## CROP BREEDING AND APPLIED BIOTECHNOLOGY

## ARTICLE

# Selection of F<sub>1</sub> genotypes of pineapple and reaction to inoculation with a *Fusarium* guttiforme isolate

José Severino de Lira Júnior<sup>1\*</sup>, Domingos Eduardo Guimarães Tavares de Andrade<sup>2</sup>, João Emmanoel Fernandes Bezerra<sup>3</sup>, Marta dos Santos Assunção<sup>3</sup>, Tereza Cristina de Assis<sup>3</sup>, Davi Theodoro Junghans<sup>4</sup> and Aristóteles Pires de Matos<sup>4</sup>

**Abstract:** Fusariosis (Fusarium guttiforme) is the most destructive disease of the pineapple crop in Brazil. The aim of this study was to evaluate the reaction to an isolate in pineapple genotypes selected for crownless fruit weight (CFW) and total soluble solids (TSS). A total of 1,125 genotypes ('BRS Imperial'×'Pérola') were evaluated. Selection for greatest CFW and TSS was performed during the sexual cycle. The isolate was inoculated on slip-type plantlets using the immersion technique. Plantlets were evaluated for disease intensity in the stem, attributing scores from 0 to 5. Eighteen genotypes were selected with values from 1.514 to 3.307 g (CFW) and from 14.6 to 19.4 °Brix (TSS). Six genotypes have agronomic traits superior to those of their parents, which qualify them to be cycled back into the plant breeding program of the Instituto Agronômico de Pernambuco - IPA.

**Keywords:** Ananas comosus var. comosus, plant breeding, resistance to fusariosis, regional adaptation

## INTRODUCTION

Pineapple (*Ananas comosus* var. *comosus*) is one of the most important fruits around the world. In 2020, production of approximately 27 million tons was harvested in about 80 countries, including an area of around 1 million ha in tropical and subtropical regions (FAO 2022). The main producing countries were the Philippines, with 2.70 million tons and 66 thousand ha; Costa Rica, with 2.62 million tons and 40 thousand ha; and Brazil, with 2.45 million tons and 64 thousand ha.

Fusariosis caused by the fungus *F. guttiforme* is among the diseases most injurious to pineapple cultivation (Nirenberg and O'Donnell 1998). The spread of this disease strongly hinders continuation of production areas or expansion into new ones. Its geographic distribution in the world is restricted, but it is the most destructive disease of the pineapple crop in Brazil. Pineapple fusariosis has been reported in other countries, such as Chile (Montealegre and Luchsinger 1990), Bolivia (Matos et al. 1992), and Cuba (Borras et al. 2001).





\*Corresponding author: E-mail: lira.junior@ipa.br ORCID: 0000-0001-5251-0844

Received: 11 August 2022 Accepted: 14 November 2022 Published: 01 February 2023

 <sup>1</sup> Instituto Agronômico de Pernambuco, Estação Experimental de Itambé, PE 75, km 32, Zona Rural, 55.920-000, Itambé, PE, Brazil
<sup>2</sup> Instituto Agronômico de Pernambuco – Pesquisa, BR 101, km 17, Zona Rural, 55900-000, Goiana, PE, Brazil
<sup>3</sup> Instituto Agronômico de Pernambuco, Sede Administrativa, Avenida General San Martin, 1371, Bongi, 50761-000, Recife, PE, Brazil
<sup>4</sup> EMBRAPA Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical, Rua Embrapa, s/n, Cruz das Almas, BA, 44380-000, Brazil

## JS Lira Júnior et al.

Pineapple fusariosis infects all parts of young or adult plant, from plantlets used as propagation material to the developing fruit. Infection of the plant results in chlorosis, curvature and shortening of the stem, reddish leaves, and wilting and death of the apical meristem (Santos et al. 2016, Sipes and Matos 2018). Fruit becomes useless for commercialization, due to soft rot of the flesh, associated with accumulation of resin or gum in the ovary locules (Cabral et al. 2009a, Ventura et al. 2009, Reinhardt et al. 2012).

Despite the availability of resistant cultivars ('BRS Imperial', 'BRS Vitória', and 'IAC Fantástico'), 'Pérola' and its variant 'Jupi', which are highly susceptible to fusariosis, predominate in Brazil. 'Pérola' is grown for the domestic market for consumption as fresh fruit. The plant has vigorous growth and spiny leaves. Its conical fruit weighs 1.6 kg on average, its white flesh contains abundant juice, and it produces an intense aroma. The total soluble solids content ranges from 13 to 16 °Brix. 'Jupi' is similar to 'Pérola', though it has a cylindrical fruit shape and may have a yellowish flesh color (Reinhardt et al. 2018, Junghans 2019).

