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ORIGINAL ARTICLE

Estimation of genetic parameters of *Plukenetia volubilis* L. seed germination

Estimativa de parâmetros genéticos de sementes de Plukenetia volubilis L.

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ABSTRACT: *Plukenetia volubilis* L. is a species that needs improvement to make commercial exploitation feasible, especially with regard to the seed quality. For this purpose, populations with rapid and uniform germination, followed by prompt seedling emergence are highly desirable for seedling production. The objectives were to study and estimate genetic parameters in germination and emergence tests of *P. volubilis* seedlings and describe the type of seed germination. Two experiments were carried out in the nursery in two seasons: in the Amazonian winter (December 2011 - January 2012) and summer (July-August 2012), evaluating 25 progenies per experiment, in a completely randomized design. The studied traits were: seedling emergence from seedling emergence from substrate (E), first emergence count (IE), emergence speed index (ESI), stem diameter (StD), hypocotyl length (HL) and shoot length (SL). Individual and combined variance analysis and the Scott Knott test were performed and genetic parameters estimated. The seedlings emerged between 19-41 days and 25-42 days in the Amazonian winter and summer, respectively. Estimates of broad-sense heritability in the analysis ranged from 9.980 (E) to 26.880 (IE). The germination of *P. volubilis* is classified as phanerocotylar-epigeal. Significant genetic variability for the traits E, GSI, SL, StD and HL was stated in the mother plants, indicating the possibility of selecting progenies with better seed and seedling quality.

RESUMO: *Plukenetia volubilis L. é uma espécie que necessita de seleção para a viabilização de sua exploração comercial, principalmente quanto à qualidade das sementes, pois populações com germinação rápida e uniforme, seguida por pronta emergência das plântulas, apresentam características altamente desejáveis na formação de mudas. O objetivo deste trabalho foi estimar parâmetros genéticos em estudos de germinação e emergência de plântulas de P. volubilis, além de descrever o tipo de germinação de suas sementes. Para tanto, foram realizados dois experimentos, em viveiro, em duas épocas: inverno (dezembro de 2011 a janeiro de 2012) e verão amazônico (julho a agosto de 2012), sendo avaliadas 25 progênies em cada ensaio, no delineamento inteiramente casualizado. As características avaliadas foram: emergência de plântulas em substrato (E); primeira contagem de emergência (PC); índice de velocidade de emergência (IVE); diâmetro do coleto (DC), e comprimento do hipocótilo (H) e da parte aérea de plântulas (CP). Foram realizadas análises de variância individual e conjunta, além de teste de Scott Knot e estimativa dos parâmetros genéticos. A emergência das plântulas ocorreu entre 19 e 41 dias, e 25 e 42 dias, respectivamente, nas épocas de inverno e verão amazônico. Os valores de herdabilidade no sentido amplo na análise conjunta oscilaram de 9,980 (E) a 26,880 (PC). A germinação de P. volubilis é classificada como do tipo epígea fanerocotiledonar e as plantas matrizes apresentam variabilidade genética significativa para os caracteres E, IVE, CP, DC e H, indicando a possibilidade de seleção de progênies com características superiores de qualidade de sementes e mudas.*

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1 Introduction

The genus *Plukenetia* comprises 17 species with pantropical distribution, 12 of which occur in America, three in Africa, one in Madagascar and one in Asia (GILLESPIE, 1993). Among these is the well-known sacha inchi (*Plukenetia volubilis* L., Euphorbiaceae family), a plant native to the Amazon. The species is a semi-perennial, woody vine and produces capsule fruits (diameter 3-5 cm); the seeds weigh between 0.8 and 1.4 g, are aleurone and oleaginous and contain about 54% oil and 27% protein (HAMAKER et al., 1992). In Manaus, sacha inchi fruit is constantly produced throughout the year, with a decline in the period from December to April.

The seed oil contains 45.2% linolenic acid (omega 3), 36.8% linoleic acid (omega 6), 9.6% oleic acid (omega 9), and 7.7% saturated fatty acid (HAMAKER et al., 1992). Polyunsaturated fatty acids (omega 3 and 6) are essential because they are not synthesized by the human body, requiring ingestion. The presence of these essential fatty acids in the body is important to prevent cardiovascular and neuromuscular diseases and also has a hypocholesterolemic effect when used as food supplement (HAMAKER et al., 1992).

