CBAB
CROP BREEDING AND
APPLIED BIOTECHNOLOGY

Crossing and diagnostic methods of cubiu hybrid plants based on genetic markers

Jean Rodrigo Pizzinato[1], Adilson Ricken Schuelter[1,2*], Antonio Teixeira do Amaral Júnior[3], Andressa Camilo de Souza Rocha[1], Jaqueline Manzatti da Siva[1], Cátia Raquel Volkweis[1], Mateus Brusco de Freitas[1], Fabiano Ricardo[2], and Diogo Oswaldo Schwantes[1]

ABSTRACT – *The objective of this study was to evaluate the effect of different artificial cross pollination techniques in cubiu (*Solanum sessiliflorum*) for breeding purposes and genetic control studies of economically important traits. The varieties Santa Luzia and Thaís, with and without protection with organza cloth, were used in natural crosses and crosses with emasculation. The methods were evaluated for pollination efficiency and fruit traits, namely: weight, length, width, length/ width ratio and mean number of seeds. RAPD analysis indicated that all progenies were derived from the controlled crosses, which confirmed the pollination efficiency of the methods applied. The pollination efficiency varied from 4.57% to 8.37% among the crosses. The use of emasculation without organza cover was statistically best for fruit yield. The variety Santa Luzia is recommended as female parent for hybridization. Crossing seems to be the predominant mechanism of reproduction in this species.*

Key words: *Solanum sessiliflorum*, controlled crosses, genetic control, allogamy.

INTRODUCTION

Solanum sessiliflorum or cubiu is a species native to the Amazon and belongs to the family *Solanaceae*, has a shrubby habit and semi-woody plants that grow quickly and reach a height of up to two meters (Silva Filho et al. 2003). The species is well-adapted to hot climate regions with high relative humidity (Silva Filho 2002). However, recently adapted cultivars of the species have been introduced in subtropical regions, such as southern Brazil (Brancher and Tagliari 2004).

The fruits are used to prepare fish, meat and salads, and to produce juice, sweets and ice cream (Silva Filho et al. 1999). The latter is particularly favorable due to

the high fruit moisture content, estimated at 88.00% to 93.00% (Silva Filho 2002). It is important to emphasize that the fruits have a low calorific value and are therefore promising as dietary products. Moreover, with the high fiber concentration, the fruit is digestive and the niacin content is high, with mean concentrations of 2.5 mg per 100 g of integral pulp, according to Pahlen (1977).

Pahlen (1977) classified cubiu as an autogamous species because it produces fruit even when grown in isolation. In contrast, Storti (1988) characterized the cubiu plant as panmitic, owing to mechanisms that favor cross-pollination, such as the presence of poricidal anthers, resulting in low pollen fertility and

[1]Universidade Paranaense, Campus Toledo, Av. Parigot de Souza, 3636, 85903-170, Toledo, PR, Brazil. *E-mail: adilson_schuelter@yahoo.com.br
[2]Master´s degree in Biotechnology applied to Agriculture/Universidade Paranaense, Praça Mascarenhas de Moraes, s/n, 87502-210, Umuarama, PR
[3]Universidade Estadual Norte Fluminense, CCTA, LMGV, Av. Alberto Lamego, 2000, Parque Califórnia, 28013-602, Campos do Goytacazes, RJ

asynchronous flowering. More recently, on 32 plants grown in a greenhouse covered with organza Luz et al. (2008) found only one seedless fruit, which is therefore probably parthenocarpic, indicating cross-pollination of the species.

Knowledge on the reproductive system in cultivated or wild plants is of fundamental importance for breeders to determine appropriate improvement strategies and to obtain gains by selection. In the particular case of cubiu, knowledge is indispensable in view of the controversial research results. The purpose of our study in this context was to determine methods of controlled pollination in *Solanum sessiliflorum* and investigate the mechanism of sexual reproduction of the species in contribution to the definition of basic breeding methods that could be to obtain advanced segregating generations.

