Production of thornless *Cnidoscolus phyllacanthus* progenies from open pollinated native trees

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Abstract - *Cnidoscolus phyllacanthus* (faveleira)*,* a caatinga xerophyte *Euphorbiaceae* tree, produces protein-rich forage consumed by ruminants. It should be used carefully due to urticaceous thorns in leaves, fine branches and fruits, and antinutritional substances present in fresh material. The narrow gene pool of the few thornless mutants should be widened by additional thornless individuals grown from open-pollinated seeds. This study checked the potential of six thorny and three thornless open-pollinated native trees in a caatinga site to produce thornless progenies, and estimated the proportion of their *thornless* progenies. The trial took place at the seed laboratory and seedling nursery facilities of UFCG/UAEF, Patos-PB, Brazil, from March 2002 to November 2003, in a completely random design with nine treatments (trees) and number of replications depending on seed and seedling quantity. Proportions of germinated seeds and thornless progenies were compared by the Student´s "t" test. Seven of the monitored trees produced thornless progenies (5.8% to 20.6%) consistently in two years. Three thorny and two thornless trees generated 15.1% to 20.6% of thornless progenies. Thornless progenies should be grown isolated to produce genetically improved seeds for the thornelss trait with a wide gene pool basis for distribution to local farmers and use to improve other traits such as forage and seed oil production. Further studies should investigate the genetic basis involved in the exhibition of this trait and if other trees in other *C. phyllacanthus* populations can produce thornless progenies as well.

Index terms*:* Spine, tree fodder, faveleira.

Produção de progênies inermes de *Cnidoscolus phyllacanthus* oriundas de árvores nativas em sistema de polinização aberta

Resumo - *Cnidoscolus phyllacanthus* (faveleira), uma Euphorbiaceae xerófila arbórea da caatinga, produz forragem protéica consumida por ruminantes. Seu uso deve ser cuidadoso pela presença de espinhos urticantes nas folhas, ramos finos e frutos, e de substâncias antinutricionais no material fresco. A estreita base genética dos raros mutantes sem espinhos deve ser ampliada com novas plantas sem espinhos obtidas de sementes de polinização livre. Este estudo confirmou a capacidade de seis árvores nativas com espinhos e três sem espinhos, submetida a polinização aberta, de gerar progênies sem espinho, e estimar as proporções de progênies sem espinho. O ensaio desenvolveu-se no laboratório de sementes e no viveiro florestal da UFCG/UAEF, Patos, PB, Brasil, de março de 2002 a novembro de 2003, no delineamento inteiramente casualizado, com nove tratamentos (árvores) e número de repetições dependendo da quantidade de sementes e mudas. As proporções de sementes germinadas e de progênie sem espinho foram comparadas pelo teste t. Todas as árvores produziram progênie sem espinho (5,5% a 20,6%) consistentemente nos dois anos. Três árvores com espinho e duas sem espinho geraram de 15,1% a 20,6% de progênie sem espinho. As progênies sem espinho devem ser cultivadas isoladamente para produzir sementes melhoradas para o caráter sem espinho com ampla base genética para distribuição aos interessados e melhoramento de outras características, tais como a produção de forragem e óleo. Estudos adicionais devem investigar as bases genéticas envolvidas na expressão deste caráter e se outras árvores em outras populações de *C. phyllacanthus* também produzem progênies inermes.

Termos para indexação*: espinho, forragem arbórea, faveleira*

Introduction

Climate conditions in the northeast semiarid region of Brazil, especially rain irregularity, make agriculture extremely risky, while cattle raising shows to be less affected. However, animal performance is harmed due to food scarcity, especially protein-rich fodder.

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Seeds, leaves and fine branches of *Cnidoscolus phyllacanthus* Pax et K. Hoffm. (faveleira), a xerophilous lactescent *Euphorbiaceae* tree of the caatinga forest, may furnish protein-rich food (Viana & Carneiro, 1991; Arriel et al. 2004; Drumond et al. 2007). However, this plant presents urticaceous thorns in the points of leaf insertion on stems, leaves and fruits (Moreira et al., 1974). These thorns are an efficient protection against browsing, and make its management and utilization very difficult. However, a few thornless *C. phyllacanthus* mutants were identified in native populations from Ceará (Moreira et al., 1974), Paraíba and Rio Grande do Norte states.

