

Evaluation of resistance in dwarf cashew to gummosis in north-eastern Brazil

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Abstract

Cashew nut is one of the most important cash crops in north-eastern Brazil where it grown mainly in coastal areas. However, cashew growing in semi-arid regions is expanding as a result of social, agronomic and yield quality potential. Environmental conditions in semi-arid zones are highly favourable for severe outbreaks of cashew gummosis, caused by *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. (*Botryodiplodia theobromae* Pat.). Management of gummosis is very difficult as no practical and adequate method has been successful under these conditions. Twenty-eight genotypes were selected from an open-pollinated population of dwarf cashew, cloned by grafting, and screened in field experiments for yield, quality and gummosis tolerance under high-disease pressure. After 3 years, four clones were selected and planted in a similar experiment with the best commercial clone as the control treatment. In both experiments, data were statistically analysed as estimated disease severity and area under disease progress curves. Gummosis was highly correlated to nut yield. Most genotypes were susceptible to gummosis and only one clone (CAPC 42) showed a consistent and stable resistance in both experiments.

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1. Introduction

Cashew nut is an important crop in north-eastern Brazil where it is grown either as a cultivated crop or in a semi-wild manner. Although most cashews are grown in the coastal zone, a great increase in the area of cashew production is occurring in semi-arid conditions, probably due to favourable conditions for higher quality of both apples and nuts than in any other region of the country. This environment is marked by very low rainfall (<350 mm/year), high daylight temperature (30–35 °C), with cool nights (18–23 °C), and low relative humidity. Cashew plants are very well adapted to this harsh environment as demonstrated by its vegetative growth and comparable yield to other regions.

Cashew gummosis, caused by *Lasiodiplodia theobromae*, is the most important disease of cashew in the semi-arid conditions of north-eastern Brazil. Severe epidemics have occurred in recent years, as a result of predisposition by water stress (Freire, 1991; Freire et al., 2002) and the predominant use of susceptible clone cultivar CP 76. Disease symptoms are characterised by swollen cankers in the trunk or woody branches, which may crack and ooze a transparent resinlike gum, thus the name gummosis (Freire et al., 2002). Gummosis damage includes reduction in water and nutrient transport, destruction of branches, reduction of photosynthesis, dieback, and plant death (Bezerra et al., 2004). Gummosis dissemination is not well understood, although the causal agent has been isolated from non-symptomatic seed and transplants (Freire and Cardoso, 1997; Freire et al., 1999), suggesting that those plant propagating materials may act as a primary source of inoculum. The involvement of the root beetle, *Marshallus bondari*, has been suggested both as vector or by facilitating

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infection (Freire et al., 2002). Under favourable conditions, cashew plants show symptoms as early as the first year after planting, although it is only after the second year that severe damage occurs (Cardoso et al., 2004). Adequate and practical measures for controlling gummosis are not known. The surgical removal of cankers followed by a copper fungicide treatment, to protect the cut surface until healing, has been recommended when infected tissue is located at the trunk (Freire, 1991). However, the prevalence of branch infections and observed trunk re-infection after 2 or 3 months (Cardoso et al., 1995) accounted for the low success of this method. Managing the root beetle by aluminium sulphite gas and sanitation did not reduce disease much.

L. theobromae is thought to be a weak parasite often associated with wounds or stress weakened plants (Cardoso, et al., 1998; Freire and Cardoso, 1997; Britton and Hendrix, 1986; Baird and Carling, 1998), therefore, differences in disease reaction may not be easily found within any host population. The search for genetic resistance has been successful in mangoes (Sangchote, 1988; Rodrigues and Mattos, 1988; Prakash and Raoof, 1989). In cashew, however, there is no report of resistance to *L. theobromae*.

As the centre of origin of cashew, Brazil presents a highly diverse genetic pool of this species to phenotypic traits such as nut quality, plant height and leaf disease reactions (Barros, 1995; Cardoso et al., 1999) and to molecular markers (Cavalcanti, 2004). A dwarf cashew type was selected and several vegetative propagated cultivars were released in Brazil to provide uniformity of crop and nut quality, precocious production, ease of crop handling and harvesting (Barros et al., 1984).

The objective of this work was to select and to evaluate dwarf cashew with resistance to gummosis.