MATERIAL AND METHODS

Plant material and cultivation conditions

Seeds of the cubiu varieties Santa Luzia and Thaís, of the Experimental Station Santa Luzia, in Guareí, São Paulo, were sown in plastic trays with 128 cells filled with substrate for seedling production, on May 3, 2005. The seedlings were replanted in plastic bags containing 1 kg of a soil: sand: cattle manure mixture (3:2:1), 45 days after sowing.

One hundred and fifty days after sowing, the seedlings were transplanted into planting holes (0.2 m x 0.2 m) in the field (plants spaced 1.00 m and rows 1.50 m apart). Plants were fertilized with 1.00 kg organic compost (manure from livestock manure), 400 g Iorin and 50 g NPK (08-20-20) per hole. Beginning two weeks after transplanting 10 g urea per plant was applied fortnightly until the beginning of flower bud growth. The other cultural treatments were performed as recommended by Silva Filho (1998).

Experimental design and evaluation of phenotypic traits

The experiment was arranged in a split plot design with six replications and the plot distribution was completely randomized; the plots consisted of plants with or without a cover of organza cloth. The subplots were represented by two forms of flower manipulation for artificial pollination: with and without emasculation of buds in the torpedo stage, when anthers and stigmas

are receptive and closed. The statistical model $Y_{ijk} = \mu + P_i + E_a + S_k + PS_{ik} + E_b$ expressed estimates of the effects of the constant (μ) of the ith plot (P_i) of the error a, the k-[th] subplot (S_k), the interaction of the ith plot with the kth subplot (PS_{ik}), and of error b. All effects except the errors were considered fixed.

The pollination efficiency of artificial crosses in each set of six plants of the varieties Thaís and Santa Luzia was evaluated by the following procedures (treatments): a) flower buds at the torpedo stage of plants fully covered with organza were emasculated and pollinated by tweezers; b) not emasculated flower buds in the torpedo stage of plants entirely covered with organza were pollinated using tweezers c) flower buds of uncovered plants at the torpedo stage were emasculated and covered with aluminum foil; and d) non-emasculated flower buds of covered plants at the torpedo stage were pollinated by tweezers. The following crosses were used for each treatment: Santa Luzia (female parent) x Thaís (pollen donor) and Thaís (male parent) x Santa Luzia (pollen donor). The crosses were then identified by labels. Six plants of each variety were used as controls, and half of these covered with organza without artificial manipulation of the flowers.

Fruit of controlled crossings and controls with yellow-orange color were collected from April to June 2006 and evaluated for length, width, ratio length / width, weight, and number of seeds.

Analysis of controlled crossings by RAPD markers and morphological traits

Young leaves of each of the varieties Santa Luzia and Thaís and the probable intercrossed progenies of a total of 53 F_1 plants were used to characterize the crosses by the RAPD technique (Williams et al. 1990, Welsh and McClleand 1990), according to the protocol described by Doyle and Doyle (1987) for DNA extraction and purification. Ten plants per assumed cross were evaluated in bulk.

The amplification reactions by the RAPD technique were performed in a laboratory of biotechnology of the Universidade Paranaense – *Campus* Toledo, in a volume of 25 µL, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl; 2.80 mM MgCl$_2$, 0.20 mM of each deoxynucleotide (dATP, dTTP, dGTP, dCTP), 0.20 µM decamer primer, 5-10 ng genomic DNA and one unit of Taq DNA polymerase per reaction. We tested five polymorphic primers for these cubiu

varieties, previously identified by Luz et al. (2008). The amplification reactions were repeated twice, testing 10 bulk samples of each assumed F_1, including the parents.

The DNA was amplified in PCR tubes (0.20 mL) in a thermocycler (Thermo-Hybaid Px2). The DNA amplification program consisted of a denaturation step at 92°C for one minute, pairing with the DNA template at 35°C for one min and an extension step at 72°C for two minutes. The products of 42 amplification cycles were separated by agarose gel electrophoresis (1%) with 0.20 mM ethidium bromide in 0.50% TBE buffer. The amplified fragments were visualized and photographed under UV light using a gel documentation system (Doc-Print, Vilber Lourmat).

The diagnosis was based on polymorphic loci for the parents and the absence of bands in the female parent to detect the crosses. Consequently, F_1 plants alternating between band presence and absence indicate cross-pollination whereas the absence of bands in all plants studied indicates the occurrence of selfing.

