Australian Journal of Crop Science AJCS

AJCS 11(10):1374-1380 (2017) ISSN:1835-2707
doi: 10.21475/ajcs.17.11.10.pne748

Evaluation of dwarf coconut (*Cocos nucifera* L.) germplasm to the damage intensity caused by foliar diseases

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Abstract

Coconut palm (*Cocos nucifera* L.) is a perennial crop with economic importance in Brazil. However, the culture is susceptible to several pathogens, such as *Camarotella acrocomiae* (large verrucosis), *C. torrendiella* (small verrucosis) and *Botryosphaeria cocogena* (leaf blight). These diseases reduce the leaf area of the plants. They usually occur as complex which are known as verrucosis leaf blight (CVLB). The objective of this study was to evaluate six accessions of dwarf coconut preserved in the active germplasm bank (AGB) of Embrapa Coastal Tablelands: BYD (Brazilian Yellow Dwarf Gramame), MYD (Malaysian Yellow Dwarf), CRD (Camaroonian Red Dwarf), BRD (Brazilian Red Dwarf Gramame), BGD (Brazilian Green Dwarf Jiqui), and MRD (Malaysian Red Dwarf). The study was carried out in a completely randomized block design with six treatments (accessions) and five replications (blocks), with 11 monthly evaluations from June 2015 to May 2016. The incidence and severity variables for the three diseases were evaluated. These six variables were jointly analyzed to describe the damage intensity. According to the non-metric multidimensional scaling ordination technique, most of the variability of the CVLB (72%) was associated with variations in damage caused by small verrucosis. A smaller portion of the variability (23%) was associated with the damage caused by large verrucosis and leaf blight, which in turn, were modulated by both the accessions and by the environmental variables. Interactions between the accessions and the seasonality of environmental conditions modulate the damage caused to dwarf coconut trees by the foliar diseases complex. These interactions, the duration of the occurrence and the magnitude of the differences between the accessions should be considered by the breeding programs.

Keywords: *Camarotella torrendiella*, *Camarotella acroccomiae*, *Lasiodiplodia theobromae*, *Cocosnucifera*, *Botryosphaeria cocogena*, genetic resources

Abbreviations: AGB_active germplasm bank; BGD_Brazilian Green Dwarf Jiqui; BRD_Brazilian Red Dwarf Gramame; BYD_Brazilian Yellow Dwarf Gramame; CRD_Camaroonian Red Dwarf; CVLB_Complex verrucosis and leaf blight; LBI_leaf blight incidence; LBS_leaf blight severity; LVI_large verrucosis incidence; LVS_large verrucosis severity; MRD_Malaysian Red Dwarf; MRPP_Multiresponse Permutation; Procedures; MYD_Malaysian Yellow Dwarf; NMS_non metric multidimensional scaling; SVI_Small verrucosis incidence; SVS_Small verrucosis severity

