Full Length Research Paper

Screening of spontaneous castor bean accesses for genetic improvement programs

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The objective of this work was to identify, through the use of multivariate analysis, the spontaneous accesses of castor bean that show promising characteristics for inclusion in genetic improvement programs of this culture in Brazil. The study was conducted at the Paulista State University, Jaboticabal Campus. The accesses of castor bean seeds obtained were identified as ACS-001-CRSP, ACS-001-JASP, ACS-001-MASP, ACS-001-SESP, ACS-002-JASP, ACS-002-SESP and ACS-003-JASP, from the municipalities of Jaboticabal, Monte Alto, Santa Ernestina and from the District of Córrego Rico. The seeds' biometric variables and germination potential were measured, in addition to the biomass accumulation of the seedling's vegetative parts. The experimental design was completely randomized, with seven treatments and four replications. The original data was standardized and submitted to multivariate analysis of principal components and grouping. Through the results obtained, it is possible to reduce the number of original variables into two latent variables with discriminant power between the castor bean accesses, being the multivariate analysis efficient in this process. The castor bean accesses ACS-001-CRSP and ACS-001-MASP are promising for introduction in genetic improvement programs of this culture.

Key words: *Ricinus communis* L., genotype, multivariate statistics, bioenergy.

INTRODUCTION

Climate change has often been featured in numerous discussions around the world, especially when emphasizing the consequences of human actions in the environmental context (Cera and Ferraz, 2015; Klein et al., 2016.). In fact, some activities developed by man are potentially harmful to the environment, for example,

processes that result in greenhouse gases emission, such as oil and gas, which are non-renewable energy sources and major precursors of CO_2 emissions into the atmosphere (Anifowose and Odubela, 2015). In this context, Shahraeeni et al. (2015) reported that the mitigation of global warming is possible through reducing atmospheric concentrations of pollutant gases.

The choice of biofuels consists of a promising strategy for environmental improvement, being from renewable sources (Lima et al., 2015; Lu et al., 2015). In this scenario, the cultivation of castor bean (*Ricinus communis* L.) can be highlighted as an alternative source of vegetable oil for biodiesel generation, and several other industrial applications with the possibility of viable fuel supply to the Brazilian energy matrix (Dutra et al., 2015; Kallamadi et al., 2015; Lara-Fioreze et al., 2015).

Brazil is characterized by its edaphoclimatic diversity, featuring varied ecosystems throughout the country, with large potential agricultural areas for the production of oilseeds. Castor bean has a high productivity capacity, high adaptability to different climate and soil, and the oil produced by the plant does not compete with edible ones, making it a promising bioenergy alternative (Paes et al., 2015). There are several varieties of castor oil plants with significant genetic diversity, making them suitable for genetic improvement (Kallamadi et al., 2015).

Therefore, research should be intensified aiming the generation of academic and scientific information, besides the possibility of introducing technologies from screening of castor-oil-plant accesses that have potential for use in genetic improvement programs in Brazil and in the world (Singh et al., 2015).

In recent studies, the use of data analysis by univariate methods is efficient, particularly when the objective is to differentiate genotypes of the same species, for example *R. communis* L. (Moraes et al., 2015). However, there is significant increase in the literature of the use of multivariate data analysis for discrimination of genotypes or accesses with desirable agronomic characteristics or for genetic improvement (Rodrigues et al., 2014).

The germplasm's genetic variability is vital for the success of a genetic improvement program, and furthermore, the diversity analysis allows the identification of divergent genitors suited to obtain hybrids of greater heterotic effect. To this end, the application of multivariate statistical techniques such as the principal component analysis (PCA) and grouping enables the differentiation between accesses and optimization of germplasm collection, in order to promote a more efficient use of available genetic resources (Reis et al., 2015).

The objective of this study was to identify, through the use of multivariate analyses, accesses of spontaneous castor bean with promising characteristics for inclusion in genetic improvement programs of this culture in Brazil, increasing the availability of raw material for biofuel production.

MATERIALS AND METHODS

The experiment was conducted between the months of July and August 2014 in a greenhouse, followed by laboratory, both belonging to the Department of Agriculture Applied Biology (DAAB) located at the Faculty of Agricultural and Veterinary Sciences (FCAV) of the Paulista State University (UNESP).

Initially, accesses castor bean seeds were obtained from the micro-region of Jaboticabal, SP. To this end, areas were identified as infested by the species (*R. communis* L.) in reproductive phase, and the racemes with 70% of fruits maturing or dry were collected and arranged inside the greenhouse for sun drying. Then, the berries were opened for removal of the seeds, which were placed in polyethylene trays staying in the greenhouse for drying and subsequent selection according to color pattern, weight, health, physical damage and pathogen attacks. The selected seeds were identified as ACS-001-CRSP, ACS-001-JASP, ACS-001-MASP, ACS-001-SESP, ACS-002-JASP, ACS-002-SESP and ACS-003-JASP, from the municipalities of Jaboticabal, Monte Alto, Santa Ernestina and District of Córrego Rico.