Chemical control of fusariosis in susceptible cultivars is expensive, due to considerable labor demands, and it is added to other crop practices, such as selection of healthy propagative material. Plants are sprayed every 7 or 10 days, and application is directed to the inflorescence, from the appearance on the leaf rosette, until the closing of the last flowers (Matos et al. 2011, Nogueira et al. 2014). Spores penetrate plant tissues mainly via floral cavities, leaf wounds, and cracks in growing fruit. These spores are spread by wind action, splashing of rain, and/or transport by insects (Ventura et al. 2009, Sipes and Matos 2018).

The susceptibility of the cultivars 'Pérola' and 'Smooth Cayenne' to fusariosis exposes the vulnerability of the Brazilian pineapple crop to disease, since the two cultivars are grown on around 95% of the commercial pineapple area in Brazil. This susceptibility has a negative impact on Brazilian production, both on yield and on fruit quality. This fragility increases the importance of plant breeding for development of cultivars resistant to fusariosis that are better adapted to different soil and climatic conditions, maintaining the focus on fruit quality.

The aim of this study was to evaluate the reaction of 'BRS Imperial' × 'Pérola' pineapple genotypes to an *F. guttiforme* isolate inoculated on slip-type lateral shoots from  $F_1$  individuals selected for greater fruit weight and higher soluble solids content under rainfed cultivation in the Zona da Mata region in the north of the state of Pernambuco, Brazil.

## **MATERIAL AND METHODS**

The field trial was carried out at the Itambé Experimental Station (lat 7° 24' 16.80" S, long 35° 10' 54.00" W, alt 190 m asl) of the Instituto Agronômico de Pernambuco - IPA. The municipality of Itambé is located in the North region of the 'Zona da Mata'. The climate of this region is hot and humid, classified as As (Köppen), with mean annual temperature of 25 °C, and mean cumulative rainfall of 1,200 mm year<sup>1</sup>.

A total of 1,125  $F_1$  genotypes from seeds (sexual cycle) of the 'BRS Imperial' × 'Pérola' progeny were evaluated. This cross was developed by the IPA plant breeding program via controlled pollinations (Lira Júnior et al. 2021a), according to the technical recommendations of Cabral et al. (2009a). Notably, the parent cultivars of this progeny have a differential response for fusariosis resistance: 'Pérola' is susceptible and 'BRS Imperial' is resistant. These parents also have contrasting and complementary characteristics for growth vigor, plant adaptability, and fruit size and quality.

## Selection of superior F<sub>1</sub> genotypes

The selection of superior genotypes considered the following variables: crownless fruit weight (CFW) and total soluble solids content (TSS). The genotypes selected were those that had fruit with CFW and TSS values equal or superior to the average of the Pérola cultivar fruit, evaluated along with the progeny BRS Imperial × Pérola.

The field trial lasted for about 26 months, from May 2017 to July 2019. This period started with planting of seedlings at the simple spacing of 1.2 m × 0.6 m, and plant were grown under rainfed conditions. Natural flowering was gradual, as there was no treatment for floral induction. Fertilization was performed following the results of soil fertility analysis, according to recommendations reported by Bezerra and Silva Júnior (2017): P = 5 mg dm<sup>-3</sup>; pH (H<sub>2</sub>O) = 5,7; Ca = 3.7 cmolc dm<sup>-3</sup>; Mg = 1,00 cmolc dm<sup>-3</sup>; Na = 0.03 cmolc dm<sup>-3</sup>; K = 0.27 cmolc dm<sup>-3</sup>; Al = 0.00 cmolc dm<sup>-3</sup>; H = 5.36 cmolc dm<sup>-3</sup>; S = 5.00 cmolc dm<sup>-3</sup>; and CEC = 10.40 cmolc dm<sup>-3</sup>. Monthly rainfall data referring to the period of evaluation are presented

as follows (mm): from May 2017 to December 2017 – 147.0, 191.0, 380.0, 83.0, 63.0, 40.0, 5.0, and 8.0; from January 2018 to December 2018 – 201.9, 217.0, 105.0, 235.0, 175.0, 136.8, 124.0, 2.0, 4.5, 3.0, 4.7, and 48.0; from January 2019 to August 2019 – 82.0, 155.0, 193.0, 144.0, 146.5, 354.0, 342.0, and 134.0.

Harvest occurred gradually, and the fruit ripening point was determined by at least 50% yellow peel color together with flattening of the fruitlets and opening of the mesh (spaces between the fruitlets). Genotypes that exhibited fruit with at least one of the following defects were not evaluated: crownless fruit, fruit with a mini crown, fruit with multiple crowns, fasciation, and seedlings (slips) inserted at the base of the fruit.

