Phenotypical, phytochemical and molecular characterization of “capim-carona” [*Elionurus muticus* (Spreng.) Kuntze] populations

FÜLLER, T.N.; TESSELE, C.; BARROS, I.B.I.; BARBOSA NETO, J.F.*
*Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves, 7712, Caixa Postal 776, CEP: 91501-970, Porto Alegre-Brasil * jfbn@ufrgs.br*

ABSTRACT: *Elionurus muticus* naturally occurs in southern Brazil and its economic potential is due to the presence of essential oils. There are few studies about this genus. Thus, the aim of the present study was to characterize native *E. muticus* populations. The study was performed with five “capim-carona” populations collected in the state of Rio Grande do Sul, totaling 50 plants grown in pots in the Agronomy School. All five *E. muticus* populations presented variability for phenotypic traits and phenolic compound concentration. The presence of citral was identified in all populations, except in that from the “Morro da Polícia” region. RAPD analysis showed high variability for these populations, allowing the separation of individuals into five groups according to their geographic origin. The highest variability occurred within each population. Based on the results, the populations from São Borja and Agronomy School can be recommended to be used in breeding programs.

Key words: genetic variability, RAPD, essential oils, phenolic compounds

RESUMO: Caracterização fenotípica, fitoquímica e molecular de populações de *Elionurus muticus* (Spreng.) Kuntze (capim-carona). *Elionurus muticus* ocorre naturalmente no sul do Brasil e o potencial econômico se deve ao fato da presença de óleos essenciais. Poucos estudos têm sido desenvolvidos para este gênero. Sendo assim, o presente trabalho teve como objetivo caracterizar populações nativas de *E. muticus*. O trabalho foi realizado com cinco populações de capim-carona coletadas no Rio Grande do Sul, totalizando 50 plantas cultivadas em vasos na Faculdade de Agronomia. As cinco populações de *E. muticus* apresentaram variabilidade para os caracteres fenotípicos e para concentração de compostos fenólicos. A presença de citral foi identificada em todas as populações, exceto a do Morro da Polícia. A análise de RAPD demonstrou elevada variabilidade para as populações, permitindo a separação dos indivíduos em cinco grupos, sendo possível, de modo geral, agrupá-los de acordo com a origem geográfica. Observou-se também que a maior variabilidade ocorreu dentro de cada população. Os resultados indicaram que as populações São Borja e Faculdade de Agronomia podem ser recomendadas para a utilização em programas de melhoramento.

Palavras-chave: variabilidade genética, RAPD, óleos essenciais, compostos fenólicos

INTRODUCTION

The genus *Elionurus*, Humb. & Bompl ex Willd, belongs to the Poaceae family, comprises about 15 species and is common in the tropical and subtropical regions of South America, Africa, Australia (Araújo, 1971; Renvoize, 1978) and Temperate Asia (Watson & Dallwitz, 2002). *Elionurus muticus* naturally occurs in Brazil, where it is known as “capim-carona” (Longhi-Wagner et al., 2001). *E. muticus* has been popularly used as a medicinal and aromatic plant since it has sudorific and fever-reducing properties (Dzingirai et al., 2007). Antioxidant and antibacterial activities of *Elionurus* sp. have been reported by some authors (Cacciabua et al., 2005; Sabini et al., 2006; Dzigirai et al., 2007; Hess et al., 2007). The essential oil of *E. candidus* is used as flavoring in cosmetics and household cleaning products (Castro & Ramos, 2003). *E. muticus* is not suitable for cattle food since its bitter taste is transmitted to the milk; however, young plants can be eaten (Castro & Ramos, 2003; Hess et al., 2007).

Recebido para publicação em 22/11/2008
Aceito para publicação em 05/05/2010

Elionurus muitcus is characterized by high variability in the chemical composition of its essential oil. In Argentina, it is classified into five chemical types according to the major compound present in its essential oil, namely neral, geranial, acorenone, iso-acorenone and 1.8-cineole. The first two have great importance to industrial purposes (Hess et al., 2007; Kolb et al., 2007). Citral is a mixture of the two geometric isomers geranial and neral. This compound has a strong citric odor (Heydorn et al., 2003) and triggers the greatest interest regarding *E. muticus* due to its wide use in aromatic, food, and cosmetic industries. Citral has been used as raw material in the pharmaceutical industry to synthesize a series of ionones. Beta-ionone has been specifically used to synthesize vitamin A (Koshima et al., 2006). The high demand for essential oil with high citral content, currently supplied by lemon grass (*Cymbopogon citratus*), encourages the use of *E. muticus* as an alternative for oil extraction (Kolb et al., 2007). The presence of neral and geranial in *E. muticus* has only been detected for Southern Brazilian populations, whereas in the remaining Brazilian regions only camphene (11.5%), E-caryophyllene (17.9%) and spathulenol (18.6%) have been reported as major oil components (Scramim & Saito, 2000).

