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Sources of resistance to *Fusarium oxysporum* f. sp. *cubense* in banana germplasm

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Abstract – Fusarium wilt (syn= Panama disease), caused by *Fusarium oxysporum* f. sp. *cubense* (FOC), is one of the most destructive diseases of banana, being genetic resistance the main management strategy for this disease. Since the pathogen is constantly evolving to supplant the genetic resistance, new sources of resistance must be investigated by genetic improvement programs aiming to developing new varieties. The objective of the present study was to identify sources of resistance from the different accessions maintained in the banana active germplasm bank (BAGB) at Embrapa Mandioca e Fruticultura. Forty-one BAGB accessions were evaluated, including 17 diploids, 21 triploids, and two tetraploids. The area under the disease progress curve, disease index, and incubation period were also evaluated. In relation to FOC resistance, there is genetic variability available among the BAGB accessions. The genotype M53 is notable for the complete resistance it expressed, and the accessions Birmanie, PA Songkla, Pirua, Imperial, Poyo, Ambei, Walebo, and Kongo FRF 1286 expressed quantitative resistance.
Index terms: *Musa* spp., Panama disease, varieties, genetic resistance.

Fontes de resistência a *Fusarium oxysporum* f. sp. in banana germosplasma

Resumo – O murcha de Fusarium, causado por Fusarium oxysporum f. sp. cubense (FOC), é uma das principais doenças da cultura da bananeira. A principal estratégia de controle da doença faz-se com o uso de variedades resistentes, sendo que, para a sua obtenção, faz-se necessário identificar genótipos para utilização nos programas de melhoramento genético da cultura. O objetivo do presente estudo foi identificar fontes de resistência em acessos do Banco Ativo de Germoplasma (BAG) de Bananeira da Embrapa Mandioca e Fruticultura. Foram avaliados 41 acessos do BAG, sendo 17 diploides, 21 triploides e dois tetraploides. Também foram avaliados a área abaixo da curva do progresso da doença, o índice interno da doença e o período de incubação. Há variabilidade genética disponível entre os acessos do Banco de Germoplasma de Bananeira para resistência ao FOC, com destaque à imunidade expressada pelo genótipo M53. Os acessos Birmanie, PA Songkla, Pirua, Imperial, Poyo, Ambei, Walebo e Kongo FRF 1286 expressaram resistência.
Termos para indexação: *Musa* spp., mal do Panamá, variedades, resistência genética.

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Introduction

Banana farming has high potential for generating jobs and income, and Brazil is one of the five largest producers of bananas in the world (FAOSTAT, 2016). However, the most commonly grown varieties in Brazil are affected by Fusarium wilt, but fortunately this disease can be efficiently controlled using resistant varieties (SILVA et al., 2016; PLOETZ, 2015).

Fusarium wilt is caused by *Fusarium oxysporum* f. sp. *cubense* (FOC), which is a fungus that inhabits the soil. The pathogen has high evolutionary potential, with 23 known vegetative compatibility groups (VCGs), three physiological races (races 1, 2 and 4) that infect banana species, and a fourth race that only infects *Heliconia* plants (FOURIE et al., 2011; PLOETZ, 2015).

Race 1 affects the cultivars Gros Michel, *Musa textilis* (abacá), 'Gros Michel' (AAB), 'Silk' or 'Maçã' (AAB), 'Pomme' or 'Prata' (AAB), 'Pisang Awak' (ABB), and 'IC2' (improved AAAA hybrid).Race 2 affects 'Bluggoe' (ABB) and improved AAAA hybrids, and has already been found in seedlings of *Musa balbisiana* (BB) and 'Gros Michel' (AAB). Race 4 affects various cultivars, including all of subgroup Cavendish (FOURIE et al, 2011; PLOETZ, 2015). The strains of this race are divided into Tropical (TR4) and Subtropical (ST4) (PLOETZ, 2006; BUTLER, 2013). FOC races 1 and 2 are distributed throughout almost all of Brazil, but TR4 is still not present in the country. However, if it was accidently introduced it could affect all the cultivars resistant to races 1 and 2 (DITA et al., 2013).