The crownless fruit weight (CFW) and total soluble solids content (TSS) characteristics were measured and used to rank genotypes in descending order. Other complementary characteristics were evaluated at harvest and recorded for selected genotypes: crown weight (CW); circumference of the fruit middle third (CFMT); diameter of the central axis (DCA); fruit shape (FS: irregular, conical, oval, cylindrical, and cylindrical base); fruitlet profile (FP: prominent, normal, and flat); flesh color (FC: cream-white, yellow, golden-yellow, and orange); plant height to the fruit base (PHFB); peduncle length (PL); peduncle diameter (PD); length of the "D" leaf (LL); width of the "D" leaf (WL); leaf color (LC: light green, dark green, purplish green, greenish purple, purple, and red); leaf margin spinescence (LMS: total, smooth or without spines, spiny tip, and irregular); and number of plantlets (NP: slip, stem shoot, and sucker).

Genotypes with CFW and TSS values greater than those of the averages of 'Pérola', the most planted cultivar in Brazil, were selected for inoculation with the *F. guttiforme* isolate in slip-type lateral shoots.

## Reaction to F. guttiforme inoculation on slip-type plantlets

Only individuals selected because of greater CFW and higher TSS were evaluated regarding reaction to the *F*. *guttiforme* isolate. The isolate was inoculated on slip-type plantlets, which is a category of vegetative propagule that sprouts from lateral buds grouped close to the base of the fruit and/or along the peduncle that supports the fruit. Around 3-4 months after fruit harvest, slips were detached from the mother plant, considering slips of approximately 30 cm in height as a standard.

The isolate IT-01 was obtained from stem and fruit portions with typical symptoms of fusariosis disease. These portions were collected in a 'Pérola' cultivar plantation in the municipality of Itambé, PE. The fungus was cultured in solid (PDA – potato, dextrose, agar) and liquid (PD – potato, dextrose) media for a period of 21 days. A suspension was obtained in a blender by trituration of the liquid medium containing the fungal colony and by removing fungal propagules from the solid culture medium formed on Petri dishes. A volume of 10 mL of sterilized distilled water was added per Petri dish and the fungal colony was scraped with a Drigalsky spatula. The final suspension was filtered through a double layer of gauze and quantified to a concentration of  $1.5 \times 10^5$  conidia.mL<sup>-1</sup> by using a Neubauer-type hemocytometer.

Inoculation was carried out by the immersion technique (Matos 1978, Matos and Cabral 2006). Three equidistant perforations with a depth of 0.5 cm were made at the base of four slip-type plantlets per genotype. Then the plantlets were immersed in a conidial suspension for 5 minutes. Subsequently, the plantlets were planted in field beds, adding 10 mL of conidial fungus suspension to the base of each treated plantlet. The substrate was kept moist and covered with a layer of approximately 2 cm of dry straw. Four plantlets of 'BRS Imperial' (resistant) and 'Pérola' (susceptible) cultivars were included as controls.

At 90 days after inoculation, the plantlets were removed from the beds and evaluated regarding development of internal and external symptoms. The following scale was used to gauge fusariosis intensity according to percent of injury in the plant stem: 0 = no symptoms; 1 = up to 3%; 2 = from 3% to 10%; 3 = from 10% to 50%; 4 = more than 50%; 5 = dead plant. The reaction of the plant was scored as follows: 0.0 (no infection) = resistant; 0.1 - 2.0 = moderately resistant; 2.1 - 5.0 = susceptible. Only genotypes with scores of 0.0 (resistant) and 0.1 - 2.0 (moderately resistant) were selected.

## **RESULTS AND DISCUSSION**

Among the 1,125 genotypes evaluated, 18 were selected considering crownless fruit weight (CFW) and total soluble solids content (TSS), because they had higher scores than those obtained by the 'Pérola' cultivar (Table 1). Among the 18 selected, six genotypes showed a high level of survival against the IT-01 isolate of *F. guttiforme*, as they did not have