Despite such a demand, little is known about the chemical composition, biological activity and cultivation of *E. muticus*. The establishment of breeding programs may improve the knowledge and allow the exploitation of existing resources, developing new populations capable of producing essential oil and favorable agronomic traits for cultivation. High genetic and morphological variability was detected for *E. muticus* (Hess et al., 2007; Kolb et al., 2007). The success of any breeding program depends basically on the genetic variability of the involved parents (Allard, 1960). Therefore, before starting the breeding itself, it is essential to characterize the genetic variability of plant populations for the target trait (Nodari & Guerra, 2000).

The aim of this study was to analyze the variability of morphological traits, phenolic compounds and citral concentration of the essential oil from five *Elionurus muticus* populations collected in southern Brazil.

MATERIAL AND METHOD

Plant material

Natural populations of *Elionurus muticus* (Spreng.) Kuntze were collected in several regions from the State of Rio Grande do Sul (Brazil). Voucher specimens for all collections were deposited at the Herbarium of the Natural Science Institute, Department of Botany, Federal University of Rio Grande do Sul (UFRGS), Rio Grande do Sul State (RS), Brazil. The number of collected individuals and herbarium vouchers are shown in Table 1. The set of individuals collected in a specific locality was considered a population and the individuals consisted of each clump. Two populations were collected in “Morro da Polícia” (MP) and “Morro Santana” (MS) regions in Porto Alegre Municipality (RS). Other two were obtained from São Borja and São Francisco de Paula Municipalities (RS). In addition, an *E. muticus* population available for teaching purposes in the Agronomy School (UFRGS Campus) was also used in the study. The collected plants were transplanted to bags containing soil and kept in a greenhouse at 20ºC mean temperature for further analysis.

Agronomic characterization

The individuals were at the same development stage when the following agronomic traits were analyzed plant height (cm), measured from the base to the apex of leaves; leaf width (mm), measured with a digital pachymeter, consisting of the largest leaf part; regrowth (cm), defined as the plant height at 30 days after cutting; shoot fresh matter (g); leaf curling; growth habit - the climbing habit was observed and received the values: 0=erect, 1=semi-prostate and 2=prostate; and inflorescence presence. The results were subjected to analysis of variance (ANOVA) and means compared according to Tukey’s test (α= 0.05).

TABLE 1. Data on the collections of *E. muticus* populations in Rio Grande do Sul State (Brazil).

Local	Number of individuals	Latitude (S)	Longitude (W)	Altitude (m)	Herbarium voucher number
Agronomy School (AS)	8	30º04’15”	51º08’22”	50	152280
“Morro da Polícia” (MP)	8	30º04’22”	51º10’07”	286	152281
“Morro Santana” (MS)	17	30º07’	51º07’	311	152279
São Borja (SB)	11	28º39’44”	56º00’15”	96	10119 - 152282
São Francisco de Paula (SFP)	6	29º20’00”	50º31’21”	922	152283

Phenolic compounds

Phenolic compounds were quantified using 3 g dry leaves homogenized with 20 mL methanol 80%. Their concentration was assessed by the Folin-Ciocaulteau method, according to Arnaldos et al. (2001). After 30 min incubation at 25°C in the dark, absorbance readings were obtained in a spectrophotometer at 765 nm (λ). Gallic acid was chosen as standard to establish the calibration curve. Three replicates per individual were used for quantification of phenolic compounds. Results were subjected to analysis of variance (ANOVA) and means compared according to Tukey's test (α= 0.05).