2. Material and methods

Data from two experimental fields were used in two stages over two periods for this study. Both fields were located in the municipal district of Pio IX, Piauí state. The first set of data was obtained at Capisa farm (6°31'30"S, 40°47'19"W, 605 m asl), from January 1995 until September 1998 (22 observations), with a group of 28 vegetatively propagated genotypes originating from previous selection from an open pollinated and seed propagated orchard of 1000 ha at the same farm. The trial was established in May 1993 in three blocks of 112 plants each, spaced 7 × 7 m apart. Each replication was a line of four plants of the same entry in each block. Disease assessments were made by recording gummosis incidence and severity at approximately 3-month intervals. Data was recorded as incidence, based on the presence of typical disease symptoms and as severity by an arbitrary numerical scale (Cardoso et al., 1998, 2004), corresponding to 0 (no symptoms), 1 (small and few cankers in trunk and branches, small cracks without gum exudation), 2 (larger, cracked cankers on

trunks and branches, reaching up to 1/3 of diameter, with little or no gum exudation), 3 (cracked cankers, larger than 1/3 of diameter with abundant gum exudation), and 4 (cracked cankers completely girdling trunk or branches, foliage yellowing; dieback and gum exudation). Both incidence and severity were evaluated at the same time. Gummosis severity (GS) was estimated by the equation $GS = \sum (x_i n_i) / n$, in which x represents disease grade ($x = 1, 2, 3$ and 4), n_i represents number of diseased plants with i th grade on the disease scale and n is the total number of diseased plants evaluated. Gummosis incidence ($GI = \sum x / N$) was the proportion of diseased plants, which consisted of the number of diseased plants (x) divided by the total number evaluated (N). Nut yield was also estimated by manually harvesting the plants fortnightly throughout the season (August to December), and computing the sum of nut yield at the end of the season.

In February 1999, four genotypes selected by yield, commercial characteristics of apple and nut (i.e. reddish-pear-shaped, high sugar and low tannin content of apple, nut and kernel mass and nut/kernel relation) and gummosis reaction were planted at Planalto farm (6°43'30"S, 40°35'19"W, 730 m asl), 10 km from Capisa, under similar soil and climatic conditions. The experiment was disposed in a randomised complete block design with four replications and 27 plants per plot (3 rows of 9 plants). The commercial and a gummosis susceptible dwarf clone CP 76 was used as the control treatment. Disease data were collected from May 2000 through September 2002 (14 observations), using the assessment method described. Cropping practices in both experiments were strictly based on local growers management which includes fungicide application (Barros et al., 1984).

Data was analysed by standard analyses of variance by date of assessment and pooled as split plot design with date as subplot (Steel and Torrie, 1980) using a SAS package. For Capisa data, mean comparisons were also done by the Scott and Knott (1974) method at 5% level of probability. Disease progress was plotted to obtain epidemic estimates among genotypes and examine trends of resistance using Excel (Microsoft, Seattle, WA). The GI values of each 3-month period were used to calculate the area under disease progress curve (AUDPC) according to the equation (Campbell and Madden, 1990)

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

where n is the number of evaluations, Y is the GI value, and t is the number of 3-month periods. The AUDPC values were used to present disease progress throughout the whole experiment. Both GS and AUDPC values were used to examine disease reactions among treatments using ANOVA procedure and applying the Tukey test at $P = 0.05$.

3. Results

At Capisa farm, gummosis symptoms were first observed 20 months after transplanting (January 1995). Gummosis incidence and severity analysis revealed highly significant differences ($P < 0.01$) among selections and time of evaluation. The main criteria used to assess gummosis reaction were AUDPC and GS score, which indicated the extent of disease development. Differences in disease reaction among tested genotypes were noted at 20 months. GS bulked from data of 22 evaluations was used to construct a disease progress curve and to estimate AUDPC (Table 1).

The first yield was recorded within the second year after transplanting (20–26 months). A great genotypic variation, in terms of yield, was observed among cashew clones (Table 1). The analysis of the yield data from second and third year divided genotypes into three groups, according to the Scott and Knott method (1974). GS and AUDPC values significantly divided genotypes into six and nine groups, respectively. A very high correlation ($R^2 = 0.855$) between yield and gummosis (AUDPC) was observed. The genotypes CAPC 35, CAPC 38, CAPC 40 and CAPC 42

were selected after the third growing season based on yield, marketable nut quality and disease reaction. The clone CAPC 42 had significantly higher yield than the others in all seasons and was placed in the lowest group in both GS and AUDPC. Clones CAPC 35, CAPC 38 and CAPC 40 were all intermediate in these parameters, but were chosen for good commercial qualities of nuts and apples.