Introduction

Coconut palm (*Cocos nucifera* L.) is a perennial fruit, found in most Brazilian states, especially in the Northeast. However, in recent years, the crop has expanded to the North and Southeast regions. The Green Dwarf variety is predominant due to its good performance in terms of yield and water quality. Southwest Asia is considered as the most likely region of coconut's origin (Purseglove, 1972). Its geographic distribution covers tropical regions, mostly warm climates with high humidity. In Brazil, the species was introduced in 1553 in Bahia by the Portuguese, who brought it from the islands of Cape Verde. Asia is the largest producer continent, with 51,582,214 tons of fruit, followed by America, where Brazil is the leader, corresponding to 2,890,286 tons of fruit in 2013 (FAO, 2016). According to Liyanage (1958), this species is composed of the varieties Typica (Tall) and Nana (Dwarf). Variety Nana, known as the dwarf coconut, is divided into three sub-varieties, according to the color of the seedling, petiole, inflorescence and epicarp of the immature fruit: green, yellow and red. The red dwarf group consists of two ecotypes, Malaysian and Cameroon, which are phenotypically distinguished by the shape and color of the fruits (Child, 1974; Pereira et al., 2006). Variety Typica Nar., known as tall coconut, is predominantly cross-pollinated, since its male flowers are formed before female flowers (FOALE, 2005). Plants have stem with mean circumference of 84 centimeters, and on average reaches 18 meters height. The leaves are long, with mean length of 5.5 meters. Fruits vary in size from medium to large. The copra of this variety is of good quality. Plants are adapted to different types of soils and climates. Under favorable conditions, yield period can reach 60 years (Siqueira et al., 2002). Dwarf varieties are the most used in Brazil for commercial purposes, such as for the production of coconut water, with sensorial quality superior to the other cultivars; and for the production of foods and/or the *in natura* dry fruit by the agroindustry, with higher yield of pulp. This variety may be a promising alternative for dry coconut producers, since it not only becomes a more commercial variety, but also reduces the deficit of pulp production currently observed in crops with hybrid and tall coconut cultivars (Aragão, 2007). The "green dwarf" is the most commercialized variety for coconut water due to the acceptance of the flavor, when compared with the "red" variety, for instance, as shown by Aroucha et al. (2014), which studied the sensory analysis of green dwarf and red dwarf coconut water. This culture is susceptible to diseases that hinder its cultivation. Among the leaf diseases, the large and the small verrucosis have as etiological agents the fungi *Camarotella acrocomiae* (Mont.) Hyde and Cannon, and *Camarotella torrendiella* (Batista) Bezerra and Vitória, respectively. According to Mariano (2011), these diseases first occurred in the state of Pernambuco, in 1945, and are currently found in all areas of coconut production in Brazil. Small verrucosis is also found in French Guiana (Warwick and Talamini, 2009). Small verrucosis is characterized by the formation of stromas adhered to the adaxial part of the leaflet, in an organized manner, similar to a diamond. A large verrucosis is characterized by the formation of stromas, which grow in the limbus and raque leaf in a disorderly way, and can be easily removed (Warwick and Talamini, 2009).

Leaf blight is a disease caused by the fungus *Botryosphaeria cocogena*, having the fungus *Lasiodiplodia theobromae* (Pat.) (Griffon and Maub.) (sin. *Botryodiplodia theobromae)* as the teleomorph. Although it is considered as an opportunistic pathogen, the attack of this fungus results in great damage to plants. It penetrates the leaves of the coconut palm through wounds and through lesions caused by large and small verrucosis (Warwick and Talamini, 2009). In the plant, symptoms begin at the leaf end, with a V-shaped lesion, and subsequently the entire leaf is infected, resulting in necrosis and fall. With the early loss of the leaves, the immature bunches fall, since they are supported by the leaves (Warwick and Talamini, 2009).

For foliar diseases that generally occur in conjunction, the opportunistic behavior of the fungus *L. theobromae* is taken into account. The joint occurrence of such diseases is known as complex verrucosis leaf blight (CVLB).

The evaluation of coconut palm accessions regarding the intensity of these diseases is extremely important for the genetic improvement of this crop. Regarding leaf blight, Warwick et al. (1990) verified lower incidence and severity of this disease in the Brazilian accession “Green Dwarf Jiqui”.

Therefore, the objective of this work was to characterize six accessions of dwarf coconut trees in terms of the damage intensity of the diseases of the complex verrucosis leaf blight, related to environmental conditions.

Results

NMS analysis for the damage intensity caused by the complex verrucosis leaf blight among different accessions

Using a set of six descriptive variables of CVLB diseases, the NMS analysis presented a suitable 2-D graphical solution to describe the differences among the accessions (Fig 1).

For the characterization of the AGB dwarf coconut accession, the NMS graphical solution represented 95% of the total variability of the original data of the six variables (accessions) among the analyzed samples, of which 72% were associated with axis 1, and 23% were associated with axis 2.

According to the MRPP analysis, dwarf coconut accessions were divided into three distinct groups, according to the damage intensity pattern of the CVLB, which coincides in great extent with the color of the fruits. The first is formed by MYD and BYD, the second is formed by BGD, and the third is formed by BRD and MRD. CDR presented intermediate behavior in relation to the other accessions, differing only from the BRD (Fig 1).