Nobre et al. (2001) reported six thornless seedlings out of 886 (0.7%) *C. phyllacanthus* germinated seeds. Arriel et al. (2005) observed that seven out of 36 trees chosen among the many adult healthy plants in a native population of *C. phyllacanthus* were able to produce thornless progenies. These trees (trees M_{32}, M_{45}, M_{46}, M_{52}, M_{57}, M_{59} and M_{61}) were thorny except M_{61}. According to these authors, the mean number of thorns per centimeter of leaf midrib of each progeny of the six thorny trees showed to be lower than the overall mean of the progenies of the 36 studied trees. This can be a possible characteristic of trees that produce thornless progenies. However, the proportion of their thornless descendants was not determined by Arriel et al. (2005). Two additional thornless adult trees were identified in this same *C. phyllacanthus* population.

There are studies about thornless trait of other caatinga thorny plants, such as *Mimosa tenuiflora* (Willd.) Poiret. (jurema preta). In this species, the thornless trait seems to be controlled by one or few recessive genes, and two cycles of selection (seed collection from thornless trees followed by free pollination among thornless progenies in an isolated seed orchard) result in 90% seeds able to produce thornless seedlings (Arriel et al., 2000).

Cnidoscolus phyllacanthus fruits should be collected before dehiscence of seeds. Germination occurs in vermiculite (Silva et. al., 2005) or moist sand, and tegument scarification accelerates germination.

Although Sales (2008) developed a successful grafting protocol to thornless *C. phyllacanthus*, the few thornless trees from which vegetative material can be collected result in a narrow genetic pool of asexually reproduced plants. The genetic pool may be widened by finding or producing new sexually generated thornless individuals.

Thus, considering that the absence of thorns in *C. phyllacanthus* makes easier its utilization for ruminant diet and reforestation projects, and also that the genetic pool basis of the thornless population should be widened, the present study had the following objectives: to confirm the potential of six thorny and three thornless *C. phyllacanthus* trees to produce thornless progenies under open pollination within a native population, and to estimate the proportion of thornless progenies of each tree.

Material and methods

This study was carried out in Patos (PB), Brazil, and consisted of seed collection and progeny observation from nine *C. phyllacanthus* trees (M_{32}, M_{45}, M_{46}, M_{52}, M_{57}, M_{59}, M_{61}, M_{63}, e M_{80}). The trees were evaluated under natural conditions (open pollination) within a native population thriving in an 80 ha caatinga site (stretching from 07°04’21” to 07°05’01”S, and from 37°15’37” to 37°15’58” W, 254 to 262 m above sea level), located at NUPEARIDO Experimental Station. The first seven trees were selected from Arriel et al. (2005) preliminary observations related to their ability to generate thornless progenies. In addition to tree M_{61}, two other thornless trees (M_{63} and M_{80}) from the same population were included in this study.

Seed collection occurred from March to May 2002 and 2003, although seed collection from trees M_{59} or M_{80} were restricted to 2002 or 2003, respectively. In these periods, the trees were visited every day from the very beginning of fruit production until no more fruits were observed in tree canopy. During this period, the fruits showing signs of imminent dehiscence were collected by means of a hooked rod, placed inside identified paper bags, and sun dried until dehiscence. The seeds were stored in paper bags at room temperature.

Two to six months after collection, the seeds were externally sterilized during 20 minutes in a 3% sodium hipochloride solution, clean-washed three times with distilled water, scarified manually with fine sandpaper (Norton 60K2.40) in the region close to the caruncle, and germinated in a dark germination chamber with no light, 27ºC and 100% air humidity. Recipients and substrates were, respectively, plastic trays and sterilized washed sand, in 2002, or gerboxes and sterilized germitest paper initially moistened with an antibiotic solution of nistatine, in 2003 to control fungi development observed in wide opened plastic trays used in 2002. Washed sand and germitest paper were kept moist during the month of daily observation and germination data collection.

Seeds presenting radicle emergence were transplanted to conic plastic containers (5 cm diameter, 15 cm deep), with approximately 300 cm^3 of a homogenized substrate composed of two parts of subsoil material and one part of bovine manure (v/v).