Evaluations were performed for longitudinal diameter (SLD, mm), transverse diameter (STD, mm) and thickness (ST, mm) using a digital caliper, and fresh mass of a hundred seeds (HSM, g) in analytical scale, according to the criteria of Embrapa (2006). The germination potential of the seeds was carried out according to the rules established in Brasil (2009). The evaluation of germination percentage (GP, %), emergency speed index (ESI), mean germination time (MGT, days), and germination rate (GR, days), were performed as described in Carvalho and Carvalho (2009). For this purpose, the seeds were sown in August 3, 2015, and 14 days after sowing, five uniform seedlings were collected per share, which was separated into root, stem and leaves. In order to obtain the dry mass of the roots (RDM, g), stems (SDM, g) and leaves (LDM, g), these vegetative parts were placed in paper bags and dried in a forced air oven at 60°C until a constant weight.

The experimental design was completely randomized, with treatments consisting of seven castor bean accesses with four replications of 25 seeds. The data were subjected to standardization in such a way to make the mean zero and the variance unitary. The multivariate structure of the results was evaluated by means of PCA in order to condense the amount of relevant information in the original set of data into a smaller number of dimensions (principal components, PC), resulting from linear combinations of the original variables generated from the higher eigenvalues in the covariance matrix. For each PC, we proceeded to cluster analysis by hierarchical method, Ward's minimum variance, considering the variables relevant in the composition of each principal component (Hair et al., 2009).

RESULTS AND DISCUSSION

Through the use of PCA, it was possible to condense the number of original variables into two main components (PC1 and PC2), which together hold 79% of the total accumulated variance. The choice of these PCs was

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Table 1. Loads of variables, eigenvalues and cumulative variance associated with the two principal components formed from 11 variables measured in seven castor bean accesses.

PCs	SLD	STD	ST	HSM	GP	ESI	MGT	GR	RDM	SDM	LDM	λ	σ²
PC1	0.58	0.52	0.79	0.55	-0.60	-0.52	0.68	-0.75	-0.91	-0.85	-0.83	5.42	49.26
PC2	0.78	0.82	0.57	0.78	0.04	0.62	-0.50	0.40	0.28	0.27	0.35	3.29	29.95

PC, Principal component; SLD, seed longitudinal diameter; STD, seed transverse diameter; ST, seed thickness; HSM, a hundred seed mass; GP, germination percentage; ESI, emergency speed index; MGT mean germination time; GR, germination rate; RDM, root dry mass; SDM, stem dry mass; LDM, leaf dry mass; λ, eigenvalue; σ², variance.
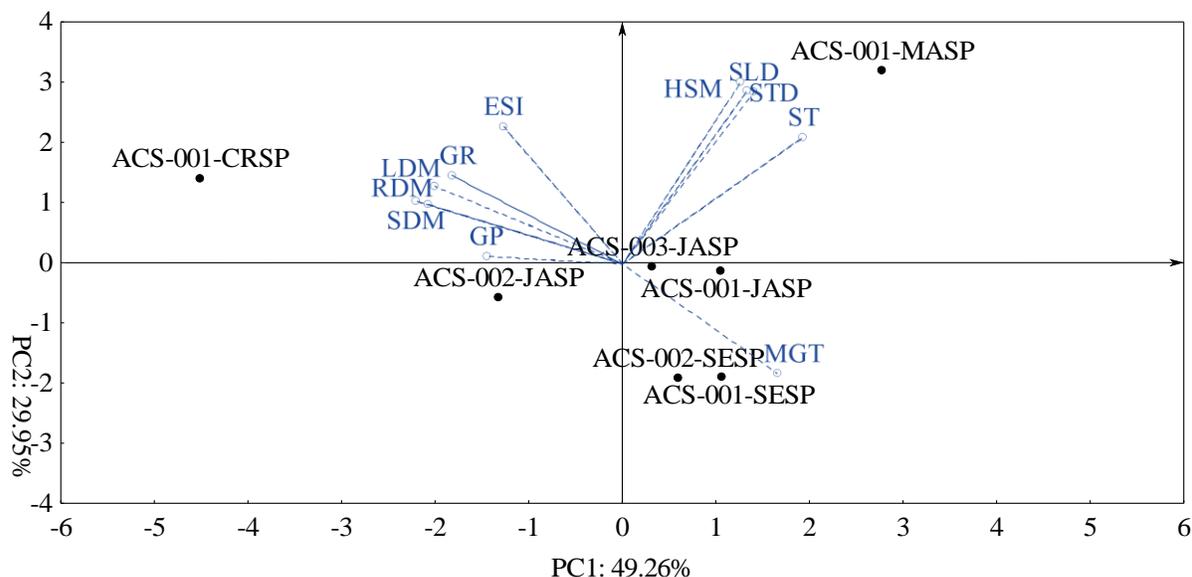

Figure 1. Two-dimensional projection (biplot) of castor bean accesses and the variables in the two principal components (PC1 and PC2).

based on the eigenvalues ≥ 1.00. For the selection of the variables, values ≥ 0.60 were adopted (absolute values) according to the Kaiser criteria (Table 1).