Essential oil

Essential oil was extracted from shoots by hydrodistillation in a Clevenger-type device for three hours. The aqueous phase was extracted with ethyl ether and the oil was dried with magnesium sulfate. The oil content was analyzed through a Hewlett-Packard 5890 gas chromatograph with flame ionization detector and HP17A (30 m x 0.25 mm x 0.25 mm) capillary column, using hydrogen as the carrier gas (1.0 mL min^{-1}) and programmed temperatures of 50ºC for five minutes to 250ºC for 30 minutes, at a rate of 15ºC per minute. The oil was also analyzed and identified by mass spectrometry in a Shimadzu GC-17A gas chromatograph attached to a Shimadzu GCMS-QP5050 selective mass detector, using a HP17A (30 m x 0.25 mm x 0.25 mm) capillary column and hydrogen as the carrier gas (1.0 mL min^{-1}) under the conditions reported above. The chemical compounds were identified by retention indices, which were obtained by coinjection of oil samples with a C11 - C24 linear hydrocarbon mixture. Mass spectrometry data were also obtained and compared with those available in the literature (Adams, 1995); furthermore, a complementary computerized comparison between the apparatus library and the literature was done. Quantitative analysis of each oil component (expressed as percentage) was carried out by peak area normalization.

Molecular analysis

DNA was extracted from leaves according to the method described by Harberer (1998) and quantified in 1.6% agarose gel, with standard solutions prepared at 2 ng μL^{-1}. RAPD reactions were amplified through the method adapted from Ferreira & Gratapagllia (1998). DNA was amplified by using the following program: 40 cycles of 1min30s at 94ºC, 50s at 94ºC, 1min at 35.5ºC, 2min at 72ºC, 10min at 72ºC, and 24h at 4ºC. The 100bp DNA Ladder (Invitrogen) was used as molecular weight standard. The amplified DNA fragments were separated in 1.6% agarose gel, with 3h migration in an electrophoresis horizontal cube. The fragments were stained with ethidium bromide (0.5 μm mL^{-1}), visualized under ultraviolet light and photographed. The studied individuals were genotyped based on the band presence. The genetic variability for each population was estimated according to the proportion of polymorphic loci (P, 0.95 criterion), the observed (Ho) and expected (He) average heterozygosity per locus and the F value (inbreeding coefficient or FIS), according to Weir & Cockerham (1984). F-statistics (Wright, 1978) were estimated in order to quantify genetic diversity levels within and among populations and to infer the degree of population subdivision. Analyses were performed by using the TFPGA software package (Miller, 1997). The similarity among genotypes was estimated through the Jaccard coefficient, and the UPGMA clustering method was performed by using the NTSYS software (Rohlf, 1997). Partitioning variability among and within populations was estimated by analysis of molecular variance (AMOVA) (Excoffier et al., 1992). Analyses were carried out by following a model to correct allele frequencies estimated based on dominant data (Lynch & Milligan, 1994).

RESULT AND DISCUSSION

E. muticus has been scarcely characterized, especially in southern Brazil, where studies have been mostly related to taxonomic descriptions (Araújo, 1971; Renvoize, 1978; Longhi-Wagner, 2001; Watson & Dallwitz, 1994). In the present study, five assessed populations showed phenotypic variability and could be differentiated based on phenotype. Of the evaluated traits, only plant height did not significantly differ among populations (Table 2). The remaining traits were significantly different, evidencing the morphological variability for populations collected in different regions. The standard deviation observed for most traits was higher than the mean values, indicating variability among individuals within each population.

Agronomy School (AS), "Morro da Polícia" (MP) and São Borja (SB) populations had the narrowest leaves (Table 2). Leaf curling variability has been reported for other species (Soster et al., 2004; Rosa et al., 2006). São Borja population presented the fastest regrowth, whereas the slowest one was detected for the population from "Morro da Polícia". The standard deviation was higher for those populations with slower regrowth (Table 2). Significant differences for regrowth have also been reported for other species (Simioni et al., 1999; Scheffer-Basso et al., 2001). The plants from São Francisco de Paula population presented the highest mean shoot fresh matter values, but did not statistically differ from those of São Borja (Table 2). However, a high phenotypic standard deviation was detected for this trait, demonstrating high variability among individuals. Total

TABLE 2. Mean and standard deviation of plant height (cm), leaf width (mm), regrowth (cm), shoot fresh matter (g) phenolic compounds (mg g^{-1}) and essential oil yield (%) of five *E. muticus* populations collected in Agronomy School (AS), "Morro da Polícia" (MP), "Morro Santana" (MS), São Borja (SB) and São Francisco de Paula (SFP).