The banana active germplasm bank (BAGB) from Embrapa comprises 323 accessions with a wide variety of characteristics of agronomic importance, and includes valuable sources of resistance to the main banana diseases. For this germplasm to meet the demands of banana genetic improvement programs, collection, characterization, and maintenance activities are indispensable to preserve and increase the existing genetic variability.

The first step to develop new improved varieties is through is the identification of sources of resistance (SILVA et al., 2016). Testing the resistance of genotypes can be done in an experimental field previously infested with FOC isolates. In this case, the evaluation process is long, lasting, on average, two years. However, tests conducted in a greenhouse reduce the evaluation time up to three months (RIBEIRO et al., 2015) and allow the use of different isolates. The objective of the present work was to identify banana accessions with resistance to *Fusarium oxysporum* f. sp. *cubense* race 1 to use to genetically improve the crop.

Material and Methods

Two experiments were performed in a greenhouse conditions at Embrapa Mandioca e Fruticultura in Cruz das Almas, Bahia, with 41 banana accessions including wild genotypes, improved diploids, triploids, and commercial tetraploids (Table 1). The plants were micropropagated using the *in vitro* culture technique followed by acclimatization for 90 days in a greenhouse.

The pathogenicity tests were conducted for the FOC isolate 'CPMF0801' (race 1), and the inoculum produced in a sand:cornmeal (SC) mixture (5:1), with an additional 150 mL of distilled sterilized water. The mixture was placed in plastic bags and autoclaved at 120 °C for two hours, two times, with an interval of 24 hours between each sterilization procedures. Disc of PDA (potato dextrose agar) containing the FOC growth were transferred to the SC substrate, after cooled, and the plastic bags were maintained in a growth chamber at 25 ± 3 °C for 15 days. To quantify the concentration of inoculum, serial dilutions were used, with an adjustment to 10^6 colony-forming units of FOC.g^{-1} of substrate.

For the first trial the following genotypes were evaluated: S.A; Mambee Thu; Pisang Rojo Uter; Pisang Pipit; Poyo; Birmanie; Pirua; Imperial; PA Songkla; Walebo; Pisang Nangkla; Pisang Jaran; Pisang Berlin; 2803-01; Tjau Lagada; Mangana; Ambei and Gros Michel.

In the second trial the genotypes evaluated were: Grande Naine; P. Formoso; Malaccensis; Pisang Tongat; Figo Cinza; M61; Nanicão Magário; FRP 1292; Tuugia; Buitenzorg; Platina; Nanica; M53; Kongo; FRF 1286; PV03-76; Pacovan; Pisang Ustrali; Fako Fako; Figue Rose Naine; Prata-Anã 2; Robusta; *Musa Ornata* x *Velutina*; Prata Maceió; Prata-Anã and Markatooa.

The plants were inoculated by adding ten grams of the SC substrate containing the inoculum in four holes in the soil around the seedlings. As control (mock), the same accessions listed previously wereplanted in pots with non-infested soil. Eight repetitions per genotype were used, in a completely randomized design.

The incubation period (IP) was considered as the amount of time between inoculation and the appearance of symptoms in at least 50% of the plants. The severity of the disease was evaluated through the external appearance of symptoms, assessed every three days up to 85 days after inoculation, and was based on the following rate scale (MOHAMMED, 1999):

0: no symptoms;
1: initial yellowing of old leaves;
2: yellowing of old leaves and initial discoloration of young leaves:
3: intense yellowing of all leaves;
4: dead plant.

Table 1- Description of the banana genotypes used in the genetic resistance test to *Fusarium oxysporum* f. sp. c*ubense* race 1 in a greenhouse. Embrapa, 2017.