## JS Lira Júnior et al.

Genotype	CFW (g)	TSS (ºBrix)	<b>TA</b> (%)	TSS/TA Ratio	Score	Level of reaction
IPA 6.5-140	3,307	14.6	0.95	15.37	3.6	S
IPA 6.1-11	2,881	16.0	0.66	24.24	1.0	MR
IPA 6.3-46	2,640	15.2	0.77	19.74	4.6	S
IPA 6.5-44	2,557	14.8	0.69	21.45	0.0	R
IPA 6.4-17	2,331	16.2	0.62	26.13	4.8	S
IPA 6.3-110	2,261	19.0	0.69	27.54	1.0	MR
IPA 6.4-16	2,155	19.2	0.73	26.30	0.0	R
IPA 6.4-21	2,111	17.6	0.65	27.08	3.6	S
IPA 6.1-30	2,101	14.6	0.59	24.75	0.0	R
IPA 6.5-95	2,087	16.0	0.92	17.39	0.0	R
IPA 6.1-15	2,002	16.0	0.71	22.54	0.0	R
IPA 6.4-09	1,822	16.0	0.42	38.10	0.0	R
IPA 6.4-04	1,807	19.6	0.75	26.13	3.6	S
IPA 6.5-127	1,791	14.4	0.85	16.94	1.2	MR
IPA 6.3-78	1,715	19.4	0.83	23.37	1.4	MR
IPA 6.3-81	1,559	17.4	0.64	27.19	3.6	S
IPA 6.2-09	1,549	18.0	0.73	24.66	4.6	S
IPA 6.3-99	1,514	18.0	0.58	31.03	0.8	MR
Average of selected	2,105.89	16.68	0.71	24,44	-	-
'Pérola' <sup>1</sup>	1,506.75	14.40	0.69	20,86	5.0	S
'BRS Imperial'2	753.81	16.20	0.58	27,93	0.0	R
Overall average	1.676.22	14.98	-	-	-	-

Table 1. Selected genotypes of the 'BRS Imperial × Pérola' progeny with their characteristics of crownless fruit weight (CFW) and total soluble solids content (TSS), TSS/TA ratio, and reaction to *F. guttiforme* 

Level of reaction: S = susceptible; MR = moderately resistant; R = resistant; crownless fruit weight (CFW) and total soluble solids content (TSS); TA = Total acidity; <sup>1</sup> susceptible control; and <sup>2</sup> resistant control.

external symptoms or internal injuries in the stem; five genotypes survived, but developed typical symptoms of the disease, especially leaves that became dull in appearance and had a brownish color, an absence of roots, and small internal lesions in the stem; and another seven genotypes showed high susceptibility to the IT-01 isolate, with plantlet death or development of severe symptoms of internal stem rot and leaf rosette death.

## Selection of genotypes with superior crownless fruit weight (CFW) and total soluble solids content (TSS)

Natural flowering occurred gradually between June and August 2018. Harvest lasted for three months, from the middle of November 2018 until the end of January 2019. This long harvest period occurred in this progeny due to the nature of the plant material evaluated in the sexual cycle, composed of hundreds of individuals with different genotypic combinations, which had different responses to the same growth environment.

Admittedly, parent cultivars of this progeny have a differential response for natural flowering: 'Pérola' is more sensitive and 'BRS Imperial' is resistent to this physiological characteristic. For example, 'BRS Imperial' ('Perolera' × 'Smooth Cayenne') shows relatively slow growth and is more resistent to natural flowering, requiring that flower initiation (artificial stimulation) be performed 12 months after planting. 'Pérola' has greater vigor, faster plant growth, and wide adaptation to different edaphic and climatic regions, even under conditions of water deficit, which allows artificial stimulation as of 9 months after planting (Bartholomew 2014, Junghans 2019).

Eighteen genotypes were selected that achieved averages higher than the averages of the 'Pérola' cultivar for crownless fruit weight of 1,506.74 g and for total soluble solids content of 14.4 <sup>o</sup>Brix (Table 1). These results revealed the excellent potential of a group of promising individuals identified at the beginning of this first stage of screening in relation to the 'Pérola' cultivar most widely planted in Brazil. These promising genotypes should be evaluated for use *per se* and also included as parents in future IPA hybridization programs to increase the frequency of alleles favorable to quantitative characteristics related to higher yield and better fruit quality.

Regarding CFW, the IPA 6.5-140, IPA 6.1-11, IPA 6.3-46, and IPA 6.5-44 genotypes stood out, which expressed values higher than 2,500 g. As for TSS, the IPA 6.4-04, IPA 6.3-78, IPA 6.4-16, and IPA 6.3-110 genotypes achieved values between 19.0 and 19.6 °Brix. The IPA-6.4-09 genotype had the lowest total acidity, at 0.42%, and the highest ratio (TTS/TA), 38.1 (Table 1). Ratio is an important measure for pineapple breeding, especially when the objective of the program is development of cultivars for the in natura market, that is, for consumption as fresh fruit. Ratio defines flesh flavor and serves as a useful indicator to determine the time of harvest (Paull et al. 2020).

Lira Júnior et al. (2021b) evaluated 429 F1 genotypes from a cross between 'Pérola' and a pollen mixture of the 'MD-2', 'BRS Imperial', and 'BRS Vitória' cultivars, which exhibited variations from 382 g to 5,393 g for crownless fruit weight, and from 9.6 °Brix to 26.4 °Brix for total soluble solids content. This wide variability is probably due to the different male parents and the interaction between parents, including transgressive segregation for the characteristics considered.