The distribution of the samples along axis 1 was positively correlated with the incidence ($r = +0.933$, $p < 0.001$) and severity ($r = +0.878$, $p < 0.001$) of small verrucosis, indicating an increase of damage in the samples from the left to the right of this axis, in the direction of BRD to BYD. In the case of axis 2, the distribution of the samples was positively correlated with the incidence ($r = +0.932$, $p < 0.001$) and severity ($r = +0.902$, $p < 0.001$) of large verrucosis, indicating an increase of damage from MYD to BYD. Although positive correlation was also observed with axis 2 for leaf blight incidence ($r = +0.313$, $p < 0.001$), this result was significantly lower than that observed for large verrucosis variables, and was not followed by significant leaf blight severity.

Regarding the association with climatic variables, axis 2 was positively correlated with temperature ($r = +0.453$, $p < 0.001$), and negatively correlated with rainfall ($r = -0.540$, $p < 0.001$) and relative humidity ($r = -0.340$, $p < 0.001$).

In summary, correlation analysis indicated that the variability in the intensity of CVLB damage on dwarf coconut accessions had two strong orthogonal components. One represented by the damage caused by small verrucosis, and another by the combination of the damage caused by large verrucosis and leaf blight. Variations in this second component were also strongly associated with seasonal variations, as evidenced by the correlations with climatic variables.

Interaction between the seasonal variation and dwarf coconut accessions regarding the damage intensity caused by the diseases of the complex verrucosis leaf blight

Due to the correlations observed between the ordination of the samples along axis 2 of the NMS and the variables incidence and severity of large verrucosis and leaf blight, this axis was used to describe the damage intensity caused by these diseases, and to model this intensity using the multivariate regression tree model. However, according to the results observed in the NMS analysis, the variability along this axis is dominated by the descriptors of large verrucosis to the detriment of those of the leaf blight. According to this analysis, a tree model with eight terminal nodes was able to explain 70% of the variability of this variable. The factors month and accessions explained 61% and 9% of the total variability of the damage intensity of large verrucosis and leaf blight, respectively, (Fig 2).

High damage intensity was observed from January to June, when the mean values for large verrucosis incidence (LVI), large verrucosis severity (LVS), leaf blight incidence (LBI) and leaf blight severity (LBS) were 95, 46, 42 and 22, respectively, contrasting with values of 49, 19, 39 and 21, observed for the same variables from July to December. This last period was subdivided into two others: the first one, between July and October, characterized by the smaller damage caused by large verrucosis and leaf blight, observed for all the accessions. The second one, from November and December, was characterized by intermediate damage caused by these diseases. From July to October, LVI, LVS, LBI and LBS value were of 38, 14, 37 and 21; From November and December LVI, LVS, LBI and LBS values were of 67, 27, 43 and 24.

In the period of high damage intensity, the red dwarfs BRD and MRD were more susceptible than the other accessions, with LVI, LVS, LBI and LBS values of 98, 54, 43 and 23 for the first group, and of 38, 14, 37 and 21 for the second group. In the period of intermediate damage intensity, the three red dwarf accessions and BYD showed greater susceptibility to large verrucosis and leaf blight (LVI, LVS, LBI and LBS of 77, 33, 43 and 24, respectively) than MYD and the BGD (LVI, LVS, LBI and LBS of 53, 17, 42 and 24, respectively).

Under low prevalence condition of these diseases, differences between the accessions were observed mainly in large verrucosis damage. The BRD presented greater susceptibility (LVI, LVS, LBI and LBS of 61, 27, 36 and 24, respectively) than the other accessions (LVI, LVS, LBI and LBS of 33, 11, 37 and 21). In addition, BRD presented stable indices during all months under this condition, contrasting with the other accessions that presented minimum variation in September (LVI, LVS, LBI and LBS of 14, 6, 36 and 21). The most significant differences in the variables that describe the leaf blight damage were associated with variations between seasons, not between accessions.

A multivariate regression tree model with seven terminal nodes explained 32% of the variability in the damage intensity caused by small verrucosis, which were described by the ordination of the samples along axis 1 of the NMS (Fig 3). The factors months and accessions explained 21 and 11% of the variability, respectively.