Seedlings in the plastic containers were arranged in a completely random design with nine treatments (nine trees) with different number of replications according to the number of seeds or surviving seedlings of each tree. Seedlings were kept in a shed, covered and protected laterally by a 50%-reduction-solar-radiation plastic screen, during 30 days, when the definitive leaves showed clearly the presence or absence of thorns. During this period, the seedlings were irrigated twice daily.

The proportion of germinated seeds that produced a viable seedling and the proportion of thornless progenies at day 30 after germination, of each tree, were compared by a Student's t test according to the least significant difference (LSD) (P<0.05) approximated by

$$2 * \sqrt{\frac{p_1 * (1 - p_1)}{n_1 - 1} + \frac{p_2 * (1 - p_2)}{n_2 - 1}}$$ (Steel and Torrie, 1960).

Results and discussion

At the first seed collection, trees M_{32}, M_{45}, M_{46}, M_{52}, M_{57}, M_{59}, M_{61}, M_{63} and M_{80} were 6.0, 7.5, 5.0, 8.5, 7.0, 7.5, 5.0, 4.1 and 6.5 meter high, respectively, and showed a basal diameter of 25.5, 39.0, 32.0, 38.0, 24.0, 37.5, 26.0, 11.5 and 40.5 cm, respectively.

The number of collected seeds from each tree ranged from 30 to 419 in a single year, and between 119 and 664 seeds / 2 years (Table 1 and Figure 1), and showed no correlation with tree height (r = 0.21) (P>0.05) and basal diameter (r = -0.04) (P>0.05) (Figure 1). Trees M_{32}, M_{52}, M_{57} and M_{61} trended to produce more seeds in both years, while trees M_{45} and M_{63} showed a high seed production in 2002, but reduced it considerably to one third in the following year. It is clear from data in Table 1 that seed production, and certainly other traits as well, is variable among trees and years.

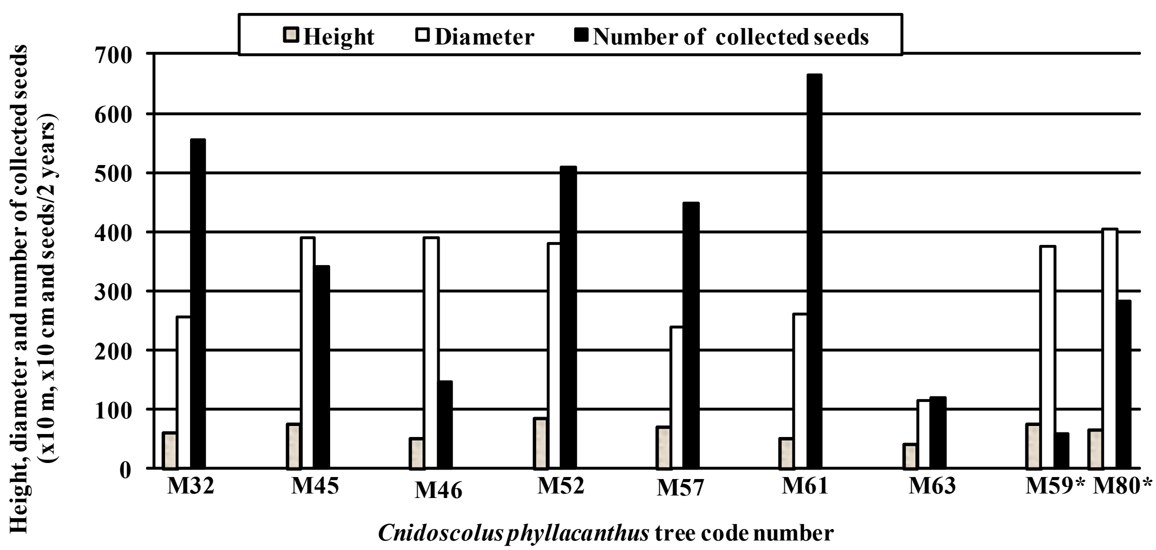

Figure 1. Height (m), diameter (cm) and total number of collected seeds in two consecutive years of nine *Cnidoscolus phyllacanthus* trees, in Patos, PB, Brazil (*number of collected seeds in one year multiplied by 2).

Table 1. Number of collected seeds, number of produced seedlings and thornless seedlings, proportion of thornless seedlings, and standard error of the proportion of thornless seedlings obtained from seeds collected from *Cnidoscolus phyllacanthus* trees under an open pollination regimen in two years (2002 and 2003), in Patos, PB.