	Plant height		Leaf width		Regrowth		Shoot fresh matter		Phenolic compounds		Essential oil yield	
Population	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
AS	77.5	19.5	1.90 b*	0.36	59.3 bc	20.5	48.8 b	22.2	9.34 a	2.05	0.70 a	0.24
MP	64.0	20.1	2.63 ab	0.38	55.3 c	16.2	48.2 b	20.0	9.81 a	3.25	0.64 a	0.24
MS	69.0	15.1	3.21 a	0.84	62.5 abc	10.0	59.8 b	38.2	9.35 a	2.19	0.74 a	0.33
SB	81.0	19.6	2.16 a	0.41	75.1 a	12.5	66.2 ab	33.7	8.21 a	2.52	0.71 a	0.18
SFP	70.0	14.7	3.49 a	0.24	71.4 b	14.6	128.0 a	53.2	5.04 b	1.48	0.33 b	0.09

* Means followed by the same letters did not significantly differ according to Tukey's test ($p<0.05$).

shoot matter is related to the plant architecture, and it is important to note that the studied populations showed no variation in height. Therefore, this difference in matter can be justified by the great leaf curling observed for São Francisco de Paula population. Similarly, the expected direct relationship between plant height and shoot fresh matter was not detected by other authors, who associated the difference in shoot fresh matter with other traits related to the lateral plant expansion (Blank et al., 2004; Bortolini et al., 2006). There was a predominance of curled leaves for Agronomy School and São Borja populations. The remaining populations presented about 20% individuals with curled leaves. All individuals from Agronomy School population and 93.3% from "Morro Santana" presented prostrate habit. In contrast, São Francisco de Paula population did not present any individual with prostrate habit, and the remaining populations had about 50%. Variability in this trait has been reported by other authors (Bortolini et al., 2006).

No population presented 100% flowering individuals. Plants from São Francisco de Paula population did not flower during the study period, and the largest number of flowering plants was observed for São Borja population (82%). San Francisco de Paula is the location of highest altitude and lowest mean temperature among all sites studied in this work. Some plants require exposure to suitable environmental conditions to flower. The main environmental factors affecting flowering are day length and temperature (Taiz & Zeiger, 2006). *E. muticus* is a grass that blooms in spring, a compliant behavior for a species that requires chilling hours during the winter to induce flowering. Therefore, since populations were analyzed in a warm environment, São Francisco de Paula population may not have received the number of chilling hours needed to induce flowering, and plants remained in the vegetative stage. Phenotype is resultant from the interaction between genotype and environment (Allard, 1960). Therefore, the environmental influence is reduced, and the observed differences in these phenotypic traits may be attributed to the effect of genotype.

The concentration of phenolic compounds varied depending on the assessed population. The lowest values were detected for São Francisco de Paula (Table 2). The remaining populations did not statistically differ and presented means around 9.4 mg g^{-1}. The obtained concentrations differed from those reported by other authors. Dzingirai et al. (2007) found a concentration of 46.8 mg g^{-1} total phenolic compounds analyzing whole *E. muticus* plants. On the other hand, the same authors detected 0.68 mg g^{-1} from the whole plant and 0.41 mg g^{-1} from the root of *E. muticus* (Spreng.) Kuntze. These data show the high variability in the phenolic compound concentration, which is dependent on the environment (Kutchan, 2001) and the plant genotype (Asami et al., 2003).

Essential oil yield was similar among the studied populations, except for that from São Francisco de Paula, which presented a lower yield (Table 2). The remaining populations had mean values around 0.70%. Similar results were reported by Silou et al. (2006) for shoot fresh matter of *E. hensii* K. Schum. The authors also assessed the oil yield of flowers and roots, which presented 1.0% and 0.4% mean yield, respectively. Other authors have reported lower oil yield for *Elionurus* species. Mevy et al. (2002) obtained 0.45% mean yield for shoot fresh matter of *E. elegans* Kunth. Hess et al. (2007) investigated differences in *E. muticus* oil yield according to the year season and observed the highest mean values during the spring (0.37%), followed by winter (0.29%), summer (0.25%) and fall (0.23%). These results were similar to those reported for São Francisco de Paula population. Variability in essential oil yield values has also been reported for other aromatic species such as *Cymbopogon* spp. Spreng. (Khanuja et al., 2005) and *Ocimum basilicum* L. (Blank et al., 2004). A preliminary chemical oil analysis identified citral for the oil from all populations, except for that from "Morro da Polícia" (Table 3).

Citral has been reported for *Elionurus* by other authors, but these compounds were more frequent in Argentina and Uruguay (Mevy et al., 2002; Cacciabua et al., 2005; Kolb et al., 2006; Sabini et al., 2006). Studies in Brazil have been concentrated in the Central-West Region and to date there are no reports on the presence of citral in the plants studied in these regions (Scramim et al., 2000; Hess et al., 2007). In the present study, the plants were collected in the state of Rio Grande do Sul, where climatic conditions are similar to those of Argentina and Uruguay because of the geographic location. These conditions (colder regions) probably favor the production of these compounds by plants.