The low prevalence condition of damage caused by small verrucosis was observed from July to September, regardless of the accession, which presented mean values of small verrucosis incidence (SVI) and small verrucosis severity (SVS) of 7 and 2. Intermediate damage intensity of small verrucosis was observed from January to June. In June, accessions presented mean values of SVI and SVS of 29 and 6. From January to May, BGD, BRD and MRD presented small verrucosis indices (SVI and SVS of 7 and 1) very similar to those of the minimum prevalence condition of the disease, and were less susceptible than CDR and the two yellow dwarfs (SVI and SVS of 13 and 4). From October to December, the most susceptible accession was BYD (SVI and SVS of 41 and 15), followed by MYD, BGD and CDR (SVI and SVS of 23 and 6) and in red dwarfs (SVI and SVS of 7 and 1). The accessions MRD, BRD and CDR presented values that were constantly lower than the others throughout the evaluated period.

Discussion

Differences of the CVLB damage intensity patterns between the investigated dwarf coconut accessions were coincided with the fruit color. In addition, high similarity of these patterns was observed among the yellow dwarfs MYD and BYD, and among the dwarfs MRD and BRDG. These patterns presented high congruence with the groups and subgroups of accessions formed by the multivariate analysis of 38 quantitative morphoagronomic descriptors analyzed by Sobral (2010). According to the author, three distinct groups were formed, which includes (i) BGDJ, (ii) CDR and (iii) MYD, BYD, MRD and BRD. Within the latter, two subgroups composed of MYD and BYD and MRD and BRD were later separated in function of the high similarity between their members. In fact, some cultivars are in different populations, like the case of BYD, in relation to MYD (Batugal and Jayashree, 2005).

Variations in CVLB damage intensity were described by two orthogonal components, one related to the damage of small verrucosis, and another by the combination of large verrucosis and leaf blight. In order to evaluate lack of correlation between small verrucosis and the other CVLB diseases, data from the studies published by Monteiro et al. (2013) and by Leal et al. (1997) were selected and analyzed. Coefficient of correlation close to zero was obtained from the mean data of the number of small verrucosis lesions and leaf blight incidence in fungicide-treated green dwarf plants, in evaluations (n = 5) carried out by Monteiro et al. (2013), between February 2011 and March 2012 in Rio de Janeiro. From the work of Leal et al. (1997), monthly monitoring data were obtained in 1989/90 regarding the number of stroma of large verrucosis and small verrucosis in CDR and MYD. Coefficients of correlation between these two verrucosis were zero for MYD, and 0.51 ($p > 0.10$) for CDR. These analyses corroborate with the data of the present study, evidencing the independence of the seasonal variation between small verrucosis and the other CVLB components for different genotypes.

Characterization of these six accessions for susceptibility to large verrucosis and small verrucosis (Leal et al., 1997) and to leaf blight (Warwick et al., 1990) were previously carried out in Sergipe. The results obtained in the present study are in accordance with the greater susceptibility of MRD and BRD to large verrucosis found by Leal et al. (1997). In fact, BRDG was sensitive to damage caused by large verrucosis, even in conditions that damage to the other accessions were relatively low. However, unlike the data presented by Leal et al. (1997), BGDJ showed levels of resistance to large verrucosis damage more similar to those of the yellow dwarfs than to those of MRD and BRD. Although differences were observed in the general susceptibility to large verrucosis between the accessions, they greatly reduced during the six months of high prevalence of the disease (January to June).

High susceptibility of the yellow dwarfs to small verrucosis reported in this study also differed from the data of Leal et al. (1997). In relation to this disease, BRD and MRD presented much lower susceptibility than other accessions, even in the three months (October to December) of greater damage intensity caused by small verrucosis. The BRD and MRD accessions, as well as BGD, were also less susceptible than the yellow dwarfs and CDR during the five months of intermediate damage intensity (January to May). The relatively long extension of the differences between the accessions for small verrucosis damage, especially considering periods of intermediate and high prevalence of this disease, may be indicative of the potential of BRD and MRD for breeding programs. However, these two accessions were the most sensitive to large verrucosis.