Aside from the molecular analysis, the fruit shape of the progenies derived from Santa Luzia (rounded fruit) and Thaís (elongated fruit) was used to confirm the controlled crossings, by measuring the ratio fruit length/ width in parents and progenies.

Statistical analysis of phenotypic traits

For each trait, the mean squares of treatments as well as the coefficient of variation were estimated by the F test, and mean comparison tests were performed by Tukey's method at 5% probability (Steel and Torrie 1980). The efficiency of pollination methods was evaluated based on the survival rate. Furthermore, a non-parametric analysis was performed using the Chi-square test (χ^2), in an attempt to detect possible differences between the methods evaluated and corroborate results of the parametric analysis.

Data were statistically analyzed using the computer resources of software SISVAR (Ferreira 2003).

RESULTS AND DISCUSSION

A total of 53 fruits were harvested from all 777 controlled crossings and analyzed in a morphological and molecular diagnosis to verify the effectiveness of the methods tested. RAPD analysis revealed that the plants grown from the 53 fruits were originated from the cross between Santa Luzia and Thaís (Figure 1a). In a visual evaluation of the fruit shape (Figure 1b) the fruit shape of all progenies was classified as elongated but shorter than the parents, independently of the female parent. Fruits of the variety Thaís (LW = 1.72) were more elongated than of Santa Luzia (LW = 1.44).

The finding of reduced fruit size in the F_1 generation (Figure 1b) allows the hypothesis of the existence of incomplete dominance for fruit shape. Further studies are however needed to confirm this assumption. Moreover, the conclusion was drawn that the pollination was efficient in all pollination methods, validating the study of effectiveness of methods for controlled cross-pollination in cubiu. For *Solanum lycopersicon* the occurrence of partial dominance in fruit
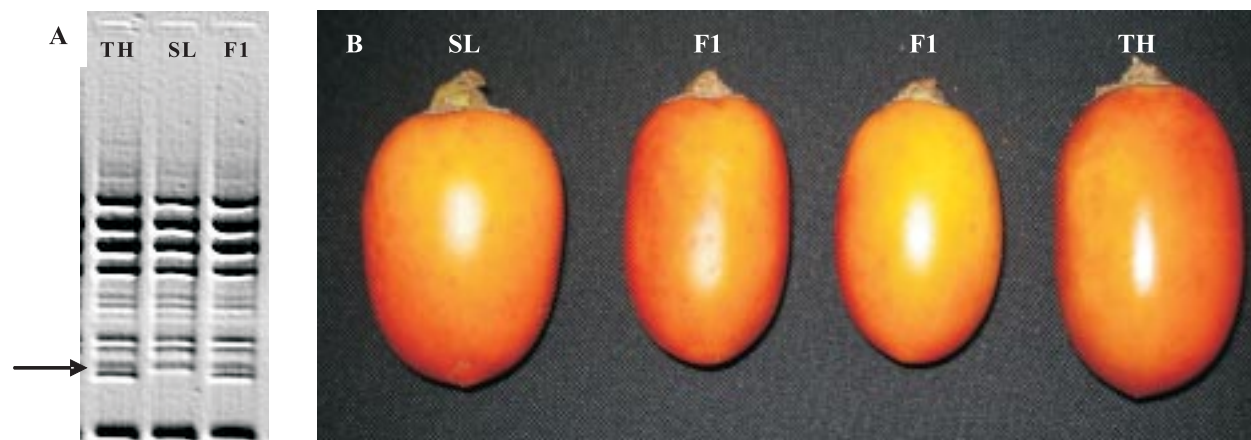
Figure 1. Characterization of cubiu crosses based on RAPD and morphological markers. A: DNA amplification of the parents (TH - Thais / male and SL –Santa Luiza / female) and of the progeny (F_1) using primer OPAB19; B: fruit shape of the parents (SL – Santa Luiza / female and TH – Thaís / male) and progeny (F_1)

traits that are intermediate between the parents has been demonstrated in several studies, e.g., Freitas et al. (1998), Amaral Junior et al. (1999) and Andrade Junior et al. (2005).