Sacha inchi was first planted near Manaus, Amazonia, and spread out in the Upper Solimões region, but there is little information in the literature concerning the cultivation of this crop. The species deserves more attention with the breeding of superior genotypes for agriculture. For this purpose, the behavior of the species must be assessed in different seasons, as done by Santi et al. (2012) in the period of regional rainfall (Amazonian winter) and dry period (Amazonian summer).

A rapid and uniform seed emergence is a desirable features in the seedling production for the establishment of plantations (RAMOS et al., 2011). For the selection of germination traits the genetic variability in the population must be known, which can be determined by estimating the genetic parameters in the progeny tests in experimental designs (CRUZ; CARNEIRO, 2006).

In some studies, considerable genetic variability was found for germination traits and seed vigor of different plant species, e.g., in studies with *Passiflora edulis* progenies (ALEXANDRE et al., 2004). In the evaluation of *Oenocarpus mapora* and *O. distichus* progenies, wide variability was observed for emergence *percentage* and mean emergence time, favoring selection for these traits (SILVA; MOTA; FARIAS NETO, 2009). A study on germination of *Jatropha ribifolia* also reported significant genetic variability for the selection of superior plants (LYRA et al., 2012).

Studies estimating the genetic parameters of sacha inchi germination can provide conclusions on the genetic variability for selection for rapid and uniform seed emergence, paving the way to domestication and improvement.

The objective was to estimate genetic parameters in studies of germination and seedling emergence of *P. volubilis* and describe the germination type of its seeds.

2 Materials and Methods

The progenies under study were derived from 25 subsamples of the germplasm bank of Embrapa Amazônia Ocidental and the experiments were carried out in the sector of medicinal plants and horticulture of this company, located in Manaus, in the State of Amazonas (3° 8' S and 59° 52' W), in a nursery with 70% shading and irrigation twice a day.

The seeds of different sancha inchi plants were obtained from fruits with dark brown color, at the stage of maximum physiological maturity before fruit dehiscence. To avoid any aging effect on germination and subsequent seedling emergence, the fruits of all plants were harvested on the same day, 27 days before planting.

Two experiments, sown on 10/12/2011 in the Amazonian winter and the second in 25/07/2012, under summer conditions, were carried out. The prevailing environmental conditions during the study were monitored (Table 1).

The seeds were planted hilum down (depth 1 cm) in styrofoam trays with 72 cells containing substrate of biostabilized pine bark and vermiculite, with the following chemical composition, analyzed by Mehlich procedures: 5.2 pH (H_2O), 440 mg dm^{-3} Ca, 324 mg dm^{-3} Mg, 51 mg dm^{-3} Na, 211 mg dm^{-3} K, and 11 mg dm^{-3} P.

The two experiments were conducted in a completely randomized design, evaluating 25 matrices of *P. volubilis* from the germplasm bank of Embrapa Amazônia Ocidental, with four replications of five seeds per plot. *Plukenetia. volubilis* is an Amazonian species for which it is difficult to obtain a large numbers of seeds with uniform physiological maturity in a same period, which was a limiting factor for increasing the number of plants per plot.

The period to the total seedling development was assessed in both experiments, as the period from sowing until open cotyledons of the seedlings, first pair of fully developed leaves, and upright hypocotyl. To estimate the parameters the following traits were evaluated:

Seedling emergence development (E) - The seedlings were counted every two days from the 13th to the 31st day after sowing, considering the seedlings with normal emergence and with part of the hypocotyl visible above the substrate. Normal seedlings, considered as those equipped with all complete, healthy and proportionate essential structures, or with damages

Table 1. Monthly meteorological data of the experimental periods (Amazonian winter and summer).

Season of evaluation	Month and year	Temperature (°C)			Relative moisture (%)	Evaporation (mL)	Sunshine (hours)
		Maximum mean	Minimum mean	Means			
Amazonian winter	December 2011	31.3	20.4	25.9	89.0	57.9	122.3
	January 2012	30.5	20.2	25.3	92.7	39.3	59.9
Amazonian summer	July 2012	32.9	22.8	27.8	83.9	62.2	173.9
	August 2012	34.2	22.6	28.4	81.5	71.6	183.2

irrelevant to the plant development, were characterized and the results were expressed as percentages.

The first emerged seedlings were counted 15 days after sowing, when the emergence of normal seedlings was uniform in each treatment, and the results expressed as percentage.

Emergence speed index (ESI) - assessed by daily counting of seedlings that emerged from zero until 31 days after sowing, calculated by the GSI equation proposed by Maguire (1962).

Stem diameter (StD) – measurement of the diameter (mm) with a caliper 38 days after sowing.