Leaf blight variability observed in the present study was more associated with seasonality than with differences between dwarf coconut accessions. This observation is in disagreement with the results obtained by Warwick et al. (1990), who observed differences between accessions. According to the authors, BGD was superior to the other accessions regarding resistance to leaf blight, since it presented lower incidence of the disease and smaller size and growth of the lesions. Increases in leaf blight in the present study were associated with the months with lower monthly average rainfall in the first months of the rainy season (November to June). This observation is in accordance with Warwick et al. (1993), who observed significantly higher growth rate of lesions caused by leaf blight in coconut hybrids in the dry season. The anemophilic dispersion of *L. theobromae* conidia has been reported to be strongly

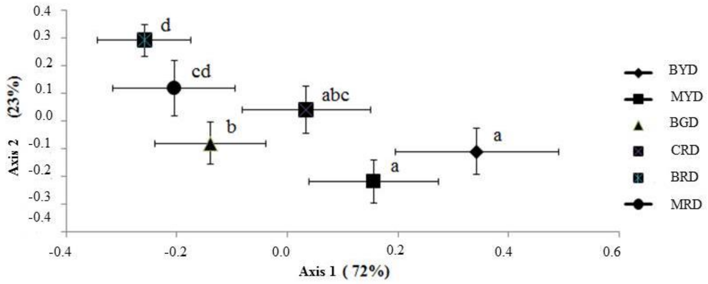

Fig 1. Two-dimensional NMS graph showing the distribution of the variability between the accessions along axes 1 and 2, as a function of the environmental variables (temperature, rainfall, and relative humidity). Symbols followed by the same letter do not differ from each other.

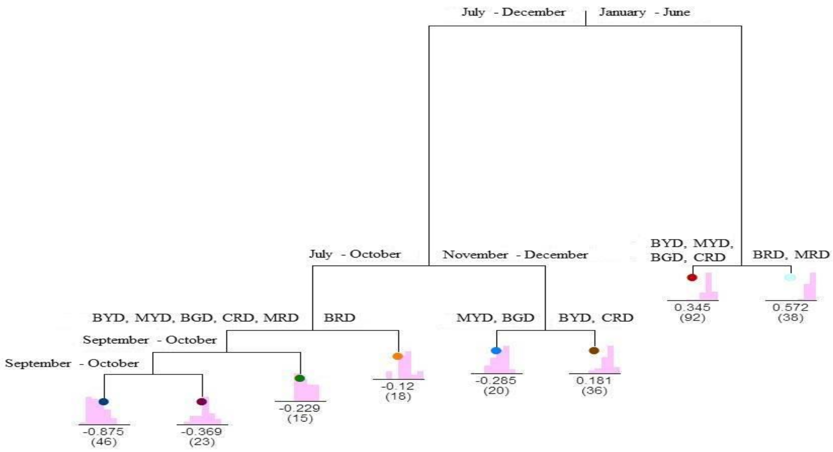

Fig 2. Multivariate regression tree model of the scores of axis 2 as a function of danage index large verrucosis, leaf blight and evaluated accessions. The BYD (Brazilian Yellow Dwarf Gramame), MYD (Malaysian Yellow Dwarf), BGD (Brazilian Green Dwarf Jiqui), CDR (Camaroonian Red Dwarf), MRD (Malaysian Red Dwarf), and BRD (Brazilian Red Dwarf Gramame). Means values of observations showed in parenthesis.

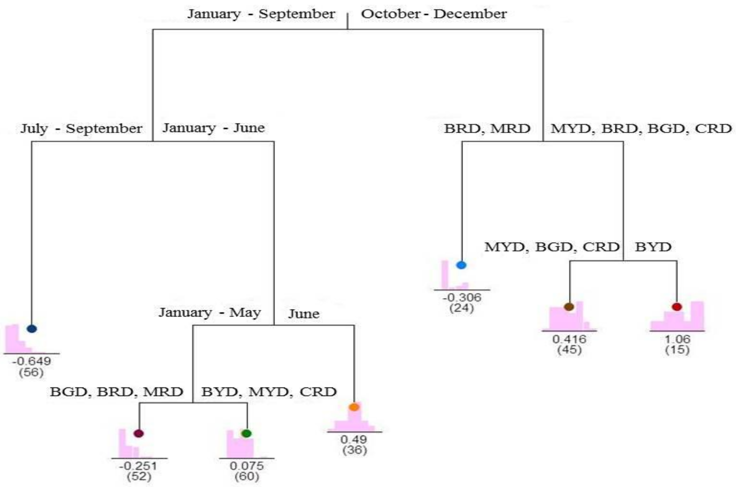

Fig 3. Multivariate regression tree model of the scores of axis 1 as a function of danage index of small verrucosis, leaf blight and evaluated accessions. BYD (Brazilian Yellow Dwarf Gramame), MYD (Malayan Yellow Dwarf), BGD (Brazilian Green Dwarf Jiqui), CDR (Camaroonian Red Dwarf), MRD (Malaysian Red Dwarf), and BRD (Brazilian Red Dwarf Gramame). Means values of observations showed in parenthesis.

