con características vérticas y oxidico caoliniticas. *Agronomía Costarricense* 38(1), 107-131. <https://doi.org/10.15517/rac.v38i1.15161>
- Anuagasi CL, Onuorah JA, Okigbo RN (2017) Fungal pathogens affecting seedlings of *Gmelina arborea* Roxb and *Tectona grandis* Lf and effect of three plant extracts. *International Journal of Agricultural Technology* 13(3), 307-330.
- Arguedas M, Rodríguez-Solís M, Moya R, Berrocal A (2018) *Gmelina arborea* “death disease” in fast-growth plantations: Effects of soil and climatic conditions on severity and incidence and its implications for wood quality. *Forest Systems* 27(1), 003. <https://doi.org/10.5424/fs/2018271-12236>
- Ávila-Arias C, Salas-Rodríguez A, Murillo-Cruz R (2016) Selección de genotipos superiores de *Gmelina arborea* Roxb. por su heredabilidad genética a la tolerancia de la enfermedad de pudrición del tronco, Pacífico sur de Costa Rica. *Revista Forestal Mesoamericana Kurú*, 13(32), pág. 11-20. <https://doi.org/10.18845/rfmk.v0i0.2548>
- Cavallini LFA (1998) Fitopatología: un enfoque agroecológico. San José, Costa Rica. Editorial Universidad de Costa Rica. 467 pp.
- Ferreira EM, Harrington TC, Thorpe DJ, Alfenas AC (2010) Genetic diversity and interfertility among highly differentiated populations of *Ceratocystis fimbriata* in Brazil. *Plant Pathology* Vol 59 (4): 721-735.
- Harrington TC, Thorpe DJ, Alfenas AC (2011) Genetic variation and variation in aggressiveness to native and exotic hosts among Brazilian populations of *Ceratocystis fimbriata*. *Phytopathology* Vol 101 (5): 555-566. <https://doi.org/10.1094/phyto-08-10-0228>
- Harrington TC, Kazmi MR, Al-Sadi AM, Ismail SI (2014) Intraspecific and intragenomic variability of ITS rDNA sequences reveals taxonomic problems in *Ceratocystis fimbriata* sensu stricto. *Mycologia* Vol 106 (2): 224-242. <https://doi.org/10.3852/13-189>
- He DC, Zhan JS, Xie LH (2016) Problems, challenges and future of plant disease management: from an ecological point of view. *Journal of Integrative Agriculture*, 15 (4), 705-715. [https://doi.org/10.1016/s2095-3119\(15\)61300-4](https://doi.org/10.1016/s2095-3119(15)61300-4)
- Hernández W, Badilla Y, Murillo O (2021a) Estimación de parámetros genéticos de *Gmelina arborea* Roxb. (melina) en el Caribe de Costa Rica. *Uniciencia* Vol. 35(1) 352-366, January-June, 2021.
- Hernández W, Badilla Y, Murillo O (2021b) Selección temprana en ensayos clonales de melina (*Gmelina arborea* Roxb.) en Costa Rica. *Agronomía Mesoamericana*. Volumen 32(1):93-106. <https://doi.org/10.15517/am.v32i1.42069>
- Hernández W, Badilla Y, Esquivel E, Murillo O (2021) Comportamiento de clones de melina (*Gmelina arborea* Roxb.) en condiciones de suelo ácidos. *Rev. Ciencias Ambientales* Vol 55 (2):229-249. <https://doi.org/10.15359/rca.55-2.11>
- Hogde GR, Dvorak WS (2004) The CAMCORE international provenance/progeny trials of *Gmelina arborea*: Genetic parameters and potential gain. *New Forests*, 28(2/3), 147-166. <https://doi.org/10.1023/B:NEFO.0000040942.34566.a7>
- Holland LA, Lawrence DP, Nouri MT, Travadon R, Harrington TC, Trouillas FP (2019) Taxonomic revision and multi-locus phylogeny of the North American clade of *Ceratocystis* Fungal Systematics and Evolution Jun (3): 135-156.

- Kumar A (2007) Growth performance and variability in different clones of *Gmelina arborea* (Roxb.). *Silvae Genetica*, 56(1-6), 32-36.
