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Genetic selection of fig tree (*Ficus carica* L.) varieties using phenotypic characteristics of rooting and development of softwood cutting

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Abstract

Although fig tree cultivation has been expanding in Brazil, there is paucity in literature on improvements necessary to propagate it at different environmental conditions. Therefore, this study aimed at assessing quality and genetic potential of thirty fig tree varieties from the germplasm bank for phenotypic analysis of root and softwood cuttings development. The softwood fig cuttings collected from pruning branches of selected varieties. We standardized cuttings of 20 cm in length having straight cut at the basal part and a bevel at the apex. They were placed into the polyethylene box (50 x 17 cm) filled with medium textured expanded vermiculite in a vegetable stove. We used intermittent nebulization irrigation with a flow rate of 0.012 m^3s^{-1} of water in 20 seconds each 3 minutes. After 60 days, cuttings were transplanted into plastic bags (30 x 20 cm) with capacity of 1.2 L filled with 1/3 soil and 2/3 organic compounds, where placed in a greenhouse, protected with 50 % shade black polypropylene mesh, with intermittent irrigation for 15 minutes with a flow rate of 0.243m^3s^{-1} every 4 times a day. After 105 days, evaluations consisted of cuttings survival number, length of the largest root, root fresh and dry matter, shoot number, length of the largest shoot, shoot fresh and dry matter. The results of analyses showed Roxo de Valhinhos, Ilha Solteira, Brunswick, IAC, Genoveso, IAC, varieties 39, 41 and 42 as high performance varieties. In addition, results indicated low genotypic coefficient of variation in relation to environmental coefficient of variation in most characters. Besides, only two characters showed superior heritability; thus, we concluded that there is no genetic variability between varieties for most measured characters. Since the studied population presented low genetic variability using morphological descriptors of roots we recommend unsuccessful selection for tested characters through breeding programs. However, cuttings survival number is of great importance to select fig genotypes.

Keywords: climatic adaptation*,* *Ficus carica* L , genetic improvement, phenotypic characterization.
Abbreviations: CSP_Cutting survival percentage; LLR_Length of the largest root; RFM_Root fresh; RDM_Dry matter; LLS_Length of the largest shoot; SFM_Shoot fresh; SDM_Dry matter; SN_Shoot number; σ_g^2_genotypic variance, experimental error variance σ_e^2_, σ_f^2_phenotypic variance, h_g^2_heritability, CV_{gi}%_individual genetic coefficient of variation; CV_e%_experimental coefficient of variation; GM_general mean.

Introduction

In fruit plants, genetic improvement studies aimed at determining genotypes and phenotypes to produce the best quality raw material and yield. These studies are scarce in fig culture. The selections of biotypes are based on phenotypic data using agronomic interests.
Although the fig tree (*Ficus carica* L.) is a temperate climate crop, it is easily adapted to different climate conditions, allowing its cultivation in regions of semi-arid to cold climate. The fig tree has lower deep dormancy than apple trees and cherry trees (Oukabli, Mekaoui, 2012).
In Brazil, fig has an important economic value. It is among top twenty fruits destined for export. It also has the third position in trading volume among temperate fruits, by producing 28,300 tons of fruits per year (Faostat, 2017). Therefore, Brazil is the world´s tenth largest producer of figs, but the second largest exporter, surpassed only by Turkey.

The propagation is often done by hardwood cuttings from the median portions of the branches and basal that is collected during winter pruning, when is its dormancy period (Karadeniz, 2003; Sousa et al., 2013). Genetic characteristic of the varieties is responsible for the diversity of rooting potential. Sirin et al. (2010) observed that the cultivar "Sarilop" has a low emergence of roots. Other factors may cause this variation such as the age of tissue, environmental condition and time of collection cuttings (Han, Zhang and Sun, 2009). Furthermore, precise information on genetic variation becomes essential for fig crop, due to its economic importance. In addition, genetic resources conservation are mainly established in Active Germplasm Banks, which has to be implemented in different populations, according to their adaptability to human-caused changes (He et al., 2016).
Germplasm banks conserve genetic variability that may develop productive genotypes (Koundinya et al., 2013). The possibility to select more productive plants is due to the cross-breeding between divergent parents for exploration of heterosis by higher lineages or cultivars (Kumar, Reddy, 2016). According to Valois et al. (1998), less than 8% of the constant resources in germplasm banks are effectively used by researchers; as plant breeders are unaware of breeding program activities and plant genetic resources (Carelli et al., 2006). A successful germplasm bank needs plant biometric data, as they have an extreme ecological value, as well as helping to determine species variability and to differentiate phenotypic parameters among individuals, which constitutes one of the most important sources of variability available for improving a culture (Melo et al., 2004; Paula, 2007). Therefore, the aim of this work was to estimate the quantitative genetic parameters for the characteristics related to rooting of fig cuttings. Thirty fig tree cultivars were selected to verify the magnitudes of the genetic correlation between rooting and other characters, as well as to analyze strategies to select and improve crops.