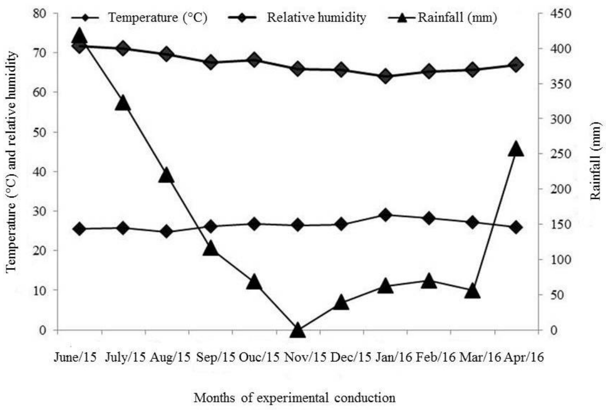

Fig 4. Environmental variations of the experimental area reported during the experiment.

stimulated by rainfall, especially between 25 and 80 mm of monthly average rainfall, according to monitoring in the coconut production field (Correia and Costa, 2005). In the present study, the period from October to March presented the most favorable monthly averages rainfall for spore dispersion, as reported by Correia and Costa (2005), which in a large extent coincides with the period of greatest damage caused by leaf blight. According to the CVLB damage intensity pattern, the evaluated dwarf coconut accessions were divided into three groups. These patterns coincide with the color of the fruits. The first is formed by MYD and BYD, the second, by BGD, and the third, by BRD and MRD. CDR shows intermediate behavior compared to other accessions, differing only from BRD. The damage intensity of the complex verrucosis leaf blight can be described by two relatively independent components, one composed by the damage of the combination between large verrucosis and leaf blight, and another by the damage caused by small verrucosis.

Materials and methods

Description of the experimental area and plant materials

The experiment was carried out at the Experimental Field of Itaporanga d'Ajuda-Embrapa Coastal Tablelands, from June 2015 to May 2016, located in the municipality of Itaporanga d'Ajuda, Sergipe, on SE 100, km 3 (lat. 11°07'S, long. 37°11'W), in the Southern Coast of Sergipe, 28 km from Aracaju, where planting is carried out in Quartzarenic Neosol. Fig 4. shows the environmental data collected during the experiment. Six accessions of dwarf coconut were evaluated. These accessions were collected from the Active Germplasm Bank (AGB) of Embrapa Coastal Tablelands, where large and small verrucosis and leaf blight naturally occurs, being: BYD (Brazilian Yellow Dwarf-Gramame), MYD (Malaysian Yellow Dwarf), CDR (Red Dwarf Cameroon), BRD (Brazilian Red Dwarf-Gramame), BGD (Brazilian Green Dwarf), and MRD (Malaysian Red Dwarf).
The plants corresponding to the dwarf AGB were 13 years of age, and planted in an equilateral triangle scheme, spaced 7.5 × 7.5 m, under micro sprinkler irrigation condition. For chemical treatments, the area received application of cotton oil in the fruits for mite control purposes. All the plants received normal cultural treatments (irrigation, fertilization) according to the conventional indications for coconut plantation. Weed control was carried out by mechanical weeding.

Evaluation of incidence and leaf blight severity

Leaf blight incidence and severity were evaluated following a methodology proposed by Talamini et al. (2013), with modifications. To evaluate the incidence, the total number of leaves and the number of diseased leaves were counted in each plant and the data obtained were expressed as percentage. Disease severity was evaluated according to the score scale and all the diseased leaves received a score from 0 to 4, in which: 0 = asymptomatic leaf; 1 = 25% of the leaf with symptom; 2 = 50% of the leaf with symptom; 3 = 75% of the leaf with symptom; and 4= 100% of the leaf with symptom or completely dead. Subsequently, scale indices were subjected to the following formula (Talamini et al., 2013):

$LBDS(\%)\frac{\Sigma(\text{n x f})}{(\text{Z x N})}\ x\ 100$, in which:

Where; LBDS (%) is the leaf blight damage severity;
n=the score attributed to the leaf;
f= the number of leaves evaluated with score n;
Z=the maximum value of the scale, in this case, 4.;
and N is the total number of leaves evaluated.