Hypocotyl length (H) - length (cm) of the hypocotyl of the seedlings, measured with a ruler 38 days after sowing.

Shoot length of (SL) - length (cm) of shoots of the seedlings, measured with a ruler 38 days after sowing.

In the statistical and genetic analyses, the data of emergence percentage and first emergence count were arcsine transformed $(x.100^{-1})^{0.5}$. The data were subjected to individual and combined analysis of variance and the following parameters were estimated: genetic and phenotypic variance, genetic coefficient of variation, coefficient of broad-sense heritability, and coefficient b (ratio between the coefficient of genetic variation and environmental variation coefficient), as proposed by Cruz and Carneiro (2006). Means were compared by the Scott-Knott test at 5% probability. Analyses of variance were performed, using the genetics-specific statistical software Genes.

3 Results and Discussion

The early emergence was characterized by the growth of the curvature of the green hypocotyl (Figure 1a) from 7 to 30 days after sowing (= 18.5 days). Within two days after emergence of the hypocotyls, most cotyledons stood out above the surface of the substrate (Figure 1b). It was also observed that some seedlings maintained dark seed coats as the cotyledons grew above the substrate (Figure 1c). The cotyledons were enwrapped in cream-colored endospermatic tissue (Figures 1b and c).

Approximately four to six days after hypocotyl emergence, the dark seed coat that covered the cotyledon dropped and the development of the cotyledonary leaves was advanced (Figure 1d). In some cases, the endospermatic (cream-colored) tissue remained in the cotyledonary leaves which were not opened, while the early development of the first pair of normal leaves took place at this stage (Figure 1e). The cotyledonary leaves that opened were freed from the cotyledons and fully formed between 9-10 days after hypocotyl emergence, and the beginning of the development of the first pair of normal leaves was also noted (Figure 1f). The total seedling development in the experiment conducted in the Amazonian winter lasted 19 to 41 days after sowing and in the summer the variation was 25 to 42 days.

The type of germination of *P. volubilis* is epigeous, since the cotyledons rose above the ground level, and