Results and Discussion

Root analysis

Regarding to cutting survival rate, the mean percentage was 56%. This is lower than the reference value of 70 % for a satisfactory vegetative propagation (Chagas et al., 2008; Cardoso et al., 2011; Radmann et al., 2014). However, varieties 27, 42 and 'Roxo de Valinhos IS' were the cultivars with best performance (Figure 1). As already known, the rooting potential of cuttings varies depending on the species and cultivars. For example, the sugar and hormone concentration are different in the tissue of diverse cultivars (Fachinello et al., 2005; Vignolo et al., 2014). In the region of Lavras, Brunswick variety showed 100% rooting of softwood fig cuttings, as reported by Bisi (2015). Besides that, Troiano variety presented 70.5% of rooting, which is lower than the value obtained in the current study. There was a significant difference among genotypes for root fresh and dry matter, as well as shoot fresh matter (Table 2). Furthermore, the mean root length was 24.92 cm, but variety 41 presented 38.5 cm, variety 33 performed 34.02 cm, while Brunswick had 32.66 cm. Therefore, these varieties obtained higher values than 33 cm (Figure 2A). In peach tree cuttings, Zanandrea et al. (2017) also verified different length of root in five cultivars. Moreover, this study demonstrated that rooting and growth factors, such as sugars and hormones concentration act differently among fruit cultivars. Fachinello et al. (1995) reinforced that root system development is due to the carbohydrate reserve accumulation. This implies a correlation between rooting and cutting survival rate, since auxin requires carbon source for nucleic acids and proteins biosynthesis. According to Moubaydin et al. (2010) the development occurs when the apical meristem prevail the cellular division on differentiation, causing the auxin concentration to promote the division rather than cytokinin in the differentiation.
Regarding to root fresh matter, the mean was 5.28g. However, some genotypes performed above mean value, such as varieties 28 (18.77g), 39 (14.06g), and 42 (30.96g) (Table 3). For root dry matter, the mean was 1.75g. The highest values were observed in Nobile (4.14g), variety 42 (6.03g) and 'Mini Fig IAC' (6.58g) (Table 3). Additionally, these variations in root system traits are responsible for nutrient and water absorption by plant. Therefore, better nutrition and seedling development may be related to growth and development of clonal seedlings of figs. A more developed root system contains a great number of roots and is measured by the weight of its fresh matter, resulting in more vigorous seedling, great absorption capacity, as well as adaptation to poor water-and-nutrient soils (Silva et al., 2008).

Shoot analysis

For the length of the largest shoot, the mean was 23.12cm, but 41.50cm was observed in variety 33, 31.91cm in Brunswick and 30.27cm in Nobile (Figure 2B). The length of shoot may be correlated with photosynthetic energy, concentration of auxin, stock of nutrients, responsible for the shooting cells extension and division (Taiz et al. 2017)
Moreover, the longest shoot length may be justified for the high temperature, which increases cellular division (Hartmann et al., 2011) in view of the same climate condition of the region that enables cultivar adaptation.
For shoot fresh matter, the mean was 18.76g, but some genotypes were superior to this value, e.g. Nobile (31.82g), varieties 28 (37.37g), 33 (38.04g) and 42 (70.01g) (Table 3). For shoot dry matter, a general mean of 5.96g was found, but the best results were observed in variety 42 (17.53g) and Nobile (14.76g) (Figure 2C). Additionally, varieties that presented higher means in root length did not have the same performance in shoot dry matter, as their values were close to or below average. The results were corroborated with Souza (2017) that noted more actuation of carbohydrate in the rooting than in the aerial part of Fig tree cultivar "Roxo de Valinhos". For shoot number, the genotypes Bonato, variety 28 and 39 obtained detachable means, as compared to mean genotypes that was 3.39 shoots per cuttings (Figure 2D). This value is similar to 3.19 cuttings per shoots that was observed by Souza (2017), working with cuttings of Fige Tree cultivar "Roxo de Valinhos" in Botucatu, state of São Paulo. The number of shoot in varieties was related to high carbohydrate reserves that increased the number of shoots (Agbo, Obi, 2007). Pacheco and Franco (2008) classified the differences of carbohydrate content into fine, medium and coarse cuttings. Besides, the lignification in plant cell walls could influence sprouting; thus, these authors stated that