The variations in RAPD band profiles of the 50 studied genotypes resulted in 188 consistent molecular markers for analysis. This number of bands was obtained by amplification of 16 primers that generated a mean number of 11.7 markers per primer. "Morro Santana" population had the highest percentage of polymorphic molecular markers, whereas São Francisco de Paula and Agronomy School populations presented the lowest percentages (Table 4). The genetic variation observed for *E. muticus* was high, and heterozigosity (Nei's diversity index) was also high for all populations (from 0.12 to 0.37) (Table 4). F results (from 0.027 to 0.105) indicated that the populations are panmictic.

Based on the Jaccard coefficient, the dendrogram showed that in general there was a relationship depending on the geographic origin (Figure 1). The mean similarity among all genotypes (0.33) was the cutoff point used for dendrogram analysis and indicated the formation of five groups. There was a clear subdivision in the second and largest group, which reflected its geographic origin. The cophenetic correlation coefficient was 0.85. The most similar individuals (0.85) were MP32 and MP33, from "Morro da Polícia". The relationship between genetic variability and geographic distribution has been observed for several aromatic plants such as *Hesperozygis ringens* Benth. (Fracaro & Echeverrigaray, 2006), littleseed canarygrass (*Phalaris minor* Retz.) (McRoberts et al., 2005) and *Camellia sinensis* (L.) Kuntze (Yao et al., 2008).

Analysis of molecular variance showed that the highest partitioning variability was observed within each population, among its individuals, representing 94% total variance (Table 5). The high intrapopulational variability suggests the occurrence of allogamy for the studied species. Several authors have reported high intrapopulational variability with cross-fertilization (Yao et al., 2008; Silva et al., 2006). Populations significantly differentiated from each other (FST = 0.30), indicating that there was gene flow reduction among them, which induced an increase in genetic differentiation (Table 4). High genetic differentiation in natural populations has been reported for other species (Ferreyra et al., 2004; Ross-Ibarra et al., 2008). Inbreeding coefficient (F) values were low for populations, suggesting cross-fertilization.

The studied *E. muticus* populations presented variability in agronomic and chemical traits. The population from São Francisco de Paula had the greatest differences. This could be observed in the phenotypic analysis, in which San Francisco de Paula population presented, in most cases, different values from those of other populations. Thus, such a population would not be recommended for a breeding

TABLE 3. Mean percentage of geranial and neral found in the volatile oil of five *E. muticus* populations collected in Agronomy School (AS), "Morro da Polícia" (MP), "Morro Santana" (MS), São Borja (SB) and São Francisco de Paula (SFP).

Population	Geranial (%)		Neral (%)	
AS	16.40	a	9.00	a
MP	-		-	
MS	1.06	b	0.67	b
SB	12.02	a	8.48	a
SFP	0.54	b	0.20	b

* Means followed by the same letters did not significantly differ according to Tukey's test ($p<0.05$).

TABLE 4. Genetic diversity estimates and inbreeding coefficient (F) for five *E. muticus* populations collected in Agronomy School (AS), "Morro da Polícia" (MP), "Morro Santana" (MS), São Borja (SB) and São Francisco de Paula (SFP).

Population	N	P (95%)	Ho	He	F
AS	8	30.32	0.11	0.12	0.083
MP	8	61.70	0.25	0.26	0.038
MS	17	82.98	0.36	0.37	0.027
SB	11	65.42	0.26	0.28	0.071
SFP	6	43.08	0.17	0.19	0.105
Total	50	97.87	0.40	0.40	0.000

N (sample size), P (proportion of polymorphic loci), Ho (observed heterozygosity), He (expected heterozygosity) and F (inbreeding coefficient).