Genotype	Genome	Classification
2803-01	AA	Resistant
Ambei	AA	Resistant
Birmanie	AA	Resistant
Buitenzorg	AA	Resistant
Fako Fako	AA	Susceptible
Figo Cinza	ABB	Resistant
Figue Rose Naine	AAB	Resistant
Grande Naine P. Formoso	AAA	Resistant
Gros Michel	AAA	Susceptible
Imperial	AAA	Resistant
Kongo FRF 1286	AAA	Resistant
M53	AA	Resistant
M-61	AAA	Resistant
Malaccensis	AA	Resistant
Mambee Thu	AA	Resistant
Mangana	AA	Resistant
Markatooa	AAA	Resistant
Musa ornata x Musa velutina	ES	Susceptible
Nanica	AAA	Resistant
Nanicão Magario FRF 1292	AAA	Resistant
PA Songkla	AA	Resistant
Pacovan	AAB	Resistant
Pirua	AAA	Resistant
Pisang Berlin	AA	Resistant
Pisang Jaran	AA	Resistant
Pisang Nangka	AAB	Resistant
Pisang Pipit	AAA	Resistant
Pisang Rojo Uter	AA	Resistant
Pisang Tongat	AA	Resistant
Pisang Ustrali	AAB	Resistant
Platina	AAAB	Resistant
Poyo	AAA	Resistant
Prata-Anã	AAB	Susceptible
Prata-Anã 2	AAB	Susceptible
Prata Maceió	AAB	Susceptible
PV03-79	AAAB	Resistant
Robusta	AAA	Resistant
S.A	AA	Susceptible
Tjau Lagada	AA	Resistant
Tuugia	AA	Resistant
Walebo	AAA	Resistant

Eighty-five days after inoculation, the plants were removed from the substrate and the discoloration of the rhizome was evaluated based on the rate scale described by Cordeiro et. al. (1993):

0: no symptoms
1: isolated areas of infection;
2: discoloration in up to 1/3 of the ring formed by the origin region of the roots;
3: discoloration in 1/3 to 2/3 of the ring;
4: discoloration in over 2/3 of the ring;
5: discoloration throughout rhizome.

Based on these notes disease indices (DI) were calculated for external (EDI) and internal (IDI) symptoms using the formula proposed by McKinney (1923): $ID\ (\%) = 100.\sum[(f.v)/(n.x)]$; where DI is the disease index; f is the number of plants with the same note; v is the observed note; n is the number of plants evaluated; and x is the maximum note from the scale.

In addition, the area under the disease progress curve (AUDPC) was calculated, as proposed by Madden et al. (2007):

$$AUDPC - \sum_{i=1}^{n} \frac{(y_i+y_{i+1})}{2}(t_{i+1} - t_i)$$

where:

y_i is the severity of the disease (based on the DI) in the observed i;

y_{i+1} is the severity of the disease at the time of the subsequent evaluation i + 1;

t_i is the time (days) at the time of observation i;

t_{i+1} is time (days) at the time of the subsequent evaluation i + 1;

n is total number of evaluations.

All statistical analyses were conducted using the software R (RCORE TEAM R, 2014). The k-means and PCA analyses wereused to group the treatments and the Pearson correlation test was used to measure the correlation between the variables.

Results and Discussion

The BAGB accessions evaluated in the two experiments, were grouped in two categories regarding the genetic resistance: susceptible (S)and resistant(R). For the first trial five genotypes were classified as susceptible (S) and 17 as resistant to the Fusarium wilt (Figure 1). For the second trial a total do 15 genotypes were considered as 'R' and only three as 'S' (Figure 2).

Although classified as resistant, the internal disease index (IDI) values of the resistant accessions varied from 0.0 for the genotype M53 to 56.7%. The diploid 2803-01 had an IDI value of, the highest among the resistant accessions, and a long incubation period (64 days).

Positive correlations were observed between variables (Figure 3) in different experiments. For the first trial, positive correlations were noticed between the AUDPC and number of dead plants (0.86, P<0.001); AUDPC and IDI (0.84, P<0.01); and also between IDI and number of dead plants (0.88, P<0.001). No significant correlation was found between the other variables (Figure 3A).

In the second experiment, there were positive correlations for the same variables described for the first trial, with positive correlation between AUDPC and number of dead plants (0.77, P<0.01); between IDI and number of dead plants (0.56, P<0.05), and positive but no significant correlation between AUDPC and IDI (0.43, ns). No significant correlation was found between the other variables (Figure 3B).