Table 1. Identification of fig tree varieties (*Ficus carica* L), Selvíria, state of Mato Grosso do Sul, 2016.

	Name of Varieties	Origin
1	Nobile	IAC*
2	Genoveso	IAC
3	Roxo de Valinhos SSP	São Sebastião do Paraíso
4	Stanford	IAC
5	White Adriatic	IAC
6	Bonato	IAC
7	White Genova SJRP	São José do Rio Preto
8	White Genova IAC	IAC
9	Smyrna IAC	IAC
10	Smyrna SJRP	São José do Rio Preto
11	Brunswick	São José do Rio Preto
12	Caprifig IAC	IAC
13	Troyano	IAC
14	Pingo de Mel	IAC
15	Palestino	Campinas
16	Mission	Piracicaba
17	Variety 25	Monte Alto
18	Variety 27	Monte Alto
19	Variety 28	Monte Alto
20	Variety 29	Monte Alto
21	Variety 30	Monte Alto
22	Variety 31	Monte Alto
23	Variety 33	Monte Alto
24	Variety 39	Monte Alto
25	Variety 41	Monte Alto
26	Variety 42	Monte Alto
27	Roxo de Valinhos IS	Ilha Solteira
28	Mini Fig	IAC
29	Caprifig IS	Ilha Solteira
30	Variety X	Monte Alto

*IAC: Agronomic Institute of Campinas, Brazil

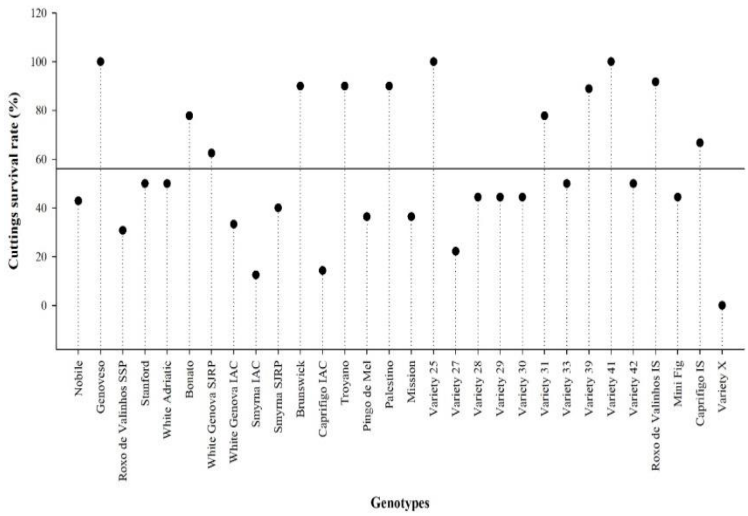

Fig 1. Cutting surviving rate (%) in relation of average of fig tree genotypes, Ilha Solteira, state of São Paulo, Brazil.

Table 2. Analysis of variances for length of the largest root; root fresh and dry matter; length of the largest shoot; shoot fresh and dry matter; and shoot number of figs genotype in Ilha Solteira, state of São Paulo.

Variation source	LLR (cm)	RFM (g)	RDM (g)
	p-Value		
Genotypes	0.55^{ns}	0.00*	0.03**
General mean	52.05	5.28	1.75
CV (%)	24.92	112.89	133.80

Variation source	LLS (cm)	SFM (g)	SDM (g)	SN
	p-Value			
Genotypes	0.09^{ns}	<0.01*	0.27^{ns}	0.52^{ns}
General mean	23.12	18.76	5.96	3.38
CV (%)	50.24	85.76	96.22	90.08

^{ns}not significant, *significant at 1% and ** significant at 5% probability by F test.