The variance analysis by the F test indicated significance for the mean squares of pollination efficiency and the mean seed number in relation to the effect of flower manipulation. On the other hand, F for plant protection was not significant for any of the traits evaluated (Table 1). Therefore, it appears that the emasculation of flowers of plants with or without a cover of organza was the procedure that most influenced the production of fruits with hybrid seeds. However, the mean number of seeds produced was higher by the pollination method without emasculation. Nevertheless, emasculation proved to be the most favorable procedure to obtain hybrid seeds, since the values of pollination

efficiency in crosses with emasculation were 1.83 and 1.42 times higher than in the parallel procedures without emasculation, respectively, with or without organza cover.

The values of pollination efficiency (Table 2) varied from 4.57% to 8.37% in the different procedures, which is considered low. The percentages of the control plants - of the varieties Santa Luzia and Thaís - grown without organza were 3.25% and 4.11%, respectively, which is lower than by the pollination methods, confirmed by the χ^2 test (Table 3). These results indicate the loss of a large number of flowers, which culminated in a mean of 56 to 65 fruits per plant varieties for Santa Luzia and Thaís, respectively. Silva Filho et al. (2005) stated a variation of 4 to 89 fruits per plant in 29 cubiu varieties grown in Amazonian conditions, a result that agrees with the range found here.

Table 1. Analysis of variance for pollination efficiency (PE), mean number of seeds (NS), fruit weight (FW), fruit width (FW), fruit length (FL) and ratio fruit length/ width (LW) according to the different controlled pollination methodologies involving the varieties Santa Luzia (female parent) and Thaís (male parent)

SV	df	Mean squares					
		PE	NS	FW	FW	FL	LW
Plant protection (PP)	1	0.003^{ns}	49.795^{ns}	1536.16^{ns}	0.074^{ns}	0.226^{ns}	0.037^{ns}
Error a	10	0.019	42027.301	581.451	0.260	0.409	0.019
Flower treatment (FT)	1	0.009^{*}	98269.443^{*}	135.043^{ns}	0.108^{ns}	0.044^{ns}	0.002^{ns}
PP X FT	1	0.001^{ns}	76703.296^{ns}	473.748^{ns}	0.001^{ns}	0.119^{ns}	0.003^{ns}
Error b	10	0.001	18402.027	418.304	0.060	0.060	0.004
CV (%) - PP		4.11	25.05	39.99	12.05	10.70	9.70
CV (%) - FT		3.78	16.58	33.91	5.81	4.04	4.34
Overall mean		0.11	818.29	60.30	4.23	5.98	1.42

[*] = Significant at 5% probability by the F test; and [ns] = non-significant at 1% probability by the F test

Table 2. Summary of number of crosses, number of fruits, pollination efficiency, mean number of seeds for the pollination methods with the cubiu varieties Thaís and Santa Luiza

Methods	Number of Crosses	Number of fruits per plant	Pollination efficiency	Mean number of seeds
I - With organza and with emasculation	239	20	8.37	805.35±258.99
II - With organza and without emasculation	197	09	4.57	830.15±320.69
III - Without organza and with emasculation	184	15	8.15	753.8±356.14
IV - Without organza and without emasculation	157	09	5.73	896.77±390.16
Controls				
Variety Santa Luzia (with organza)	1,024	0	0	0
Variety Thaís (with organza)	915	0	0	0
Variety Santa Luzia (without organza)	1,720	56	3.25	1.720±366.23
Variety Thaís (without organza)	1,580	65	4.11	1.580±163.17

[ns] = Insignificant at 5% probability by the Chi-square test (χ^2).