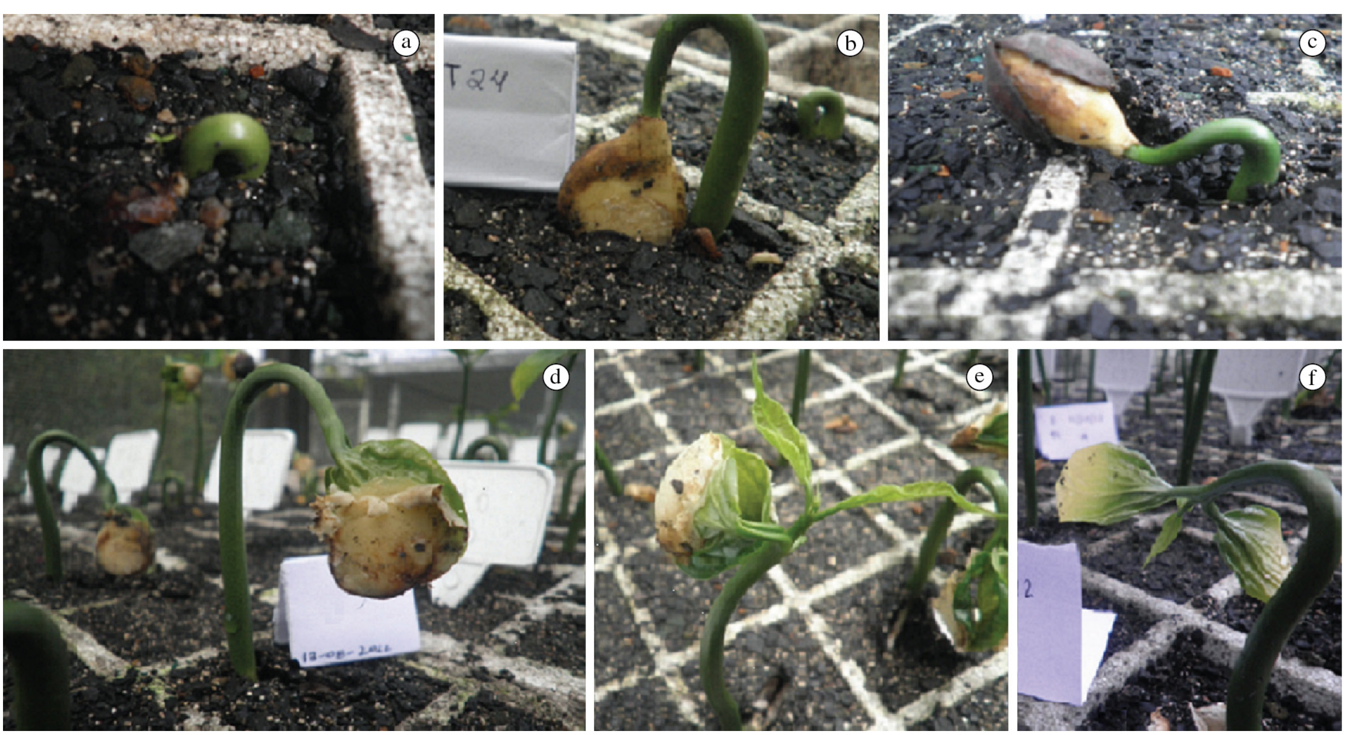

Figure 1. a - Early stage of *Plukenetia volubilis* seedling emergence, with soil disruption and formation of the hypocotyl hook; b - Growth of the cotyledon over the substrate two days after emergence; c - opening of the hypocotyl hook and appearance of the burst dark integument; d - advanced stages of *Plukenetia volubilis* seedling emergence where foliaceous cotyledons are fixed to the cream-colored endosperm; e - cotyledonary leaves fixed to the remaining endosperm; f - seedling with endosperm-free foliaceous cotyledons, partially chlorotic and early development of the first leaf pair.

phanerocotyledonary, because the cotyledons were completely tegument-free.

The period for the complete seedling formation can vary, due to environmental alterations, from one species to another and even between individuals within the same species, depending on the existing genetic variability for emergence and growth speed. Somewhat similar values were reported by Añez et al. (2005) and Nunes et al. (2009) for *Jatropha elliptica* and *Jatropha curcas*, a species of the same family as *P. volubilis*, which completed seedling formation 20-32 and 15-30 days after sowing, respectively.

When planted in the Amazonian winter, the emergence of *P. volubilis* seedlings began six days earlier than in summer. The differential performance of the seeds may have been caused by the reduced sunshine in the winter and by the lower temperatures, three degrees below those in the summer. In the Amazonian winter, the mean minimum and maximum temperatures and number of hours of sunshine per month were lower than in the summer (Table 1). Although the study was carried out in a 70% shaded greenhouse, an effect of sunshine cannot be ruled out.

In the individual and combined analyses of variance of the experiments, a significant treatment effect ($p<0.05$) was observed for: seedling emergence in substrate (E), first emergence count (IE), emergence speed index (ESI), stem diameter (StD), and hypocotyl length (HL). In the combined analysis, there was a significant effect ($p<0.05$) of sowing date, due to changes in the mean GSI and IE of the progenies between the first and the second experiment. A significant effect ($p<0.05$) of the interaction genotype - sowing date on E, GSI and IE was observed.

The significant treatment effect in the individual and combined analyses of variance in the experiments indicated genetic variability in the progenies for the traits: seedling emergence from substrate, first emergence count, germination speed index, stem diameter, and hypocotyl length. The significant effect of the interaction genotype - sowing date on the studied traits showed differentiated progeny responses in each season. This interaction may be due to differences in the climatic conditions of the evaluation periods that characterize different environments in the state of Amazonas, December 2011 and January 2012 (Amazonian winter) and August and September 2012 (Amazonian summer). The seed germination percentage can change according to the sowing date (ABREU; NOGUEIRA; MEDEIROS, 2005; COIMBRA; NAKAGAWA, 2006), a factor that must be taken into account in the planning of seed production.

The values of coefficients of environmental variation (CVe) were highest for the first emergence count and plant shoot length, while the values for the other traits were low (Tables 2, 3 and 4). Estimates of CVg were relevant to estimate the variation among genotypes (FERRÃO et al., 2008) and ranged from 4.09 to 18.73 for all traits in the individual analyses (Tables 2 and 4).

Estimates of the broad-sense heritability in the individual analyses ranged from 7.55 (shoot length) (Table 4) to 91.59 (germination speed index) (Table 2). In the combined analysis, heritability was low for all traits. A large number of superior

Table 2. Genetic parameters of the analysis of experiments in the Amazonian winter and summer for emergence (E), emergence speed index (ESI) and the first emergence count (IE) of seedlings grown from subsamples of *Plukenetia volubilis* seeds on substrate.