Tree	Year	Number of collected seeds	Number of produced seedlings (n)	Number of thornless seedlings (a)	Proportion of thornless seedlings (p=a/n)	SE_p*
M_{32}	2002	289	216	27	0.1250	
	2003	267	134	27	0.2015	
	Total	556	350	54	0.1543	0.0193
M_{45}	2002	258	245	16	0.0653	
	2003	82	52	3	0.0577	
	Total	340	297	19	0.0640	0.0141
M_{46}	2002	68	56	5	0.0893	
	2003	78	55	5	0.0909	
	Total	146	111	10	0.0901	0.0273
M_{52}	2002	169	125	9	0.0720	
	2003	339	185	13	0.0703	
	Total	508	310	22	0.0710	0.0146
M_{57}	2002	238	172	34	0.1977	
	2003	209	150	30	0.2000	
	Total	447	322	64	0.1988	
M_{59}	2002	30	16	3	0.1875	
M_{61}**	2002	419	350	72	0.2057	
	2003	245	152	23	0.1513	
	Total	664	502	95	0.1892	0.0175
M_{63}**	2002	86	58	9	0.1552	
	2003	33	25	5	0.2000	
	Total	119	83	14	0.1687	0.0414
M_{80}**	2003	141	32	4	0.1250	

*SE_p = standard error of the proportion of thornless seedlings = $\sqrt{\frac{p(1-p)}{n-1}}$

**Thornless trees

Thirty-day-old seedling survival proportions were obtained from data in Table 1, and the results are visualized in Figure 2. The values ranged from 0.23 to 0.95, or equivalently from 23 to 95% of the collected seeds from each tree in a particular year. Survival showed a tendency to be higher in 2002 than in 2003, although seed collection, processing and scarification protocols were similar in both years. It is possible that the recipient (gerbox) and the antibiotic solution (nistatine) in which the seeds were placed in 2003 contributed to the decrease of seed germination in the second year. Also, the intense *C. phyllacanthus* defoliation observed in the field in 2003 may have affected seed vigor in that year. The low (23%) M_{80}'s seedling survival corroborates this possibility, as this tree was growing in a very precarious site (thin soil over rocks), while all other trees are located in sites showing better conditions and resulted in 50% or more of seedling survival.

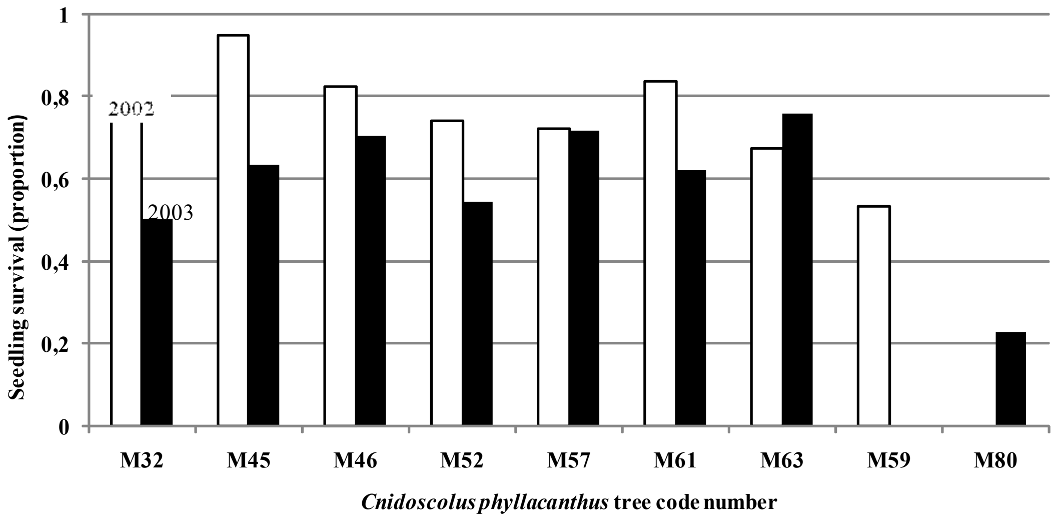

Figure 2. Survival of 30-day old *Cnidoscolus phyllacanthus* seedlings from nine native trees producing thornless progenies, in Patos, PB, Brazil, in two years (2002 and/or 2003).