Although the intensity of Fusarium wilt is diagnosed visually by analyzing external and internal symptoms, often these values are not directly proportional. A plant can exhibit external characteristics, such as yellowing caused by nutritional deficiency and excess water, but internally not exhibit discoloration in the rhizome. Thus, the most precise evaluation of the disease depends on the internal symptoms. The disease symptoms are expressed primarily in the roots; however, this depends on the genetic background of the plant.

Although in the same group, the resistant accessions (Group 2) had well differentiated AUDPC and IDI values. Most were wild diploids, evaluated as individuals with good agronomic characteristics (e.g., productivity, plant size, bunch size, flavor and appearance of the fruit, tolerance to certain pests and diseases, and adaptability to certain climate conditions), which is important to genetic improvement programs (CORDEIRO et al., 1993; AMORIM et al., 2008; SILVA et al., 2013).

The accessions 'Birmanie', 'Pirua', 'Poyo', 'Walebo', 'Imperial', 'PA Songla' and 'Pisang Nangka' had low internal disease indices and could be used as parents in crosses. A study conducted by Rebouças et al. (2015), which used microsatellite markers and evaluated the severity of the disease under greenhouse and field conditions, also observed that the accessions Birmanie and Pisang Jaran are resistant to the disease. The accession 'Tjau Lagada', classified in the resistant group, was also found to be resistant in fieldwork conducted by Cordeiro et al. (1993). Thus, the methodology of early detection of Fusarium wilt developed by Ribeiro et al. (2011) is efficient at evaluating FOC.

The genotypes 'Gros Michel' (AA), 'Mambee Thu' (AAA) and 'S.A' (AA) exhibited the highest severity of the disease due to their high susceptibility to FOC. These accessions had high internal disease indices (96.7, 86.7 and 83.3%, respectively) and short incubation periods (23 days for the first accession and 21 for the second and third). In addition, they had a high number of dead plants, with values of 62.5% for 'Gros Michel' and 50% for 'S.A' and 'Mambee Thu'. Although these accessions have only the AA genome, it was not possible to observe a relation between the genome and susceptibility to FOC.

In the second experiment, accessions were also classified as susceptible ('Prata Maceió', 'Fako Fako',

'Prata-Anã 2', '*Musa ornata* x *velutina'* and 'Prata-Anã') and resistant ('Grande Naine P. Formoso', 'Malaccensis', 'Pisang Tongat', 'Figo Cinza', 'M61', Nanicão Magário FRF 1292', 'Buitenzorg', 'Tuugia', 'Platina', 'Nanica', 'PV03-76', 'Pacovan', 'Pisang Ustrali', 'M53', 'Kongo FRF 1286', 'Markatooa' and 'Robusta') (Figure 2).

The diploid M53 was notable for not exhibiting symptoms of Fusarium wilt. This hybrid has been used as a parent in crosses to generate some cultivars (e.g., BRS Platina, BRS Princesa, BRS Preciosa and BRS Pacovan Ken) that are widely used in the internal market because they have good characteristics. This result corroborates the work developed by Cordeiro et al. (1993), to analyze BAGB banana diploids in the field.

The 'Malaccensis' accession is in the resistant group and exhibited a long incubation period (62 days) and no plant death. Subspecies *Malaccencis* belongs to the species *Musa acuminata*, is highly resistant to races 1 and 2 and tropical and subtropical race 4, and demonstrates quantitative or polygenic resistance (LI et al., 2015).

The cv. Malaccensis, Grande Naine P. Formoso and Pisang Tongat had the same incubation period value (62 days) and exhibited no symptoms of the disease. It was observed that shorter incubation periods were related to the lowest AUDPC values.