The first principal component (PC1) explained 49.26% of the total variance. In this component, the accesses ACS-001-CRSP and ACS-001-MASP were discriminated, in which the biometric variables (ST), seed vigor (GP, MGT and GR) and dry mass of seedlings (RDM, SDM and LDM) contributed to the cumulative variance for presenting factor loads above 0.60 (absolute value) (Figure 1). The second Principal Component (PC2) holds 29.95% of the remaining variance. In this component, the access ACS-001-MASP was separated from the accesses ACS-001-SESP and ACS-002-SESP, and it should be highlighted that the biometric variables (SLD, STD and HSM) and emergency speed index (ESI) of the seeds contribute with significant actor loadings in screening of these castor bean accesses (Figure 1).

The knowledge of differences in genetic constitutions within groups or between genotype groups is important to any genetic improvement program (Rodrigues et al.,

2014). Therefore, the results of this study contribute to the state of the art and scientific development, by increasing the genetic amplitude with new materials, according to the statement Cruz and Regazzi (1997), which emphasize the importance of identification of genitors with genetic differences that produce progeny of higher heterosis, thereby increasing the probability for obtaining superior individuals.

The search for genetic variability and the introduction of new genetic material into existing germplasm banks are important strategies to guarantee the goals of improvement programs (Reis et al., 2015). Most species exploited agriculturally had their genetic amplitude drastically reduced as a result of domestication, selection processes and plant improvement (Gonçalves et al., 2008).

The lower growth of plants can be considered an indicator of adaptive defense strategies, especially in situations of abiotic stresses (Freitas-Silva et al., 2016; Li et al., 2016). The variation in seedling growth, aerial part and root system denotes more or less adaptation to the
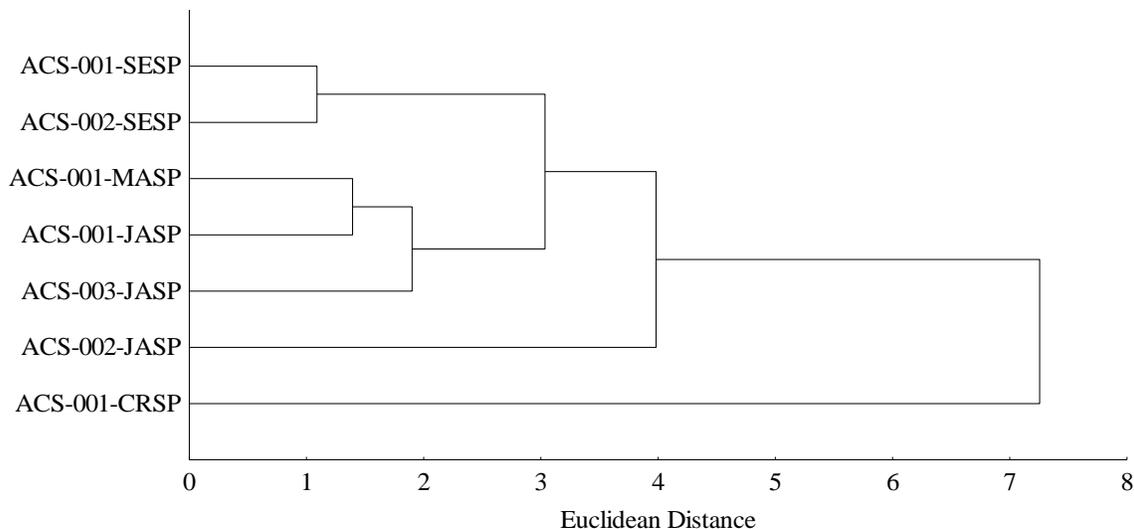
Figure 2. Dendrogram showing the structure of groups contained in the seven castor bean accesses, built with the expressive variables PC1.

environment (Mesquita et al., 2015). Based on this information, there is further need to study the ACS-001-CRSP for its capacity of adjustment in the growth of root system and aerial part, as a defense strategy to abiotic stresses such as water deficit and salt stress, which should lead to greater stability in the field.