Nhat Hang et al. (2011) studied F1 hybrids from different crosses, which exhibited averages from 1,500 to 1,760 g for fruit weight and from 18.4 to 19.4 °Brix for soluble solids. Cabral et al. (2009b) studied F1 genotypes of 8 progenies obtained from different combinations, two by two, among the 'Primavera', 'Smooth Cayenne', 'Pérola', 'Perolera', 'Porto Rico', and 'Roxo de Tefé' cultivars. The characteristics evaluated included the average weight of the fruit, which ranged from 1,102 g ('Pérola' × 'Primavera') to 1,857 g ('Perolera' × 'Puerto Rico'), and soluble solids content, which ranged from 13.2 °Brix ('Pérola' × 'Primavera') to 15.0 °Brix ('Puerto Rico' × 'Primavera').

Despite its wide acceptance by the Brazilian consumer, the fruit characteristics of the 'Pérola' cultivar are not attractive to and do not satisfy the international pineapple market. Its share in the export market is tiny, basically due to the conical shape of the fruit, together with a greenish peel at the maturation stage, creamy-white flesh, and high susceptibility to translucency (Matos and Reinhardt 2009, Reinhardt et al. 2018, Sanewski et al. 2018).

Among the genotypes selected, there was a predominance of those with the following traits: small crown with weight below 170 g; fruit with a cylindrical shape or cylindrical base; yellowish flesh color; smooth leaf margin, that is, without any spines; and satisfactory production of vegetative propagules (Tables 2 and 3), including the slip type, which is the most used plantlet category for commercial planting of the 'Pérola' pineapple in Brazil.

Constant	CFW	CFW CFMT		50			
Genotype	(g)	(cm)	(mm)	FS	FP	FL	
IPA 6.5-140	22	46.6	23	cyl. bas.	nor.	yel.	
IPA 6.1-11	80	43.4	18	con.	nor.	cre. whi.	
IPA 6.3-46	77	41.0	20	cyl. bas.	pro.	cre. whi.	
IPA 6.5-44	71	43.0	23	cyl. bas.	nor.	yel.	
IPA 6.4-17	148	41.4	32	cyl. bas.	pro.	yel.	
IPA 6.3-110	128	43.0	24	cyl. bas.	pro.	yel.	
IPA 6.4-16	75	42.4	27	cyl. bas.	pro.	yel.	
IPA 6.4-21	62	41.6	23	cyl. bas.	nor.	cre. whi.	
IPA 6.1-30	68	36.2	21	con.	pro.	gol. yel.	
IPA 6.5-95	167	45.0	25	cyl.	pro.	yel.	
IPA 6.1-15	155	40.4	22	cyl.	fla.	yel.	
IPA 6.4-09	46	37.0	30	con.	pro.	yel.	
IPA 6.4-04	102	39.0	21	cyl. bas.	nor.	gol. yel.	
IPA 6.5-127	133	41.0	22	cyl.	pro.	gol. yel.	
IPA 6.3-78	87	38.0	14	cyl.	nor.	yel.	
IPA 6.3-81	115	36.8	19	cyl.	fla.	yel.	
IPA 6.2-09	117	38.2	21	cyl.	nor.	yel.	
IPA 6.3-99	101	35.4	23	cyl. bas.	pro.	gol. yel.	

*Table 2.* Complementary characteristics of the fruit measured in the 18 genotypes from the cross 'BRS Imperial' × 'Pérola' selected for greatest crownless fruit weight (CFW) and highest total soluble solids content (TSS)

CW = crown weight; CFMT = circumference of the fruit middle third; DCA = diameter of the central axis; FS = fruit shape (irr. = irregular; con. = conical; ova. = oval; cyl = cylindrical; and cyl. bas. = cylindrical base); FP = fruitlet profile (pro. = prominent; nor. = normal; and fla. = flat); FC = flesh color (cre. whi. = cream white; yel. = yellow; gol. yel. = golden yellow; and ora. = orange).