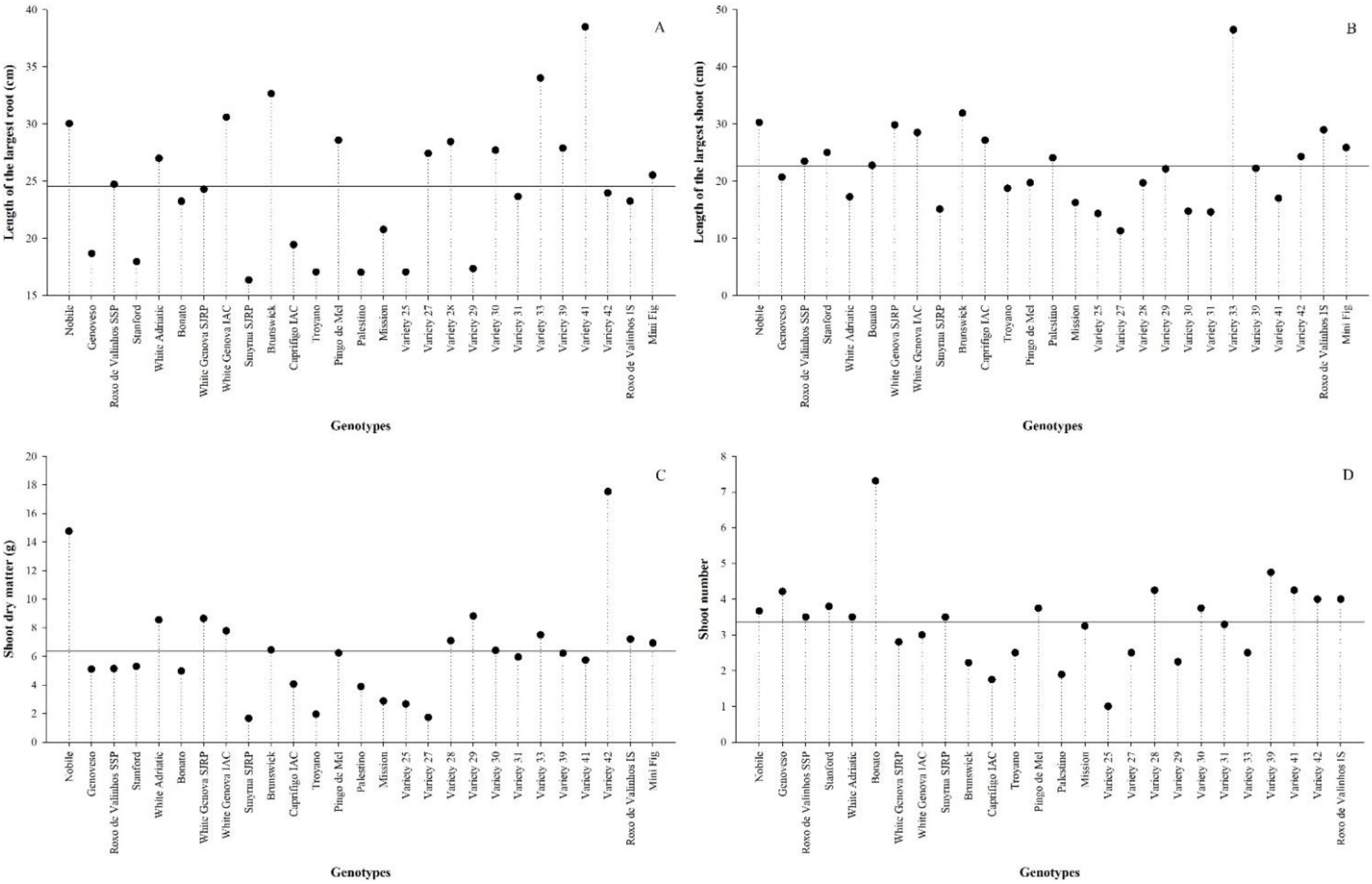

Fig 2. Length of the largest root (A) and shoot (B), shoot dry matter (C) and shoot number in relation of average of fig genotypes (D) in Ilha Solteira, state of São Paulo, Brazil.

high carbohydrate reserves enable coarse cuttings to sprout.