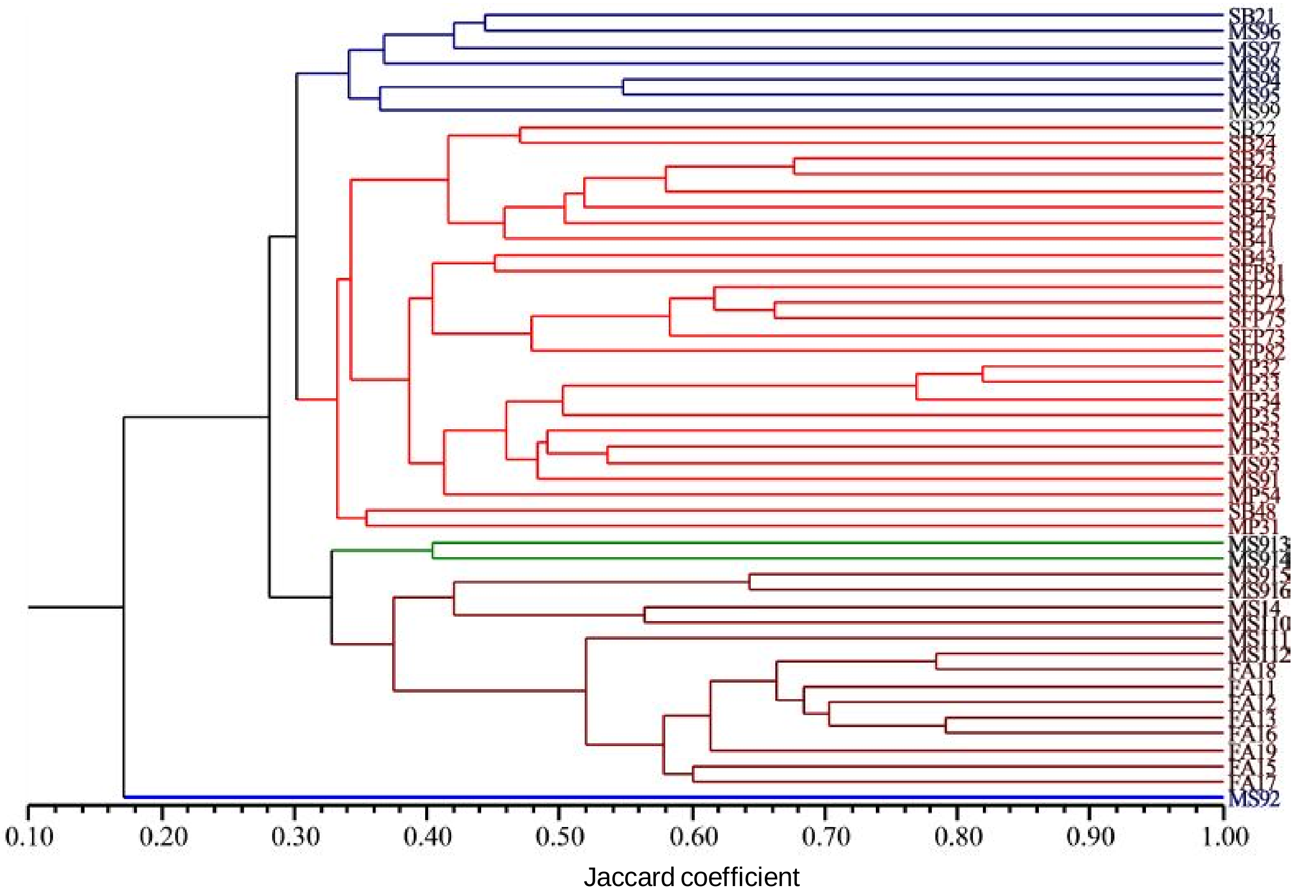

FIGURE 1. Clustering of individuals from five *E. muticus* populations collected in Agronomy School (AS), “Morro da Polícia” (MP), “Morro Santana” (MS), São Borja (SB) and São Francisco de Paula (SFP).

TABLE 5. Analysis of molecular variance (AMOVA) for five *E. muticus* populations collected in Agronomy School (AS), “Morro da Polícia” (MP), “Morro Santana” (MS), São Borja (SB) and São Francisco de Paula (SFP).

Causes of variation	Df	Mean square	Variance component	Total variance (%)
Whithin population	45	0.53	0.329	94
Among population	4	0.33	0.021	6

program. However, this was the most different population and may represent a source of variability for conservation in germplasm banks. To establish a breeding program, the breeder should concentrate efforts on São Borja and Agronomy School populations. The former presented high citral concentrations, accentuated variability and good performance for the assessed traits. Agronomy School population may also be highlighted based on the citral concentration, but it presented low variability due to a more reduced sampling.

ACKNOWLEDGMENT

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq) for financial support; Ana Carolina Nunes for the germplasm; and Drª Fernanda Bered for her valuable suggestions.

REFERENCE

ADAMS, R.P. **Identification of essential oil components by gas chromatography/mass spectroscopy**. 2.ed. Ilinois: Allured Publishing Corporation, 1995. 469p.