## JS Lira Júnior et al.

Genotype	PHFB	PL	PD	LL	WL	Leaf color	LMS	NP
	(cm)	(cm)	(mm)	(cm)	(cm)			
IPA 6.5-140	54.7	20.1	34	87.3	5.1	d. gre.	w. spi.	7-0-3
IPA 6.1-11	61.3	27.4	35	101.5	6.2	p. gre.	w. spi.	19-1-2
IPA 6.3-46	52.3	23.4	34	100.1	5.2	l. gre.	w. spi.	13-1-5
IPA 6.5-44	53.4	24.7	35	97.5	6.0	d. gre.	w. spi.	15-5-1
IPA 6.4-17	55.4	21.3	32	92.4	6.5	d. gre.	w. spi.	6-0-3
IPA 6.3-110	56.5	23.0	24	96.2	4.8	d. gre.	w. spi.	13-2-0
IPA 6.4-16	50.4	19.8	31	70.7	4.8	p. gre.	w. spi.	9-5-2
IPA 6.4-21	49.3	19.6	29	78.7	5.8	d. gre.	w. spi.	7-0-2
IPA 6.1-30	57.5	20.3	33	95.6	6.7	l. gre.	w. spi.	15-0-4
IPA 6.5-95	42.1	25.0	17	60.2	5.2	p. gre.	w. spi.	8-6-1
IPA 6.1-15	57.2	29.6	28	99.6	6.1	d. gre.	w. spi.	11-2-4
IPA 6.4-09	52.3	22.4	29	95.3	6.6	p. gre.	w. spi.	16-1-1
IPA 6.4-04	58.5	22.7	28	88.5	6.9	p. gre.	tot.	9-3-5
IPA 6.5-127	48.5	17.3	28	74.5	5.1	l. gre.	w. spi.	6-5-5
IPA 6.3-78	48.5	21.5	31	87.6	6.1	d. gre.	w. spi.	14-1-1
IPA 6.3-81	50.4	17.2	28	86.4	4.1	d. gre.	w. spi.	11-1-2
IPA 6.2-09	47.6	19.2	29	91.2	6.4	d. gre.	w. spi.	8-2-2
IPA 6.3-99	50.1	17.9	31	90.2	6.0	l. gre.	w. spi.	12-2-2

Table 3. Complementary characteristics of plants measured in the 18 genotypes from the cross 'BRS Imperial' × 'Pérola', selected for greatest crownless fruit weight (CFW) and highest total soluble solids content (TSS)

PHFB = plant height to the fruit base; PL peduncle length; PD = peduncle diameter; LL = length of the D leaf; WL = width of the D leaf; LC = leaf color (l. gre. = light green; d. gre. = dark green; p. gre. = purplish green; g. pur. = greenish purple; and pur. = purple); LMS = leaf margin spinescence (tot. = total; w. spi. = without spines; s. tip = spiny tip; and irr. = irregular); NP = number of plantlets (slip - stem shoot - sucker).

## Reaction of plantlets to inoculation with the F. guttiforme isolate

Initially, symptoms were expressed on the leaves of the susceptible control ('Pérola') between five and six weeks after inoculation. The severity of the disease advanced rapidly, and at 90 days after inoculation, all plantlets died (Figure 1A). This result confirmed infection of the IT-01 isolate (*F. guttiforme*) in slip-type plantlets of the susceptible 'Pérola' cultivar. Non-inoculated plantlets of this susceptible cultivar showed normal vegetative growth and no symptoms of the disease.

Regarding the 'BRS Imperial' cultivar, used as a resistant control, no symptoms of the disease were observed on the leaves, and no infection or internal lesion was observed in the stem. This result indicates that the IT-01 isolate did not develop to cause infection; it was not able to overcome the genetic resistance of that cultivar.

Of the 18 genotypes selected for higher CFW and TSS, six genotypes were classified in the highest level of survival (Table 1). Inoculated plantlets of these genotypes showed normal vegetative growth and did not show symptoms of fusariosis in the leaves, lesions, or internal infections in the stem at 90 days, as highlighted for the IPA 6.1-15 genotype (Figures 1B and 1C). In an intermediate position, five genotypes were classified as moderately resistant, with scores between 0.1 and 2.0, due to the appearance of typical symptoms of the disease on the leaves, such as brown leaf color and dullness, as well as the occurrence of small internal lesions in the stem. Another seven genotypes showed high susceptibility to fusariosis, with scores from 3.6 to 4.8, due to the combination of typical symptoms of the disease, such as brown leaf color or chlorosis and dullness, as well as resin exudation at the leaf base, open leaf rosette, internal stem rot, and even plant death.

The IPA 6.1-15 genotype stood out in gathering the largest number of desirable traits, including survival against the *F. guttiforme* isolate (Figures 1B and 1C), a spineless leaf margin, cylindrical fruit shape, crownless fruit weight of 2,005 g, yellowish flesh color, and total soluble solids content of 16 °Brix. In addition, this genotype inherited the flat fruitlet profile, which facilitates peeling of the fruit. The number of plantlets was also satisfactory for vegetative propagation, with 11 slips, 2 stem shoots, and 4 suckers (Table 3).



*Figure 1.* (A) Dead plantlets of the 'Pérola' cultivar (susceptible), at 90 days after inoculation; (B) plantlets of the IPA 6.1-15 genotype, without symptoms on the leaves (B) and without internal lesions in the stem (C).

The different reactions observed among the genotypes evaluated occurred due to the nature of the plant material evaluated in the sexual cycle. This variation occurs because of propagation of plantlets by seeds obtained by hybridization between two cultivars with differences in fusariosis resistance, growth vigor and plant adaptability, leaf margin spinescence, and fruit size and quality characteristics.