Phenotypic analysis

According to data obtained in this study, genetic analysis of most morphological characters presented a genotypic coefficient of variation (CVg) lower than the environmental coefficient of variation (CVe), indicating that the studied population has medium to low genetic variability. Table 4 estimates genetic parameters for these traits. The estimation of heritability ranged from 0.38 to 33.2% (shoot dry matter and root fresh matter, respectively). Only two characters exhbited heritability considerably above zero, showing that there is low genetic variability between varieties for most characters.

Moreover, the quantitative traits were strongly affected by environment, while the qualitative ones were not. This fact may have contributed to the inclusion of a non-genetic

Table 3. Length of the largest root fresh and dry matter, shoot fresh matter of fig genotype cuttings in Ilha Solteira, state of São Paulo, Brazil.

Genotypes	RFM (g)	RDM (g)	SFM (g)
Nobile	7.64 C	4.14 A	31.82 B
Genoveso	3.79 C	1.20 B	15.13 C
Roxo de Valinhos SSP	3.76 C	1.98 B	13.32 C
Stanford	5.86 C	0.79 B	15.21 C
White Adriatic	6.69 C	2.65 B	25.70 C
Bonato	3.23 C	0.96 B	17.96 C
White Genova SJRP	4.32 C	2.12 B	21.20 C
White Genova IAC	4.06 C	2.06 B	20.76 C
Smyrna SJRP	1.37 C	0.29 B	8.04 C
Brunswich	3.93 C	2.49 B	17.27 C
Caprifig IAC	1.12 C	0.58 B	9.03 C
Troyano	0.33 C	0.06 B	6.55 C
Pingo de Mel	1.72 C	0.86 B	19.32 C
Palestino	2.64 C	0.95 B	10.05 C
Mission	0.92 C	0.56 B	9.06 C
Variety 25	1.48 C	0.39 B	14.42 C
Variety 27	2.26 C	0.44 B	7.06 C
Variety 28	18.77 B	2.16 B	37.73 B
Variety 29	4.69 C	2.04 B	21.04 C
Variety 30	2.62 C	1.31 B	15.13 C
Variety 31	4.14 C	2.27 B	20.71 C
Variety 33	2.12 C	0.68 B	38.04 B
Variety 39	14.06 B	2.33 B	29.36 C
Variety 41	8.46 C	1.62 B	25.30 C
Variety 42	30.96 A	6.03 A	70.01 A
Roxo de Valinhos IS	6.11 C	2.38 B	17.46 C
Mini Fig IAC	10.99 C	6.58 A	25.25 C
General mean	5.28	1.75	18.76

*Means followed by the same uppercase letters in the column do not differ from each other by Scott-Knott test at 5% probability

Table 4. Components of variance for genetic parameters for cutting survival percentage, length of the largest root, root fresh and dry matter, length of the largest shoot, shoot fresh and dry matter, and shoot number, evaluated in relation to seedlings development by softwood cuttings.

Genetic Parameters	Analysed variables			
	CSP	LLR	RFM	RDM
σg^2	0.05	0.02	0.52	0.03
σe^2	0.20	1.51	1.05	0.40
σf^2	0.25	1.54	1.57	0.43
hg^2	20.2 (± 7.8)	1.40 (± 2.8)	33.2 (± 13.6)	7.12 (± 6.31)
CVg (%)	41.0	3.01	34.4	13.0
CVe (%)	81.5	25.2	48.8	47.1
CVg/CVe	0.50	0.12	0.70	0.28
MG	0.55	4.89	2.10	1.35
Genetic Parameters	Analysed variables			
	LLS	SFM	SDM	SN
σg^2	0.10	0.27	0.00	0.03
σe^2	1.42	3.02	1.03	0.25
σf^2	1.52	3.28	1.04	0.28
hg^2	6.63 (± 6.09)	8.11 (± 6.74)	0.38 (± 1.47)	11.0 (± 7.84)
CVg (%)	6.77	12.8	2.72	9.33
CVe (%)	25.4	42.9	43.7	26.6
CVg/CVe	0.27	0.30	0.06	0.35
GM	4.69	4.04	2.32	1.89