The cv. BRS Platina was classified in the group of individuals resistant of FOC race 1. This cultivar is a tetraploid hybrid (AAAB) from a cross between the triploid Prata-Anã, which is susceptible and has an AAB genetic constitution, and the diploid M53, which is resistant and has an AA genetic constitution. This genotype was developed by Embrapa Mandioca e Fruticultura (SILVA et al., 2016) and has good production characteristics, such as good tillering and a medium size, and sensory characteristics similar to the cultivar Prata-Anã (SILVA et al., 2016).

Among the resistant triploids, the accessions 'Nanicão Mangário FRF 1292', 'Grande Naine P. Formoso' and 'Marakatooa' had low IDI indices and long incubation periods. This study demonstrated the existence of genetic variability in relation to FOC race 1 resistance in the BAG of banana plants at Embrapa Mandioca e Fruticultura.

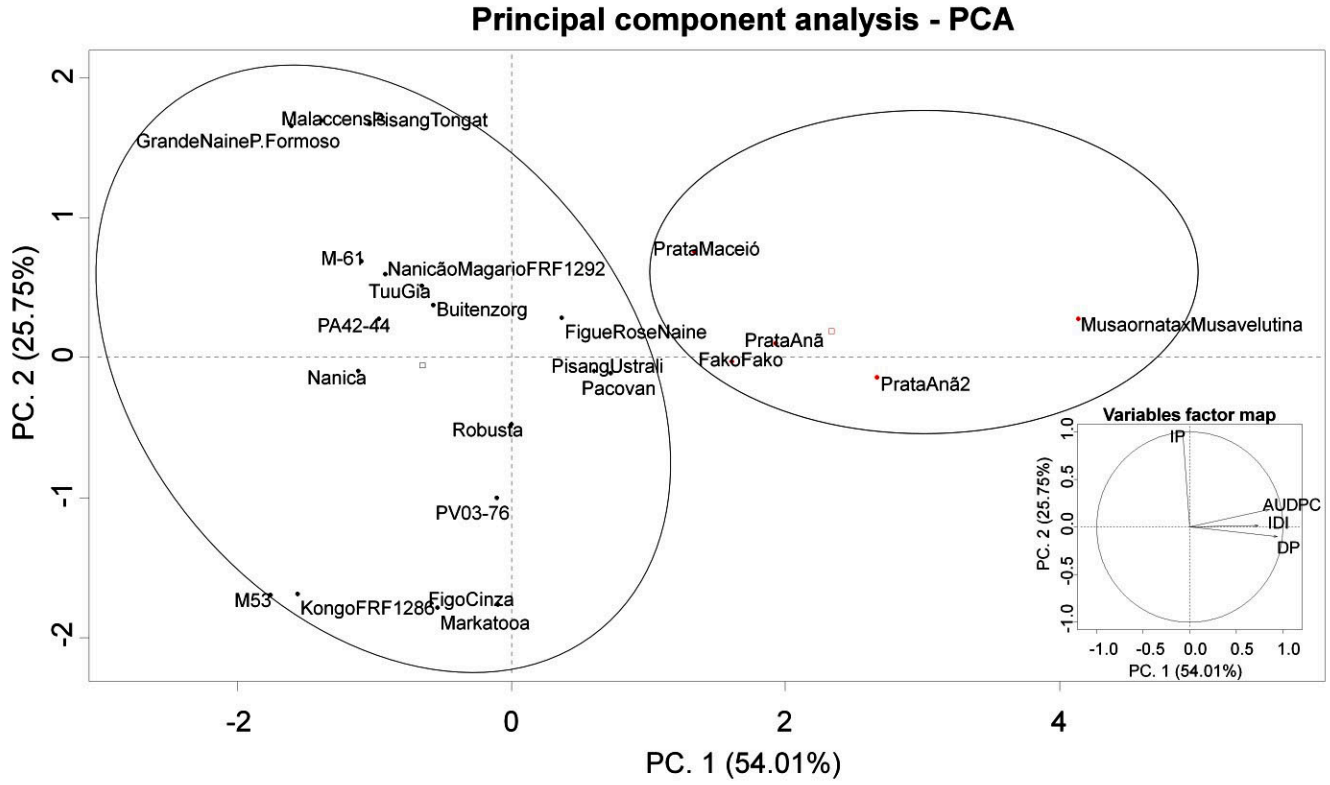

Figure 1. Principal component analysis of Experiment 1, based on the internal disease index (IDI), area under the disease progress curve (AUDPC), number of dead plants (DP) and incubation period (IP). Embrapa, 2017.