Table 3. Chi-square test ($\chi2$) for the evaluation of methods of controlled pollination involving the varieties Santa Luzia and Thaís independently of the pollen origin

Pollination Methods	Fruits		Loss		Fruits + loss	χ^2	Calculated
	Observed	Expected	Observed	Expected			Probability
I - With organza and with emasculation	20	16.30	219	222.7	239	0.90	0.34[ns]
II - With organza and without emasculation	09	13.44	188	183.56	197	1.57	0.21[ns]
III - Without organza and with emasculation	15	12.55	169	171.45	184	0.51	0.47[ns]
IV - Without organza and without emasculation	09	10.71	148	146.29	157	0.29	0.59[ns]
Total	53		724		777	3.28	0.35[ns]

[ns] = Insignificant at 5% probability by the Chi-square test ($\chi2$), considering fruits and loss

It was found that the mean number of seeds per fruit from controlled crossings ranged from 753.80 ± 258.99 to 896.77 ± 390.16, while the expression in the varieties Santa Luzia and Thaís without organza coverage was more pronounced, with values between 1580.00 ± 163.17 to 1720.00 ± 366.23, respectively (Table 2). However, the numbers of seeds in both were consistent with results of Pahlen (1977), who stated a variation of 500 to 2,000 seeds per fruit in cubiu.

Plants of the varieties Santa Luzia and Thaís covered with organza and without flower manipulation produced no fruits. It was also found that the flowers aborted about 3 to 4 days after anthesis. Pahlen (1977) concluded that cubiu can be considered an autogamous plant, because even in isolated plants the fruit yield is satisfactory, although the occurrence of natural crossings cannot be ruled out, due to the presence of solitary and social bees carrying pollen from one flower to the other. Storti (1988) however found that the cubiu flowers are visited by buzzing bees that promote pollination. In this study only a sporadic presence of carpenter bees (*Xylocopa* sp) of low frequency was observed during the pollination cycle. The bees usually visit the border plants, which usually leads to a higher number of fruit in control plants without organza. However, despite the marginal presence of pollinating insects, the absence of fruit production in the controls with organza favors the possibility of cross-pollination in the evaluated genotypes and reinforces the evidence of predominance of panmixia in the species. Several mechanisms facilitate cross-pollination, e.g., self-incompatibility, which must not be discarded as

hypothesis of pollination as main propagation form in *Solanum sessiliflorum*, although further research is needed focused on the reproduction of the species.

In the joint the evaluation of the controlled pollination methods, including the controls, differences were found by the χ^2 test, although not significant in a partial comparison analysis of the pollination methods (Table 3), indicating that the efficiency of procedures is similar. However, the use of flower buds emasculated in the torpedo stage of plants not covered with organza had advantages. One of them is the possibility of cultivating the plants without organza, since it is difficult to handle this fabric routinely. Moreover, the previous assumption in this study, that emasculation should be used to ensure the identity of the crossing is confirmed, despite the finding of Luz et al. (2008), that the anthers are closed in the torpedo stage and that the *in vitro* germination of pollen grains is quite low at this time.

The pollination efficiency was also evaluated considering the influence of the maternal genotype (Table 4). Santa Luzia produced more fruits, and in the treatment without organza and with emasculation (15.00%) production exceeded the treatment with organza and emasculation (12.00%). Variety Thaís as maternal genotype on the other hand produced less fruits than Santa Luzia. The treatment with organza and with emasculation was most efficient (4.00%), followed by the treatment with organza and without emasculation (2.00%). It is noteworthy that although no significant differences were detected at 5% probability, the treatment without organza and with emasculation

obtained the most significant result, with an estimate of 18.00% for Santa Luzia as female parent. This probability value exceeded all other methods. In the joint analysis of the chi-square test for comparison of maternal genotypes, regardless of the pollination methods, probability values of 15.25% and 5.96% were verified for Santa Luzia and Thaís, respectively, indicating the presence of maternal effect, especially for Thaís as female parent.

These results indicate that Santa Luzia should be used in future crosses as female parent. In this context, Allard (1971), Lewontin (2000), Bueno et al. (2001) and Borém and Miranda (2005) emphasize that cultivated plants differ widely in response to environmental factors, be it at the inter-species level, among cultivars of the same species and even between development stages of the cultivar. However, according to Tomé et al. (2007), in a study on the pollen viability and meiotic analysis of *Solanum commersonii* Dun., *Solanum commersonii malmeanum* Bitt. and *Solanum tuberosum* L., the occurrence of early chromosome migration in metaphases I and II and changes in chromosome pairing were the main causes of pollen unviability in the species studied.