Studies indicate that genetic control of fusariosis resistance in the pineapple plant basically adjusts to the single inheritance model, linked to one or a few genes, and that resistance is dominant over susceptibility (Cabral et al. 1997, Junghans et al. 2005). This inheritance model allows selection of resistant individuals in a segregating progeny. The first generation is obtained via genetic crosses between cultivars with contrasting characteristics for resistance to fusariosis and for complementary traits of size, shape, and fruit quality.

Souza et al. (2011) evaluated resistance to fusariosis in 40 ornamental pineapple hybrids (*A. comosus* var. *erectifolius*; *A. comosus* var. *ananassoides*; and *A. comosus* var. *bracteatus*), among which 14 were resistant, 15 were moderately resistant, and 11 were susceptible. These results are according to Matos and Souto (1984) and Cabral et al. (1985), who obtained different reactions regarding inoculation with *F. subglutinans* f. sp. *ananas* on several botanical varieties of the *Ananas* genus.

Matos and Cabral (2006) reported different responses of resistance to fusariosis in 211 pineapple accessions maintained in the Embrapa Active Germplasm Bank (*A. comosus* var. *comosus*, *A. comosus* var. *ananassoides*, *A. comosus* var. *bracteatus*, *A. comosus* var. *erectifolius*, *A.* sp., *Bromelia* sp., intraspecific hybrids, and intravarietal and interspecific hybrids). Of this total, 100 genotypes did not express disease symptoms, and the other 111 genotypes responded as susceptible.

In Brazil, development of pineapple cultivars has focused on the recombination of genes that control qualitative characteristics, such as spineless leaf margins (Lira Júnior et al. 2021a) and fusariosis resistance (Cabral et al. 1997, Junghans et al. 2005), that exhibit a weak effect or no effect from the environment. Considering the extensive area of pineapple cultivation in Brazil, as well as the different regional cultivation systems, characteristics of a quantitative nature should be included in selection strategies, as they are greatly affected by environmental factors and their variations over the years.

## CONCLUSION

Selected F<sub>1</sub> genotypes have values for crownless fruit weight and total soluble solids content higher than the average values of the 'Pérola' cultivar (1,506 g and 14.4 °Brix, respectively). For the selected genotypes, crownless fruit weight ranges from 1,514 to 3,307 g, and total soluble solids content ranges from 14.4 to 19.4 °Brix. Regarding inoculation with the *F. guttiforme* isolate IT-01, six genotypes showed a survival reaction similar to the response of the resistant cultivar 'BRS Imperial'. The selected genotypes predominantly have spineless leaves, fruits of cylindrical shape or cylindrical base, and yellowish flesh color, with satisfactory production of vegetative propagules. The IPA 6.1-15 genotype stands out for the largest number of desirable plant and fruit traits, including survival against the IT-01 isolate of *F. guttiforme*, spineless leaf margins, cylindrical shape of the fruit, crownless fruit weight of 2,005 g, yellowish flesh color, total soluble solids content of 16 °Brix, and a flat fruitlet profile, as well as a satisfactory amount of propagation material. Selected genotypes have superior agronomic traits in relation to their parents, which qualify them to be cycled back into the IPA plant breeding program and advance to performance evaluation per se, considering a larger number of plantlets per experimental plot.

### ACKNOWLEDGMENTS

Our thanks to the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for financial support.

## REFERENCES

- Bartholomew DP (2014) History and perspectives on the role of ethylene in pineapple flowering. Acta Horticulturae 1042: 269-283.
- Bezerra JEF and Silva Júnior JF (2017) Abacaxi. In Cavalcanti FJA (ed) Fertilizer recommendations for the Pernambuco State: 2ª aproximação. IPA – Instituto Agronômico de Pernambuco, Recife, 83p.
- Borras O, Santos R, Matos AP, Cabral JRS and Arzola M (2001) A first attempt to use a *Fusarium subglutinans* culture filtrate for the selection of pineapple cultivars resistant to fusariose disease. Plant Breeding 120: 435-438.
- Cabral JRS, Matos AP and Souto GF (1985) Reação de germoplasma de abacaxi à inoculação com *Fusarium moniliforme* var. *subglutinans*. **Pesquisa Agropecuária Brasileira 20**: 787-791.
- Cabral JRS, Matos AP and Coppens D'Eeckenbrugge G (1997) Segregation for resistance to fusariose, leaf margin type, and leaf color from the Embrapa pineapple hybridization programme. Acta Horticulturae 425: 193-200.
- Cabral JRS, Ledo CAS, Caldas RC and Junghans DT (2009b) Characters variation in pineapple hybrids obtained by different crosses. **Revista Brasileira de Fruticultura 31**: 1129-1134.
- Cabral JRS, Matos AP, Jughans DT and Souza FVD (2009a) Pineapple genetic improvement in Brazil. Acta Horticulturae 822: 39-46.
- FAO Food and Agriculture Organization FAOSTAT (2022) Crops. Pineapples. Available at <a href="https://www.fao.org/faostat/en/#data/ucl-">https://www.fao.org/faostat/en/#data/ucl-</a>. Accessed on March 14, 2022.
- Junghans DT, Brasileiro HS, Santos VJ, Cabral JRS and Matos AP (2005) Herança da resistência à fusariose em abacaxizeiro. Fitopatologia Brasileira 30: S123-S123.
- Junghans DT (2019) Cultivares. In Santos CEM and Borém A (eds) Abacaxi

do plantio à colheita. Editora UFV, Viçosa, p. 40-52.