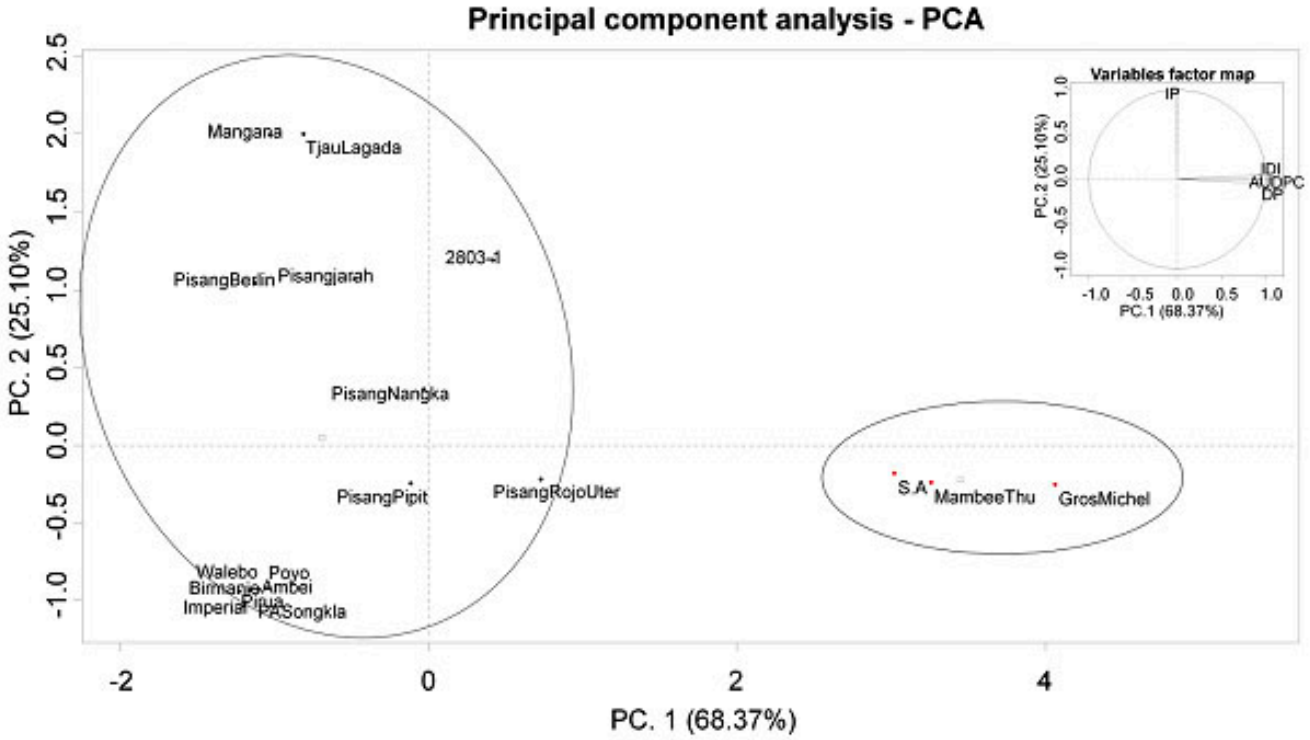

Figure 2. Principal component analysis of Experiment 2, based on the internal disease index (IDI), area under the disease progress curve (AUDPC), number of dead plants (DP) and incubation period (IP). Embrapa, 2017.