According to Singh (2003), in *Solanum*, the presence of univalent and trivalent chromosomes reduces the proportion of homologous chromosome recombination and consequently, fertility. In the species, small chromosomes have a low chiasma frequency (Hermsen 1984), which together with the early terminalization or presence or absence of synaptic mutants in Prophase I, result in the generation of univalent chromosomes (Tomé et al. 2007).

It is possible that an analogous situation to *Solanum sessiliflorum* could explain the low survival rate (6.82%) in this study, despite the controlled crossings. Cytogenetic evaluations of the species could therefore provide more detailed explanations of low fertility inherent to the crosses made here. Moreover, studies related to mechanisms favoring cross-pollination, such as self-incompatibility, may be of interest to elucidate the inability of cubiu plants with hermaphrodite flowers to produce zygotes by self-pollination.

Table 4. Effect of maternal genotype on survival rate of planted fruits

Pollination methods	Santa Luzia					Thaís				
	Realized crosses	Harvested Fruits	Pollination efficiency	χ^2 Calculated	Probability	Realized crosses	Harvested Fruits	Pollination efficiency	χ^2 Calculated	Probability
I - With organza and with emasculation	120	15	0.12	0.28	0.60 [ns]	119	5	0.04	2.94	0.09 [ns]
II - With organza and without emasculation	98	7	0.07	1.49	0.22 [ns]	99	2	0.02	0.00	0.99 [ns]
III - Without organza and with emasculation	91	14	0.15	1.79	0.18 [ns]	93	1	0.01	0.40	0.52 [ns]
IV- Without organza and without emasculation	78	8	0.10	0.04	0.83 [ns]	79	1	0.01	0.22	0.64 [ns]
Total	387	43		3.542	0.31 [ns]	390	10		3.38	0.34 [ns]

CONCLUSIONS

The employment of emasculation of flowers results in a higher pollination efficiency, but in a lower mean number of seeds per fruit.

There is strong evidence that *Solanum sessiliflorum* species is predominantly panmitic.

ACKNOWLEDGEMENTS

Métodos de cruzamento e diagnóstico de plantas híbridas em cubiu por meio de marcadores genéticos

RESUMO – *Avaliou-se o efeito de diferentes métodos de cruzamento artificial em cubiu para fins de melhoramento para estudos de controle genético de características de importância econômica. Genótipos das variedades Santa Luzia e Thaís, com e sem cobertura com organza, foram utilizados em cruzamentos naturais e com emasculação. Os métodos foram quantificados quanto à eficiência de cruzamentos e por características de frutos, a saber: peso, comprimento, largura, relação comprimento/largura e número médio de sementes. Pelos marcadores RAPD todas as progênies originaram-se dos cruzamentos controlados, revelando a eficiência dos métodos empregados. A eficiência de cruzamentos variou de 4,57% a 8,37%. O uso da emasculação sem a cobertura com organza foi estatisticamente superior na obtenção de frutos. Recomenda-se a variedade Santa Luzia como genótipo materno em procedimentos de hibridação. O cruzamento parece ser o mecanismo de reprodução preponderante na espécie.*

Palavras-chave: *Solanum sessiliflorum*, cruzamentos controlados, controle genético, alogamia.

REFERENCES

Allard RW (1971) **Princípios do melhoramento genético das plantas**. Edgard Blücher: São Paulo, 381p.

Amaral Júnior AT, Casali, VWD, Cruz CD and Finger FL (1999) Inferências genéticas na produção e qualidade de tomateiro sob cruzamento dialélico. **Pesquisa Agropecuária Brasileira 34**: 1407-1416.

Andrade Júnior VC, Maluf WR and Faria MV (2005) Produção e qualidade de frutos de tomateiros portadores de alelos mutantes de amadurecimento e coloração. **Pesquisa Agropecuária Brasileira 40**: 555-561.

Borém A and Miranda GV (2005) **Melhoramento de Plantas**. Editora UFV, Viçosa, 525p.