- Lira Júnior JS, Bezerra JEF and Andrade DEGT (2021a) Genetic control of leaf spinescence in BRS Imperial, Pérola, and Pico de Rosa pineapple cultivars. Crop Breeding and Applied Biotechnology 21: e33492112.
- Lira Júnior JS, Bezerra JEF, Canuto VTB and Santos DA (2021b) Variation among pineapple half-sibs and selecting genitors based on potential genetic divergence. World Journal of Advanced Research and Reviews 10: 289-301.
- Matos AP (1978) Métodos de inoculação com *Fusarium moniliforme* var. *subglutinans* Wr. e Rg. emabacaxizeiro 'Pérola'. **Revista Brasileira de Fruticultura 1**:37-41.
- Matos AP and Reinhardt DH (2009) Pineapple in Brazil: Characteristics, research and perspectives. Acta Horticulturae 822: 25-36.
- Matos AP and Cabral JRS (2006) Evaluation of pineapple genotypes for resistance to *Fusarium subglutinans*. Acta Horticulturae **702**: 73-77.
- Matos AP and Souto GF (1984) Avaliação de técnicas de inoculação para detectar resistência à fusariose em plântulas de abacaxi. **Revista Brasileira Fruticultura 6**: 39-47.
- Matos AP, Mourichon X and Pinon A (1992) Occurrence of *Fusarium* moniliforme var. subglutinans on pineapple in Bolivia. **Fruits 47**: 33.
- Matos AP, Sanches NF, Teixeira FA and Simão AH (2011) Pineapple integrated pest management - an overview. Acta Horticulturae 902: 339-347.
- Montealegre JR and Luchsinger LE (1990) Postharvest rot of pineapple fruits marketed in Chile. Fitopatologia 25: 51-53.
- Nhat Hang NT, Diem NTN and Chau NM (2011) Pineapple breeding for quality improvement in South Vietnam. Acta Horticulture 902: 115-121.
- Nirenberg HI and O'Donnell K (1998) New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex.

#### Selection of F, genotypes of pineapple and reaction to inoculation with a Fusarium guttiforme isolate

#### Mycologia 90: 434-458.

- Nogueira SR, Lima FSO, Rocha EM and Araújo DHM (2014) Fungicidas no controle de fusariose do abacaxi no estado de Tocantins, Brasil. Revista de Ciências Agrárias 37: 447-455.
- Paull RE, Uruu G and Chen NJ (2020) Rapid field assay for pineapple fruit acidity. HortTechnology 30: 593-596.
- Reinhardt DH, Cabral JRS, Matos AP and Junghans DT (2012) 'BRS Ajubá', a new pineapple cultivar resistant to fusariosis and adapted to subtropical conditions. Acta Horticulturae 928: 75-79.
- Reinhardt DHRC, Bartholomew DP, Souza FVD, Carvalho ACPP, Pádua TRP, Junghans DT and Matos AP (2018) Advances in pineapple plant propagation. **Revista Brasileira de Fruticultura 40**: e-302.
- Sanewski GM, Coppensd'Eeckenbrugge G and Junghans DT (2018) Varieties and breeding. In Sanewski GM, Bartholomew DP and

Paull RE (eds) The pineapple: botany, production and uses. CAB International, Boston, p. 42-84.

- Santos C, Ventura JA and Lima N (2016) New insights for diagnosis of pineapple fusariosis by MALDI-TOF MS technique. **Current Microbiology 73**: 206-213.
- Sipes B and Matos AP (2018) Pests, diseases and weeds. In Sanewski GM, Bartholomew DP and Paull RE (eds) **The pineapple: botany**, **production and uses**. CABI, Boston, p. 269-294.
- Souza EH, Matos AP and Souza FVD (2011) Evaluation of ornamental pineapple hybrids for resistance to *Fusarium subglutinans* f. sp. *ananas.* Acta Horticulturae 902: 381-386.
- Ventura JA, Costa H, Cabral JRS and Matos AP (2009) 'Vitória': new pineapple cultivar resistant to fusariosis. Acta Horticulturae 822: 51-56.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.