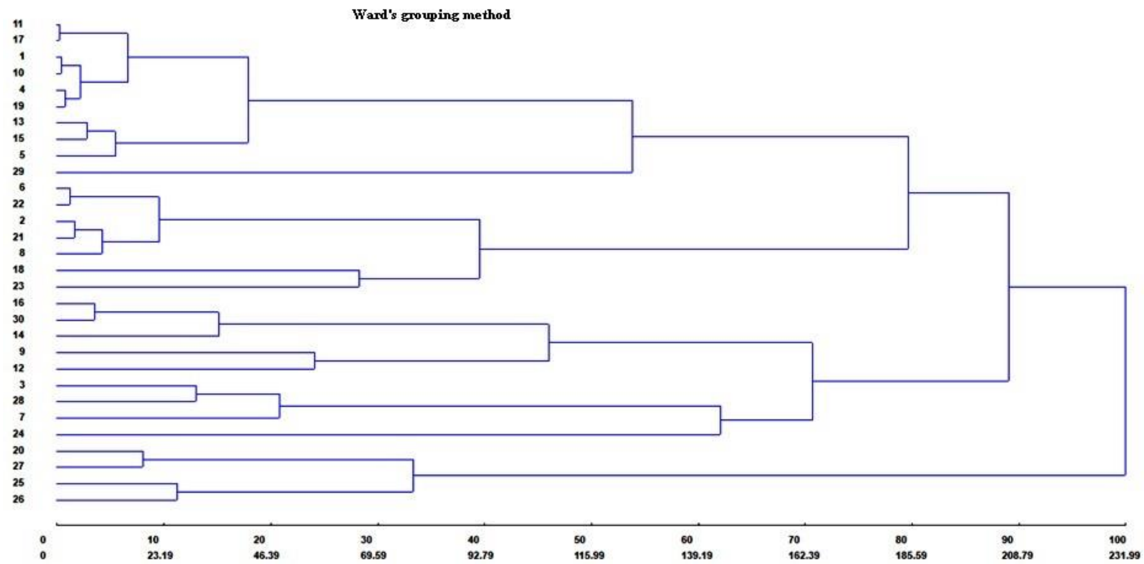

Fig 3. Dendrogram obtained from generalized Mahalanobis distance of 8 characters (cutting survival percentage, length of the largest root, root fresh and dry matter length of the largest shoot, shoot fresh and dry matter, and shoot number) in relation to seedlings development through rooting of softwood cuttings by Ward's method.

variation to the quantitative data. However, in a study on genetic diversity of cassava germplasm, Kawuki et al. (2011) evaluated 29 qualitative and 4 quantitative traits, in which root dry matter was included, showing that qualitative traits presented low power for discrimination of cassava varieties, while quantitative traits presented high genetic variation, which were adequate to obtain high genetic gains in breeding programs. According to Hunt (1990), growth analysis aims at describing and interpreting species performance. Oliveira et al. (2006) evaluated the components of variance and heritability for root traits in half-sib families of baru (*Dipteryx alata*) seedlings, reporting heritability around 100%. In a study of genetic parameters and diversity in macaúba (*Acrocomiaaculeate*) progenies, Domiciano et al. (2015) reported that the estimation of heritability ranged from 50.24 to 71.9% (height and length of the rachis, respectively), besides authors considered these values to be high, especially when analyzed together with those obtained for genetic and environment coefficients of variation, the ratio of which in most cases was close to 0.5. In this case, the same authors concluded that species breeding is possible to be achieved through selection of promising genotypes for morphological characteristics. Oliveira Filho (2014) evaluated genetic diversity in cassava root system and concluded that phenotypic variation based on morph agronomic traits may demonstrate the possibility of dividing cassava germplasm into different groups. Furthermore, they stated that joint analysis of qualitative and quantitative traits can assist to cluster differences in varieties. In the current study, cutting survival rate and root fresh matter were the only ones that presented mean heritability (20.2 and 33.2), with a CVg/CVe ratio of 0.5 and 0.7, respectively; thus showing as promising characters. In this sense, 'Roxo de Valinhos IS' and variety 27 stood out for first character, with 100% cuttings survival. For root fresh matter, varieties 42, 28, 39, 'Mini Fig IAC' and 'Nobile' stood out with statistically significant values of 30.96, 18.77, 14.06, 10.99 and 7.64 g, respectively.