Brancher A and Tagliari PS (2004) Cubiu: uma fruta amazônica no litoral catarinense. **Revista Agropecuária Catarinense 17**: 43-45.

Bueno LCS, Mendes ANG and Carvalho SP (2001) **Melhoramento genético de plantas**. Editora UFLA, Lavras, 282p.

Doyle JJ and Doyle JL (1987) Isolation of plant DNA from fresh tissue. **Focus 12**: 13-15.

Ferreira DF (2003) **Sisvar**. Editora DEX/UFLA, Lavras, 53 p.

Freitas JA, Maluf WR, Gomes LAA, Oliveira ACB, Martins WS and Braga RS (1998) Padrão de amadurecimento e conservação pós-colheita de frutos de tomateiro, em função das diferentes constituições genotípicas no loco alcobaça. **Revista Brasileira de Fisiologia Vegetal 10**: 191-196.

Hermsen JG (1984) Mechanisms and genetic implications of 2n gamete formation. **Iowa State Journal of Research 58**: 421-434.

Lewontin, R (2000) **The triplex helix**. Harvard University Press, USA, 129 p.

Luz CL, Schuelter AR, da Luz CL, D'almaso A, Vieira ESN and Barreto RR (2008) Germinação *in vitro* de grãos de pólen, receptividade do estigma e efeito da proteção das plantas na frutificação de cubiu (*Solanum sessiliflorum* Dunal). **Acta Scientiarum Agronomy 30**: 1-5.

Pahlen AVD (1077) Cubiu (*Solanum topiro* Hum. & Bonpl.), uma fruteira da Amazônia. **Acta Amazônica 7**: 301-307.

Silva Filho DF, Anunciação Filho CJ and Noda H (1998) Variação fenotípica em frutos de doze introduções de cubiu (*Solamum sessiliflorum Dunal*) da Amazônia. **Revista Brasileira de Olericultura 20**: 60-67.

Silva Filho DF, Andrade JS, Clement CR, Machado FM and Noda H (1999) Correlações fenotípicas, genéticas e ambientais entre descritores morfológicos e químicos em frutos de cubiu (*Solanum sessiliflorum* Dunal) da Amazônia. **Acta Amazônica 29**: 503-511.

Silva Filho DF (2002) **Discriminação de etnovariedades de cubiu (*Solanum sessiliflorum* Dunal, Solanaceae) da Amazônia, com base em suas características morfológicas e químicas**. PhD Thesis, Universidade Federal do Amazonas, Manaus, 123p.

Silva Filho DF, Noda H, Yuyama K, Yuyama LKO, Aguiar JPL and Machado FM (2003) Cubiu (*Solanum sessiliflorum* DUNAL): uma planta medicinal nativa da Amazônia em processo de seleção para o cultivo em Manaus, Amazonas, Brasil. **Revista Brasileira de Plantas Medicinais 5**: 65-70.

Silva Filho DF, Yuyama LKO, Aguiar JPL, Oliveira MC and Martins MC (2005) Caracterização e avaliação do potencial agronômico e nutricional de etnovariedades de cubiu (*Solanum sessiliflorum* Dunal) da Amazônia. **Acta Amazônica 35**: 399-406.

Singh RJ (2003) **Plant cytogenetics**. CRC Press, London, 391p.

Steel RGD and Torrie JH (1980) **Principles and procedures of statistics: a biometrical approach**. McGraw Hill Book Company, New York, 1980. 633p.

Storti EF (1988) Biologia floral de *Solanum sessiliflorum* Dunal var. *sessiliflorum,* na região de Manaus. **Acta Amazônica 18**: 55-65.

Tomé LG, Davide LC, Pereira Pinto CA, Alves AA and Salgado CC (2007) Pollen viability and meiotic analysis of *Solanum commersonii* Dun., *Solanum commersonii malmeanum* Bitt. and *Solanum tuberosum* L. **Crop Breeding and Applied Biotechnology 7**: 387-393.

Welsh J and McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. **Nucleic Acids Research 1**: 7213–7218.

Williams JGK, Rafalski JA and Tingey SV (1990) Genetic analysis using RAPD markers. **Methods of Enzymology 218**: 704-740.