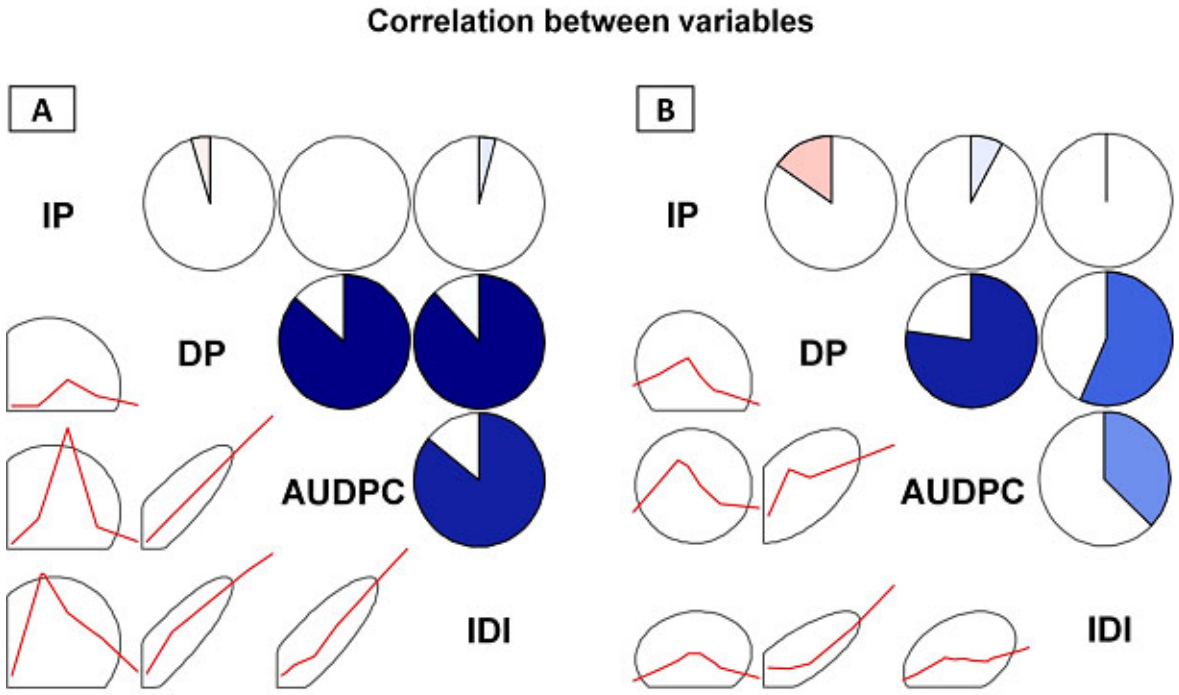

Figure 3. Correlogram displays for the evaluated parameters: internal disease index (IDI), area under the disease progress curve (AUDPC), number of dead plants (DP) and incubation period (IP). Above diagonal depicts pie chart for the Pearson's correlation coefficient.Shades of blue indicating positive correlations and shades of red indicating negative correlations. Dark blue corresponds to significant values at $P<0.01$, medium blue corresponds to a significance of $P<0.05$, light blue and pink corresponds to no significant positive and negative correlation, respectively. Embrapa, 2017.

Conclusion

There is genetic variability available among the BAGB accessions in relation to FOC CNPMF0801 race 1 resistance. Notable plants were the genotype M53 for not exhibiting symptoms of Fusarium wilt, the accessions 'Birmanie', 'PA Songkla', 'Pirua', 'Imperial', 'Poyo', 'Ambei', 'Walebo' e 'Kongo FRF 1286' that were resistant because they had low disease index values, and the accessions Birmanie', 'Pisang Nangkla', 'Pisang Jaran', 'Pisang Berlin', 'Tjau Lagada', 'Mangana', 'Pisang Pipit', 'Pisang Rojo Uter' and the selection 2803-1 that had low values for all of the variables.

For the second experiment, the accessions 'Grande Naine P. Formoso', 'Malaccensis', 'Pisang Tongat', 'Figo Cinza', 'M61', 'Nanicão Magário FRF 1292', 'Buitenzorg', 'Tuugia', 'Platina', 'Nanica', 'PV03-76', 'Pacovan', 'Pisang'Ustrali', 'Kongo FRF 1286', 'Markatooa' and 'Robusta' were notable.

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