The proportion of thornless progenies ranged from 5.77 to 20.57% (Table 1). This is higher than the 0.7% thornless progeny obtained from seeds collected from thorny *C. phyllacanthus* trees in a native population by Nobre et al. (2001). Despite of the nine trees were surrounded by numerous thorny trees, the values in Table 1 were not very different from the ones reported by Arriel et al. (2000) in the first selection cycle of thornless *M. tenuiflora* (31%), when seeds were collected from thornless trees surrounded by thorny individuals in an average rate of 17% thornless and 83% thorny plants. These authors report 90% of thornless progenies for *M. tenuiflora* in seed orchard formed by thornless plants only. If the thornless trait in C. *phyllacanthus* is regulated by one or few recessive genes as it seems to be in *M. tenuiflora,* it is reasonable to expect the thornless trait in 90% or more of *C. phyllacanthus* progenies if thornless trees are left to reproduce in an isolated seed orchard.

Five trees from which seeds were collected in this study presented thorns, while Arriel et al. (2000) collected seeds only from thornless trees in the first selection cycle. This same strategy may be used in the future for *C. phyllacanthus*. However, there is an estimative of 16.7% (6/36) of thorny individuals heterozygous for the thornless trait with the potential to produce thornless progenies in the sampled population. Half of them produced less than 10% of thornless progenies, and the other half produced as much thornless progeny as the three thornless trees. Considering that the thornless trait in *C. phyllacanthus* is controlled by one pair of recessive alleles, it is plausible that most of the surrounding *C. phyllacanthus* trees of the first three thorny (*i.e.*: those ones that produced less than 10% of thornless progenies) and of the three thornless trees are certainly homozygous for the dominant thorny trait, while most of the surrounding trees of the thorny trees that produced as much thornless progeny than the thornless trees are heterozygous for the thornless trait.

The thornless progenies from these and other thorny *C. phyllacanthus* trees heterozygous to the thornless trait should also be considered in a breeding program, to guarantee a wider gene pool to the thornless population than if only the few thornless trees were used for seed collection and seedling production.

Trees M_{57}, M_{61} and M_{63} were noticeable because more than 15% of their seeds resulted in thornless progenies in 2002 and 2003, and to a lesser extend tree M_{32}, that showed 12.5% of thornless progeny in 2002 and 20.2%

in 2003 (Table 1). These trees, except the M_{63}, had a relatively high seed production. The performance of M_{59} should be carefully considered due to the low quantity of collected seeds. The remaining trees yielded a lower proportion of thornless progenies (from 5.8% to 9.1%) (Table 1). Even so, the overall proportion of thornless progenies of the present study was higher than the overall value (p = 0.007 reported by Nobre et al. (2001). Indeed, this should be expected, as seven trees (M_{32}, M_{45}, M_{46}, M_{52}, M_{57}, M_{59}, M_{61}) were intentionally selected because had already been reported to produce thornless progenies by Arriel et al. (2005), and the other ones (trees M_{63} and M_{80}) were chosen to show themselves no thorns.

Among the seven trees that had their seeds collected in 2002 and 2003, the proportion of thornless progenies from each tree was considered similar in both years, according to the LSD test (P<0.05) (Steel & Torrie, 1960). This means that, if the reproductive conditions are kept constant in the population, the thornless trait is transmitted to the descendants in a constant rate, showing the genetic control of the thornless trait and complying with the Hardy-Wainberg balance.

Considering the two years together, progenies from trees M_{32}, M_{57}, M_{61} and M_{63} showed more than 15% of thornless seedlings (Table 1), higher than the proportion of thornless seedlings in the progenies of the other trees (P<0.05) (Steel & Torrie, 1960).

Conclusions

1 –All nine monitored *C. phyllacanthus* trees under a regimen of open pollination within a native population are able to produce thornless progenies.

2 – The percentage of thornless progenies ranges from 5.8 to 20.6%, and shows to be stable for the seven trees monitored during two years.

3 – Three thorny and two thornless trees generate more than 15.1% thornless progenies, and this *C. phyllacanthus* population can be a source of many sexually generated thornless progenies with a reasonably wide gene pool basis. These progenies can be used in a breeding program to increase the thornless trait frequency in this species and produce seeds for distribution to local farmers, as well to improve other traits related to production, such as forage and seed oil production.

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