Disease onset in the Planalto experiment occurred 12 months after transplanting, but disease incidence and severity ($GS < 0.06$) were low. Thereafter, gummosis progressed very rapidly, reaching close to 50% after 24 months and 99% at 45 months. GS and AUDPC showed similar trends in disease reaction after four years (Fig. 1A,B). Nut yield data from 3 years (2000, 2001, and 2002) showed significant differences among clones, but the most susceptible CP 76 did not differ significantly from the most resistant CAPC 42 (Fig. 1C). The rainfall average received was 763, 475, and 686 mm in 2000, 2001 and 2002, respectively.

Table 1
Nut yields, gummosis severity (GS) and area under disease progress curve (AUDPC^a) of 28 genotypes of dwarf type cashew in the second and third year of age

Clone ^b	Nut yield (Kg ha ⁻¹) ^c	GS	AUDPC
CAPC 67	85.20 a	1.76 a	283.41 a
CAPC 24	110.43 a	1.72 a	289.00 a
CAPC 56	125.47 a	1.55 b	267.23 c
CAPC 59	82.29 a	1.55 b	262.84 c
CAPC 57	49.14 a	1.54 b	271.64 b
CAPC 38	128.17 b	1.52 b	269.44 b
CAPC 16	74.45 a	1.48 b	273.27 b
CAPC 20	148.32 b	1.41 c	248.91 d
CAPC 53	89.65 a	1.37 d	252.08 d
CAPC 04	71.85 a	1.31 d	251.24 d
CAPC 35	147.05 b	1.30 d	239.24 e
CAPC 40	138.40 a	1.27 d	242.21 e
CAPC 23	94.27 a	1.27 d	239.39 e
CAPC 12	95.98 a	1.25 d	247.02 d
CAPC 15	85.40 a	1.23 e	245.65 d
CAPC 49	125.05 b	1.22 e	232.37 e
CAPC 66	83.99 a	1.22 e	240.67 e
CAPC 62	89.45 a	1.20 e	242.31 e
CAPC 28	48.80 a	1.19 e	241.49 e
CAPC 65	61.10 a	1.19 e	230.72 f
CAPC 55	102.91 a	1.17 e	229.16 f
CAPC 19	90.59 a	1.17 e	238.43 e
CAPC 09	104.35 a	1.16 e	236.38 e
CAPC 50	151.62 b	1.16 e	221.02 g
CAPC 42	239.00 c	1.10 f	227.16 f
CAPC 54	162.21 b	1.07 f	223.72 g
CAPC 17	85.40 a	0.99 g	209.89 h
CAPC 68	83.82 a	0.96 g	201.04 h

^aData are means of 22 observations. In any column, means followed by the same letter did not differ statistically as determined by the Scott & Knott test ($P = 0.05$).

^bCAPC: stands for “Clone de cajueiro anão precoce”—CAPISA.

^cMeans from 2 years of production.