Genetic parameters	Winter experiment			Summer experiment		
	E	GSI	IE	E	GSI	IE
σ^2_g	0.01	0.012	0.03	0.01	0.01	0.025
CV_g (%)	5.18	18.62	13.80	4.09	18.73	13.09
CV_e%	12.27	11.29	32.29	11.29	12.73	29.93
h^2(%)	41.59	91.59	42.22	34.48	89.64	43.36
CV_g / CV_e	0.42	1.65	0.43	0.36	1.47	0.44

σ^2_g: genetic variance; CV_g(%): coefficient of genetic variation; CV_e(%): coefficient of environmental variation; h²: coefficient of broad-sense heritability; CV_g/CV_e: coefficient b.

Tabela 3. Genetic parameters of the analysis of Amazonian winter and summer experiments of emergence (E), emergence speed index (ESI) and the first emergence count (IE) of seedlings grown from subsamples of *Plukenetia volubilis* seeds on substrate.

Genetic parameters	E	GSI	IE
σ^2_g	0.01	0.01	0.01
CV_g (%)	1.72	5.72	8.09
CV_e(%)	11.78	12.05	31.07
h^2(%)	9.98	14.88	26.88
CV_g / CV_e	0.15	0.47	0.26

σ^2_g: genetic variance; CV_g(%): coefficient of genetic variation; CV_e(%): coefficient of environmental variation; h²: coefficient of broad-sense heritability; CV_g/CV_e: coefficient b.

Tabela 4. Genetic parameters of traits in the Amazonian summer experiment: stem diameter (StD), hypocotyl length (HL) and shoot length (SL) of seedlings grown from subsamples of *Plukenetia volubilis* seeds on substrate.

Genetic parameters	StD	HL	SL
σ^2_g	0.023	0.89	3.26
CV_g (%)	4.14	7.54	4.47
CV_e%	8.39	13.30	31.28
h^2(%)	49.34	56.22	7.55
CV_g / CV_e	0.49	0.57	0.14

σ^2_g: genetic variance; CV_g(%): coefficient of genetic variation; CV_e(%): coefficient of environmental variation; h²: coefficient of broad-sense heritability; CV_g/CV_e: coefficient b.

Table 5. Result of the grouping by the Scott Knott test of experimental means of *Plukenetia volubilis* in the Amazonian winter and summer for the traits: seedling emergence from the substrate (E), emergence speed index (ESI) and first emergence count (IE) and in the summer experiment, the traits: stem diameter (StD), hypocotyl length (HL) and shoot length (SL).