The experiment consisted of randomized blocks design, with six treatments of coconut accessions and five replications, with a missing plot (MRD accession in blocks IV and V). Each plot was formed by three plants.

Evaluation of the incidence and severity of large verrucosis and small verrucosis

The methodology proposed by Leal et al. (1998) with modifications was used to evaluate the incidence of large verrucosis and small verrucosis. Each month, between June 2015 and May 2016, six leaflets were collected from each plant and sent to the Pathology Laboratory of Embrapa Coastal Tablelands for evaluation. Incidence was calculated by the percentage of leaflets with symptoms. For the expression of severity, the score scale methodology proposed by Carvalho et al. (2003) with modifications was adopted, as follows: for small verrucosis - 0: absence of disease; 1: one to two lesions; 2: three to four lesions; 3: more than five lesions; 4: predominance of lesions with necrosis; 5: lesions with necrosis throughout the leaflet; and for large verrucosis: 0: absence of disease; 1: one to five lesions; 2: six to ten lesions; 3: more than ten lesions; 4: predominance of lesions with necrosis; 5: completely dried/necrotized leaflet. The data collected were calculated using the formula:

$VDS(\%)\frac{\Sigma(\text{n x f})}{(\text{Z x N})}\ x\ 100$, in which:
Where; VDS (%) is the verrucosis damage severity;
n is the score attributed to the leaf;
f is the number of leaves evaluated with score n;
Z is the maximum value of the scale, in this case, 5;
and N is the total number of leaves evaluated.

Statistical analysis

Multivariate analyses were used to evaluate the variations that occurred both in the severity and in the incidence of large verrucosis, small verrucosis and leaf blight. The combined effect was used as a descriptor of the "damage intensity of the complex verrucosis leaf blight". This analysis allowed interpreting the impact of commonly associated foliar diseases on areas of coconut production, also relating the possibility of using more than one descriptive variable of each disease, strengthening the evaluation of the damage caused to the plants.

In order to describe the relationship between the different accessions regarding the damage intensity caused by the complex verrucosis leaf blight, the non-metric multidimensional scaling technique (NMS) (Borg and Groenen, 1997) was used, after relativizing the data of each sample, within each variable, by the total of samples. The purpose of this operation was to minimize the undesired effect of differences in the magnitude of the expression unit of the variables on the interpretation of the results. The NMS analysis was carried out in the statistical package PC-ORD 6.0, using Sorensen distance measures by selecting the "slow and thorough" options of the "Autopilot" mode. The number of dimensions to be represented was selected based on the stress and significance criteria of the graphic solution. Sample scores in the different axes of the selected ordination were used in correlation analyses with the environmental variables to describe the factors associated with the variability in the intensity of the complex verrucosis leaf blight.

The MRPP (Multiresponse Permutation Procedures) method of comparison of multivariate means was used to test the hypothesis of difference in damage intensity caused by the CVLB among the accessions.

Multivariate regression tree models (Breiman et al., 1984) were used to evaluate the existence of accessions × climatic variables interactions, using the NMS axis scores as effect variables, since they describe the variability of one or more diseases of the complex verrucosis leaf blight. The adjustments to be done prior to the analysis were: ten for the minimum size of samples contained in a new node as a condition to allow a new partition of the sample set, and 20 for the number of sub-sample cycles for the cross-validation action. The number of terminal nodes to be interpreted was selected from the tree model indicated by the cross-validation operation.

Conclusions

Dwarf coconut accessions were susceptible to small verrucosis, according to the sequence of BRD = MRD = BGD <CDR <MYD = BYD. For the damage caused by large verrucosis, susceptibility increased in the following order: MYD = BYD = BGD <CDR <MRD = BRD. Differences in the damage intensity caused by leaf blight were associated with environmental factors, regardless of the accession. Interactions between the accessions and the seasonal variations of the environmental conditions modulated the damage caused to dwarf coconut trees by the foliar diseases complex. These interactions, as well as the duration of the occurrence and the magnitude of the differences between the accessions should be considered by breeding programs.

Acknowledgments

Capes and Embrapa Coastal Tablelands for financial support.

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