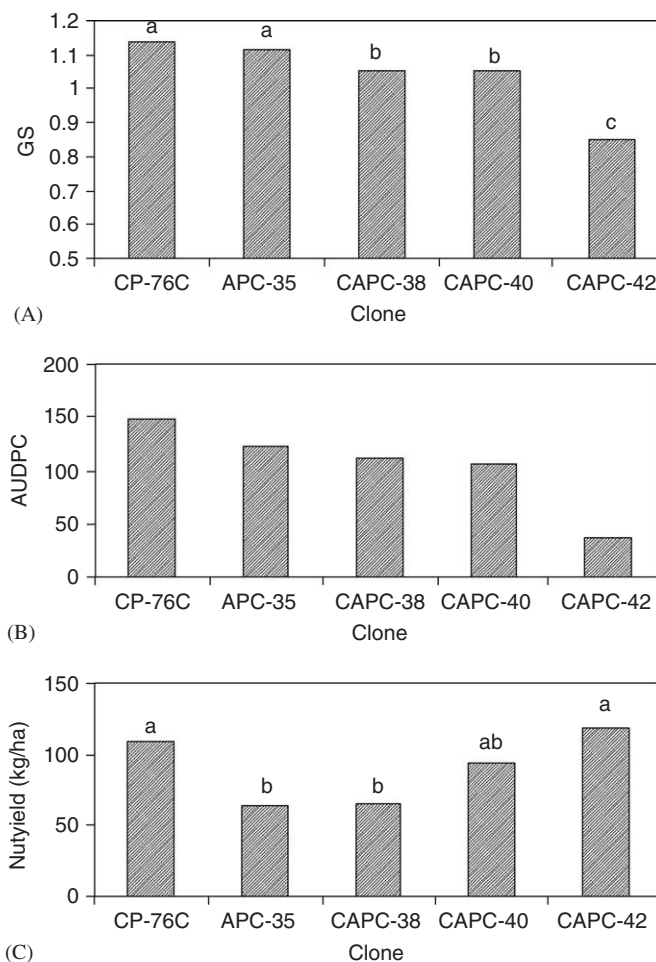


Fig. 1. (A–C) Gummosis severity (GS), the area under disease progress curve (AUDPC), and nut yield of five cashew clones under field conditions. GS varying from 0 (no symptom) to 4 (plant dead). Bars on GS and AUDPC data represent means of 14 observations (from May, 2000 to September, 2002). Nut yield present is the mean of three years (2000, 2001 and 2002). In each graph, bars with common letters over them are not significantly different as determined by Tukey test at $P = 0.05$.

4. Discussion

The environmental effect was assumed to be constantly low and uniform throughout the experiments, based on earlier results in the same orchards (Cardoso et al., 2004). Gummosis epidemics are characterised by a progressive phase for several years with no digressive phase until death rates reach high levels (Cardoso et al., 2004). The estimated AUDPC was chosen to be the descriptor for each epidemic to compare the reaction of a dwarf cashew population for gummosis resistance, as it incorporates variations in time of disease onset and finish. The presence of inoculum was also assumed to be constantly high and uniform as previously observed in similar fields (Cardoso et al., 1995, 1998, 2004; Freire et al., 1999, 2000).

The purpose of the Capisa experiment was to screen germplasm for yield, commercial traits of nuts, and gross comparisons among clones for gummosis tolerance, assuming climatic and soil conditions highly conducive for disease development.

Our results support the fact that gummosis onset can begin 12 months after planting (Cardoso et al., 2004).

The effect of gummosis on plants is mainly through blockage of nutrients and water transport, drainage of nutrients to the swollen trunk and reduction of plant canopy by death of branches (Cardoso et al., 1995; Bezerra et al., 2004). This suggests yield would be reduced following infection and the correlation coefficient between yield and gummosis severity in this study reinforced this suggestion. However, at Planalto farm, nut yield in the first 3 years did not reflect differences between the susceptible clone CP-76 and resistant CAPC-42 (Fig. 1) which may imply higher tolerance by CP-76 than other susceptible clones, such as CAPC-35.

Differences in disease reactions were first noticed at the onset of epidemics. This was surprising for a ubiquitous, non-specialised fungus such as *L. theobromae* (Punithalingam, 1976). The overall data show that most of the tested germplasm is susceptible, pointing to a polygenic or horizontal type of resistance. The characters of consistence and stability of the resistant reaction of CAPC 42 suggest an indirect mechanism of resistance, such as tolerance to water stress. Resistance to fungal pathogens in woody stems has been attributed to the formation of vascular occlusions in the case of wilt diseases, or to the formation of reaction zones, which limit colonisation by canker or decay fungi. Although the study of the mechanisms of resistance was not the purpose of the present work, the resistance to gummosis in cashew does not seem to be related to any of these causes.

Our results demonstrate very clearly the existence of genetic resistance in dwarf cashew germplasm to gummosis with special emphasis on CAPC 42 which showed a consistent and stable resistance throughout almost a decade of field observation. The data also confirmed the susceptibility of commercial clone CP 76 which might explain the increasing disease development in this region.

The source of resistance found here may be included directly into the overall approach for integrated gummosis management, used in future breeding programs, for genetic studies, and for identification of molecular markers.

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