ALLARD, R.W. **Principles of plant breeding**. 2.ed. New York: J. Wiley, 1960. 381p.

ARAÚJO, A.A. **Principais gramíneas do Rio Grande do Sul**. 3.ed. Porto Alegre: Sulina, 1971. 255p.

ARNALDOS, T.L. et al. A. Changes in phenol content during strawberry (*Fragaria ananassa* x cv. Chandler) callus culture. **Physiologia Plantarum**, v.113, p.315-22, 2001.

ASAMI, D.K. et al. Comparison of the total phenolics and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn using convencional, organic, and suistainable agricultural practices. **Journal of Agricultural and Food Chemistry**, v.51, p.1237-41, 2003.

BLANK, A.F. et al. Caracterização morfológica e agronômica de acessos de manjericão e alfavaca. **Horticultura Brasileira,** v.22, n.1, p.113-6, 2004.

BORTOLINI, F. et al. Caracterizações morfológica e agronômica e divergência genética em germoplasma de trevo-branco. **Revista Brasileira de Zootecnia**, v.35, n.4, p.1601-10, 2006.

CACCIABUE, M. et al. *Elionurus muticus* from north of Argentina: evaluation of the antibacterial activity of three essencial oil chemotypes. **Biocell**, v.29, n.2, p.223, 2005.

CASTRO, L.O.; RAMOS, R.L.D. **Principais gramíneas produtoras de óleos essenciais**: *Cymbopogon citratus* (DC.) Stapf., capim-cidró, *Cymbopogon martinii* (Rox.) J.F. Watson, palma-rosa, *Cymbopogon nardus* (L.) Rendle, citronela, *Elyonurus candidus* (Trin.) Hack., capim-limão, *Vetiveria zizanioides* (L.) Nash, vetiver. Porto Alegre: FEPAGRO, 2002. 31p. (Boletim FEPAGRO, 11)

DZINGIRAI, B. et al. Phenolic content and phospholipids peroxidation inhibition by methanolic extracts of two medicinal plants: *Elionurus muticus* and *Hypoxis hemerocallidea*. **African Journal of Biochemistry Research**, v.1, n.7, p.137-41, 2007.

EXCOFFIER, L.; SMOUSE, P.E.; QUATTRO, J.M. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. **Genetics**, v.131, p.479-91, 1992.

FERREIRA, M.E.; GRATTAPAGLIA, D. **Introdução ao uso de marcadores moleculares em análise genética**. 3.ed. Brasília: EMBRAPA-CENARGEM, 1998. 220p.

FERREYRA, L.I. et al. First report on RAPDs patterns able to differentiate some argentinean species of section algarobia (*Prosopis*, Leguminosae). **Genetica**, v.121, n.1, p.33-42, 2004.

FRACARO, F.; ECHEVERRIGARAY, S. Genetic variability in *Hesperozygis ringens* Benth. (Lamiaceae), an endangered aromatic and medicinal plant of southern Brazil. **Biochemical Genetics**, v.44, p.479-89, 2006.

HABERER, G. et al. Mapping of the nucleolus organizer region on chromosome 4 in *Arabidopsis thaliana*. **Molecular and General Genetics**, v.250, p.123-8, 1996.

HESS, S.C. et al. Evaluation of seasonal changes in chemical composition and antibacterial activity of *Elyonurus muticus* (Sprengel) O. Kuntze (Gramineae). **Química Nova**, v.30, n.2, p.370-3, 2007.

HEYDORN, S. et al. Citral, a fragrance allergen and irritant. **Contact Dermatitis**, v.49, p.32-6, 2003.

KHANUJA, S.P.S. et al. Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon* Spreng. (Poaceae). **Biochemical Systematics and Ecology**, v.33, p.171-89, 2005.

KOLB, N. et al. **Estúdio de las condiciones para la producción de aceite esencial de "espartillo"**. Missiones: Universidad Nacional de Missiones, 2006. Available from: <http://www. unam.edu.ar/ index.php?option= com_content&task= view&id=241&Itemid=123>. Accessed on: 05 abr. 2007.

KOLB, N. et al. **Evaluación de la aptitud del espartillo para su explotación comercial.** Missiones: Universidad Nacional de Missiones, 2007. Available at: <http://www.unam.edu.ar/index.php?option=com_content&task=view&id=243&Itemid=123>. Accessed on: 05 abr. 2007.