Additionally, a complementary analysis was performed to infer structure of fig tree varieties, as phenotypic characters of seedling production are shown through genetic distance performed by hierarchical clustering analysis. According to genetic similarity, the grouping of these varieties is represented by dendrogram in Figure 3.

Regards to the hierarchical clustering according to Mahalanobis distance, fig genotypes showed a high genetic variability among themselves; however, there are very similar varieties, i.e. variety 11 with 17, as well as 1 and 10; 4 and 19. In general, one can segregate the genotypes into three large groups that have high genetic diversity among them. However, the two largest groups still have a hierarchical structure of subgroups, showing that even within a given group there is still variability, even presenting very similar genotypes for the studied characters.

Materials and methods

Conduction of study

The experiment took place at the orchard of Education, Research and Extension Farm, Faculty of Engineering of Ilha Solteira (FEIS), Universidade Estadual Paulista (UNESP), Brazil. This farm is located in the city of Selviria, state of Mato Grosso do Sul, at the geographical coordinates of 20°22'S, 51°22'W and the elevation about 357 m. Besides, the climate was classified as tropical with dry winter (Aw) (Köppen, 1984). All plant material was obtained from adult plants of *Ficus carica* L. They were kept into Fig trees Germoplasma Bank, which is located at the aforementioned farm.

Experimental design

Softwood cuttings of 20 cm long were collected from pruning branches through a straight cut at the basal part and a bevel cut at the apex. Subsequently, all material was treated with fungicide Ridomil® (Metalaxyl M + Mancozeb

[4+64WP]) at 300 g L^{-1} of water. Inserting 1/3 of the cuttings into polyethylene boxes (50 x 17 cm), filled with medium textured expanded vermiculite. After 60 days, they were transplanted into plastic bags (30 x 20 cm) with capacity for 1.2 L filled with 1/3 of soil and 2/3 of sieved organic compound. The plant material were kept in a vegetable stove with an intermittent nebulization irrigation with a flow rate of 0.012 m^3s^{-1} distributing a daily quantity of water in 20 seconds each 3 minutes in a vegetation house, protected with 50 % shade black polypropylene mesh, with intermittent irrigation with a flow rate of 0.243m^3s^{-1} for 15 minutes every 4 times a day. A completely randomized design was conducted including 30 Fig genotypes, with 8 replications per genotype. Table 1 shows all varieties, as well as their place of origin.

Analyzed variables

The following evaluations were performed 105 days after experiment setup. The cuttings survival rate (%); length of the largest root (cm); root fresh and dry matter (g); shoot number; length of the largest shoot (cm); shoot fresh and dry matter (g) were measured. In order to evaluate the percentage of rooted cuttings, the total amount of rooted and sprouted cuttings and those not rooted or sprouted (dead) were counted. The length of the largest shoot and root were measured using a ruler. The weighing of fresh matter mass of shoots and roots were performed in a digital scale. The dry matter masses, placed in paper bags, were kept for 72 hours in electric stove at 65º C until reached a constant mass.

Statistical analysis

The analysis of variance was performed by F-test, besides means were submitted to Scott-Knott test at 5% significance for means comparison among evaluated genotypes through SISVAR 5.3® software.

The estimation of variance components were obtained for each variable using the Selegen-REML/BLUP (Statistical system and computer-based genetic selection via linear mixed models) (Resende, 2002). The components of variance and descriptive statistics were genotypic variance, experimental error variance, phenotypic variance, heritability, individual genetic coefficient of variation experimental coefficient of variation and general mean.

Additionally, the Mahalanobis distance was used to measure genetic divergence of varieties by root and leaf biometric characters (Cruz and Carneiro, 2003). Moreover, dendrogram was generated by Ward's method of cluster analysis among genotypes (Cruz et al., 2006).

Conclusion

Varieties "Roxo de Valhinhos of Ilha Solteira", Brunswick IAC, Genoveso IAC, varieties 39, 41 and 42, presented the best results in the analyzed variables. Genetic variability ranged from medium to low in studied population, proving to be difficult to identify materials through morphological descriptors. However, cuttings survival rate is a major character and should be considered for selecting fig genotypes.

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