Experiment in the Amazonian winter									Experiment in the Amazonian summer																	
E			GSI			IE			E			GSI			IE			StD			HL			SL		
T	Mean		T	Mean		T	Mean		T	Mean		T	Mean		T	Mean		T	Mean		T	Mean		T	Mean	
25	100.0	a	1	0.75	a	25	100.0	a	25	100.0	a	20	0.36	a	20	100.0	a	19	4.72	a	14	14.44	a	7	51.02	a
24	100.0	a	19	0.73	a	24	100.0	a	24	100.0	a	21	0.36	a	12	100.0	a	10	4.47	a	24	14.28	a	19	50.47	a
23	100.0	a	2	0.67	b	23	100.0	a	23	100.0	a	15	0.35	a	17	95.0	a	12	4.46	a	13	13.66	a	20	50.35	a
22	100.0	a	18	0.66	b	22	100.0	a	22	100.0	a	2	0.35	a	13	95.0	a	22	4.44	a	15	13.62	a	1	48.80	a
21	100.0	a	3	0.65	b	21	100.0	a	21	100.0	a	13	0.35	a	2	95.0	a	24	4.41	a	23	13.61	a	10	48.17	a
20	100.0	a	11	0.65	b	19	100.0	a	19	100.0	a	12	0.35	a	23	90.0	a	7	4.38	a	19	13.58	a	16	46.35	a
18	100.0	a	23	0.64	b	18	100.0	a	18	100.0	a	7	0.35	a	21	90.0	a	14	4.37	a	12	13.56	a	14	46.28	a
15	100.0	a	9	0.64	b	17	100.0	a	17	100.0	a	4	0.35	a	15	90.0	a	2	4.37	a	20	13.09	a	8	44.51	a
14	100.0	a	24	0.63	b	13	100.0	a	13	100.0	a	1	0.35	a	16	85.0	a	21	4.27	a	3	13.05	a	11	42.72	a
11	100.0	a	10	0.63	b	11	100.0	a	11	100.0	a	23	0.34	a	10	85.0	a	4	4.26	a	7	12.88	a	4	41.67	a
10	100.0	a	25	0.63	b	10	100.0	a	10	100.0	a	10	0.34	a	7	85.0	a	18	4.26	a	25	12.87	a	25	40.62	a
3	100.0	a	14	0.61	c	8	100.0	a	8	100.0	a	24	0.34	a	3	85.0	a	15	4.22	a	10	12.80	a	23	40.00	a
2	100.0	a	13	0.60	c	6	100.0	a	6	100.0	a	3	0.34	a	1	85.0	a	25	4.18	a	21	12.74	a	15	39.42	a
1	100.0	a	20	0.59	c	4	100.0	a	4	100.0	a	17	0.33	a	4	80.0	a	8	4.14	b	17	12.67	a	22	39.36	a
19	95.0	a	17	0.59	c	3	100.0	a	3	100.0	a	19	0.33	a	24	75.0	a	13	4.14	b	4	12.58	a	2	39.35	a
17	95.0	a	22	0.58	c	2	100.0	a	2	100.0	a	8	0.33	a	14	75.0	a	3	4.10	b	6	12.50	a	21	38.95	a
13	95.0	a	8	0.57	c	1	100.0	a	1	100.0	a	5	0.32	b	8	75.0	a	20	4.09	b	2	12.49	a	24	38.15	a
12	95.0	a	21	0.56	c	16	95.0	a	16	95.0	a	25	0.32	b	19	70.0	b	6	4.06	b	1	12.27	a	17	37.29	a
9	95.0	a	15	0.55	c	15	95.0	a	15	95.0	a	6	0.31	b	9	70.0	b	23	3.97	b	8	12.19	a	6	34.12	a
8	95.0	a	16	0.51	d	12	95.0	a	12	95.0	a	18	0.31	b	6	70.0	b	1	3.95	b	9	11.75	b	18	33.67	a
5	90.0	b	6	0.47	d	7	95.0	a	7	95.0	a	14	0.31	b	18	65.0	b	17	3.94	b	18	11.28	b	12	33.07	a
16	85.0	b	4	0.40	e	5	95.0	a	5	95.0	a	16	0.30	b	25	60.0	b	11	3.91	b	5	11.25	b	13	33.02	a
6	85.0	b	7	0.39	e	9	90.0	b	9	90.0	b	11	0.28	b	11	55.0	b	16	3.84	b	16	11.03	b	9	31.37	a
4	85.0	b	12	0.34	e	14	85.0	b	14	85.0	b	22	0.26	c	22	50.0	b	9	3.80	c	11	10.20	b	5	30.62	a
7	80.0	b	5	0.32	e	20	75.0	b	20	75.0	b	9	0.26	c	5	40.0	c	5	3.70	c	22	8.92	b	3	30.05	a

Means followed by the same letter in the column do not differ from each other by the Scott Knott test. T = treatment.

progenies was selected in the study because the heritability was low in the combined analysis for the traits E, GSI and IE and medium for StD and HL in the experimental analyses of the Amazonian winter.

For the trait seedling emergence from substrate consisting of biostabilized pine bark and vermiculite, the 17 best progenies common to the summer and winter experiments were selected (1, 2, 3, 8, 10, 11, 12, 13, 15, 17, 18, 19, 21, 22, 23, 24 and 25) (Table 5). For emergence speed index, 64% of the progenies were selected (1, 2, 3, 4, 7, 8, 10, 12, 13, 15, 17, 19, 20, 21, 23 and 24) (Table 5). For the first emergence count (IE), 60% of the progenies were selected (1, 2, 3, 4, 7, 8, 10, 12, 13, 15, 16, 17, 21, 23 and 24) considering the analysis of both experiments (Table 5).

For the shoot length of seedlings in the summer experiment there was no difference between the progeny means in the treatments (Table 5). For stem diameter and hypocotyl length, traits with medium heritability, the selection with few plants would not be recommended, excluding only the worst progenies 9 and 5 for StD and 9, 18, 5, 16, 11, and 22 for HL (Table 5). The traits SL, StD and HL were in the summer evaluated only, so a comparison with the winter was not possible.

4 Conclusions

Plukenetia volubilis has a phanerocotylar epigeal germination type.

The studied *P. volubilis* progenies contain genetic variability for seed germination traits (E, GSI, IE, StD, and HL) for the selection of superior plants.

The heritability values of the traits E, GSI, SL were lower in the combined analysis, requiring the recommendation of a greater number of superior progenies common to both environments to continue the breeding program.

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