KOSHIMA, F.A.T.; MING, L.C.; MARQUES, M.O.M. Produção de biomassa, rendimento de óleo essencial e de citral em capim-limão, *Cymbopogon citratus* (DC.) Stapf, com cobertura morta nas estações do ano. **Revista Brasileira de Plantas Medicinais**, v.8, n.4, p.112-6, 2006.

KUTCHAN, T.M. Ecological arsenal and developmental dispatcher. The paradigma of secondary metabolism. **Plant Physiology**, v.125, p.58-60, 2001.

LONGHI-WAGNER, H.M. et al. **Flora fanerogâmica do estado de São Paulo (Poaceae)**. São Paulo: Hucitec, 2001. v.1, 88p.

LYNCH, M.; MILLIGAN, B.G. Analysis of population genetic structure with RAPD markers. **Molecular Ecology**, v.3, p.91-9, 1994.

McROBERTS, N. et al. An assessment of genetic diversity within and between popualtions of *Phalaris minor* using ISSR markers. **Weed Research**, v.45, p.431-9, 2005.

MEVY, J.P. et al. Composition and some biological activities of the essential oils from an african pasture grass: *Elionurus elegans* Kunth. **Journal of Agricultural and Food Chemistry**, v.50, n.15, p.4240-3, 2002.

MILLER, M.P. **Tools for population genetic analyses (TFGPA), version 1.3:** a Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author, 1997.

NODARI, R.O.; GUERRA, M.P. Aspectos genéticos e moleculares da produção vegetal. In: SIMÕES, C.M.O. et al. **Farmacognosia:** da planta ao medicamento. 2.ed. Florianópolis: Editora da UFSC, 2000. p.25-38.

RENVOIZE, S.A. Studies in *Elionurus* (Gramineae). **Kew Bulletin**, v.32, n.3, p.666-72, 1978.

ROHLF, F.J. **Numerical taxonomy and multivariate analysis system**. 2.ed. New York: Stony Brook, 1997. 29p.

ROSA, M.S. et al. Caracterização agromorfológica interpopulacional em *Oryza glumaepatula*. **Bragantia**, v.65, n.1, p.1-10, 2006.

ROSS-IBARRA, J. et al. Patterns of polymorphism and demographic history in natural populations of *Arabidopsis lyrata*. **Plos One**, v.3, n.6, p.1-13, 2008.

SABINI, L.I. et al. Study of the citotoxic and antifungal activity of the essencial oil of *Elyonurus muticus* against *Candida* spp. **Molecular Medicinal Chemistry**, v.11, p.31-3, 2006.

SCHEFFER-BASSO, S.M. et al. Potencial de genótipos de aveia para duplo propósito. **Revista Brasileira de Agrociência**, v.7, n.1, p.22-8, 2001.

SCRAMIM, S.; SAITO, M.L. Essencial oil of *Elyonurus muticus* (Sprengel) O. Kuntze (Gramineae). **Journal of Essencial Oil Research**, v.12, p.298-300, 2000.

SILOU, T. et al. Study of essencial oil composition of *Elionurus hensii* Schum from Congo. **Journal of**

Essencial Oil Research, v.18, p.518-20, 2006.

SILVA, M.A.S. et al. Phytochemical and genetic variability of *Casearia sylvestris* Sw. from São Paulo State Atlantic Forest and Cerrado populations. **Revista Brasileira de Plantas Medicinais**, v.8, p.159-66, 2006.

SIMIONI, C. et al. Tolerância ao frio e caracterização de híbridos entre *Leucaena leucocephala* e *L. diversifolia.* **Ciência Rural**, v.29, n.3, p.453-8, 1999.

SOSTER, M.T.B. et al. Caracterização morfofisiológica de genótipos de cornichão (*Lotus corniculatus* L.). **Revista Brasileira de Zootecnia**, v.33, n.6, p.1654-61, 2004.

TAIZ, L.; ZAIGER, E. **Fisiologia vegetal**. 3.ed. Porto Alegre: Artmed, 2004. p.95-105.

WATSON, L.; DALLWITZ, M.J. **The grass for the world**. 2.ed Cambridge: CAB International, 1994. 1081p.

WEIR, B.S.; COCKERHAM, C.C .Estimating *F*-Statistics for the analysis of population structure. **Evolution**, v.38, p.1358-70, 1984

WRIGHT, S. Isolation by distance. **Genetics**, v.28, p.114-38, 1978.

YAO, M.Z. et al. Genetic diversity among tea cultivars from China, Japan and Kenya revealed by ISSR markers and its implication for prental selection in tea breeding programmes. **Plant Breeding**, v.127, p.166-72, 2008.