Variations in the biometric characteristics of the seeds can be attributed to various factors, from physiological maturity to differences in genetic make-up, so that such situations act directly on the proliferation and development of cells that constitute the embryo axis and reserve tissues (Severino et al., 2015). According to Paes et al. (2015), due to the large number of varieties, the oil content may vary from 44-55% of the seeds' dry mass. Still, these authors emphasize the relationship between biometrics and oil content in castor beans. Thus, considering the results obtained in this study, the access CCS-001-MASP can be explored for oil production purposes, mainly for standing out for the peculiar characteristics of its seeds, which gives it potential in oil accumulation.

In order to express structure groups contained in the accesses, cluster analysis was performed based on PC1 variables. Through this technique, it was verified that the ACS-001-CRSP access has high dissimilarity compared with others, followed by ACS-002-JASP, while the accesses ACS-003-JASP, ACS-001-JASP and ACS-001-MASP form an intermediate group, with significant dissimilarity regarding the accesses ACS-001-SESP and ACS-002-SESP (Figure 2).

The grouping of accesses based on the variables with high factor loadings in PC2, shows that the ACS-001-MASP access has great dissimilarity when compared with others (Figure 3). The ACS-003-JASP and ACS-001-SESP accesses form an intermediate group, while the

rest are grouped into two subdivisions consisting of ACS-001-JASP and ACS-001-CRSP; and ACS-002-JASP and ACS-002-SESP (Figure 3).

The data clustering process consists in the vectorization of similar multidimensional data in a number of clusters, and currently this type of analysis is widely used in exploratory analyses (Wang et al., 2016). The use of this technique is justified by the practicality in the visualization of the results by interest group, even when the number of genotypes involved in the research is high (Laurindo et al., 2015). Vargas et al. (2015) add that the increased use of multivariate techniques to quantify the genetic divergence is relevant, because these analyses allow considering simultaneously a large number of features, which facilitate the decision-making process based on a joint response, unlike studies that assess the variables in an isolated manner.

Rodrigues et al. (2014), studying castor bean accesses from the state of Minas Gerais, found the formation of groups and dissimilarity among the accesses with peculiar characteristics promising for improvement, and concluded that the employment of the multivariate technique (cluster analysis) proved efficient for the investigation of genetic variability among the studied materials.

Conclusions

1) The original set of variables may be reduced in two latent variables with discriminating power among the castor bean plant accesses.
2) The accesses of castor bean ACS-001-CRSP and ACS-001-MASP have high potential for introduction in genetic improvement programs of the culture.
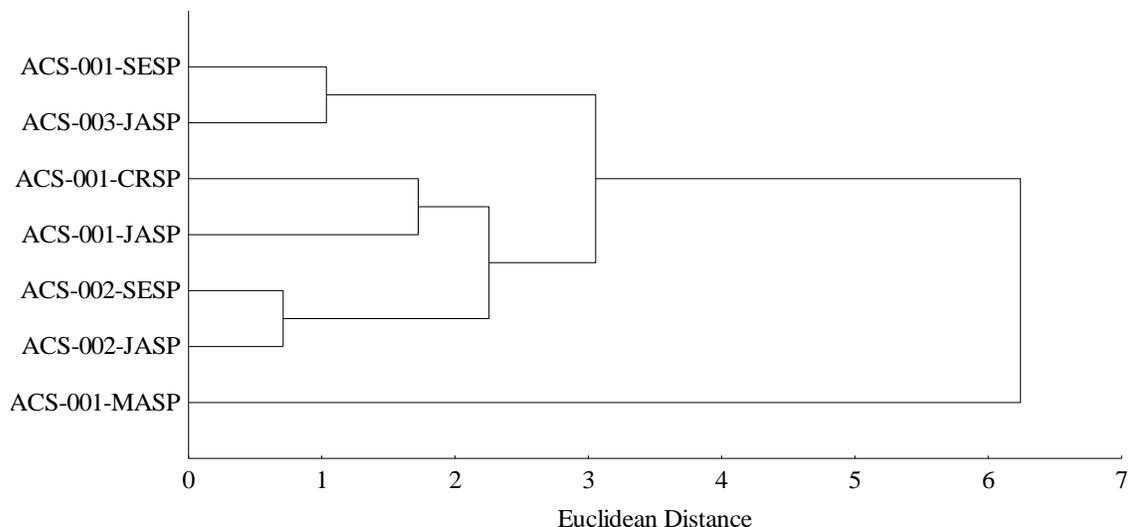
Figure 3. Dendrogram showing the structure of groups contained in the seven castor bean accesses, built with significant variables in PC2.

3) The use of multivariate data analysis shows efficiency in screening studies of castor bean accesses.

Conflict of Interests

The authors have not declared any conflict of interest.

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