<https://doi.org/10.1515/sg-2007-0005>.
- Lokmal N (1994) Genetic parameters of *Gmelina arborea*: Height and diameter growth. *J. Trop.For. Sci.* 7(2):323 – 331.
- Méndez-Báez A (2011) Evaluación de tratamientos para el manejo de *Nectria* sp. en plantaciones de *Gmelina arborea* en Santa Rosa de Pocosol, Alajuela. Tesis. Licenciatura en Ingeniería Forestal. Instituto Tecnológico de Costa Rica. Cartago, Costa Rica. 39p
- Méndez Álvarez D, Tonimara de Souza C, Couto Alfenas A, Murillo O, Badilla Y, Ferreira Alfenas R (2020) First report of *Ceratocystis fimbriata* causing wilt on *Gmelina arborea* in Costa Rica. *Forest Pathology*. doi.org/10.1111/efp.12628
- Moya R (2004) *Gmelina arborea* en Costa Rica. *Bois et Forest Des Tropiques*, 279(1). 47-57.
- Murillo O, Salas A, Murillo R, Ávila-Arias C (2016) Tasa de avance de la pudrición del tronco en melina (*Gmelina arborea* Roxb.) y posibilidades de manejo. *Revista Forestal Mesoamericana Kurú* 13 (32): 40-50. doi:10.18845/rfmk.v0i0.2551
- Murillo O, Resende MDV de, Badilla Y, Gamboa JP (2019) Genotype by environment interaction and selection in teak (*Tectona grandis* L.) in Costa Rica. *Silvae Genetica*. (68):116-121. <https://doi.org/10.2478/sg-2019-0020>
- Oliveira LS, Harrington TC, Ferreira MA, Damacena MB, Al-Sadi AM, Al-Mahmooli IH, Alfenas AC (2015) Species or genotypes? Reassessment of four recently described species of the *Ceratocystis* wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. *Phytopathology*, 105(9), 1229-1244.
<https://doi.org/10.1094/phyto-03-15-0065-r>
- Palloix A, Ayme V, Moury B (2009) Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. *New Phytologist* 183(1): 190-199.
- Pathala D, Harini A, Hegde PL (2015) A review on gambhari (*Gmelina arborea* Roxb.). *Journal of Pharmacognosy and Phytochemistry* 4(2).
- Resende MDV de (2016) Software Selegen-REML/BLUP: a useful tool for plant breeding. *Crop Breeding and Applied Biotechnology* - 16: 330-339.
<https://doi.org/10.1590/1984-70332016v16n4a49>
- Resende MDV, Murillo O, Badilla Y (2018) Genética Cuantitativa y Selección en el Mejoramiento Forestal. Editorial Tecnológica de Costa Rica. Cartago, Costa Rica. 302 pp.
- Salas A, Murillo O, Murillo-Cruz R, Ávila-Arias (2016*) Evidencia de tolerancia genética a la pudrición del tronco en clones de *Gmelina arborea* Roxb. en Costa Rica. *Revista Forestal Mesoamericana Kurú* 13(32): 30-39.
<https://doi.org/10.18845/rfmk.v0i0.2550>
- Salas A, Murillo O, Murillo R, Ávila-Arias C, Mata X (2016b) Evaluación de la severidad de la pudrición del tronco en *Gmelina arborea* Roxb. *Revista Forestal Mesoamericana Kurú* 13(32): 01-10.
<https://doi.org/10.18845/rfmk.v0i0.2547>
- Telford A, Cavers S, Ennos RA, Cottrell JE (2014) Can we protect forests by harnessing variation in resistance to pests and pathogens? *Forestry: An International Journal of Forest Research* 88(1): 3-12.
<https://doi.org/10.1093/forestry/cpu012>
- Valdetaro DCOF, Oliveira LSS, Guimaraes LMS, Harrington TC, Ferreira MA, Freitas RG, Alfenas AC (2015). Genetic variation, morphology and pathogenicity of *Ceratocystis fimbriata* in *Hevea brasiliensis* in Brazil. *Tropical Plant Pathology*, 40(3), 184-192. <http://dx.doi.org/10.1007/s40858-015-0036-6>.