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Selection among and within full-sib families of elephant grass for energy purposes

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Abstract: *Pennisetum purpureum Schum. has been a key alternative as an energy source in Brazil because of its higher dry matter accumulation and fiber content. This research aimed to select superior individuals of P. purpureum for energy purposes using among-and-within family selection. The study was carried out in Campos dos Goytacazes- RJ (Brazil), using eight full-sib families. Plants were individually assessed during two pasture cuttings, one in 2014, and another in 2015. The dry matter production (DMP) was correlated with the number of tillers, stem diameter, plant height, and neutral detergent fiber content. Plant selection criteria in both cuts were through direct and indirect selections, and Smith and Hazel index. A joint analysis of variance showed significant differences for all five traits assessed in both cuts. The results achieved with Smith and Hazel index were promising for simultaneous selection of the evaluated traits, favoring selection of superior families and individuals them.*

Key words: *Pennisetum purpureum Schum, biomass, selection gain, heritability, Smith and Hazel index.*

INTRODUCTION

After the Paris COP 21 Climate Conference in 2015 (COP 21), efforts towards carbon emission and global warming reductions have been strengthened. In this sense, vegetal biomass has become a safe and sustainable energy alternative since it is renewable, and has low production cost. In addition, unlike other sources as oil or coal, this material has no contribution to the greenhouse effect as it reduces carbon dioxide emissions through absorption during photosynthesis (Anderson et al. 2008, Lee et al. 2010).

In Brazil, the interest of researchers was aroused by elephant grass. This relevance comes from being highly efficient in fixing atmospheric CO_2 during photosynthesis (Azevedo et al. 2012, Flores et al. 2012, Rossi et al. 2014). This plant is African in origin and has traits benefiting biomass quality such as fast growth, small farm plots for cultivation, high dry matter accumulation, high fiber content, and high calorific power (Daher at al. 2014).

As elephant grass cultivation has increased for energy production, a significant raise in acreage will be consistent (Cunha et al. 2013, Pereira et al. 2017); therefore, studies on genetic improvement of this species should consider new

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selection criteria (Pereira et al. 2008, Benin et al. 2013), exploring variability within species (Cavalcante and Lira 2010).

Selection of superior phenotypes of individuals or families is an important practice in breeding programs, making it feasible since improved populations are generated from selection and recombination at individual and family levels (Cruz et al. 2014, Salgado et al. 2014). In this context, among-and-within-family selection becomes a great option, mainly for considering only one character of interest, being simple to be applied. Moreover, using selection indexes consists of providing a new trait, which is a linear combination of all traits involved, whose weighting coefficients are estimated so as to maximize the correlation between the index and a genotypic aggregate (true breeding values for selection candidates) (Cruz et al. 2012). However, to date, there have been no studies involving the selection of elephant grass full-sib families using such method.

This study had, therefore, the aim to estimate and compare the predicted genetic gains by means of direct and indirect selections, as well as through a classical selection index, thus selecting the most promising families and individuals within full-sib families of elephant grass for energy purposes.

MATERIAL AND METHODS

The experiment was conducted at the Rio de Janeiro State Research Unit for Agro-Energy and Waste Exploitation (PESAGRO) (lat 21° 19’ 23’’ S, long 41º 19’ 40’’ W and alt 20 m asl), in Campos dos Goytacazes, RJ, Brazil. According to the Köppen’s classification, the local climate is an Aw type, which stands for a hot and humid tropical climate with annual rainfall of around 1152 mm. The soil is classified as a dystrophic Argisol (Ultisol) (Santos et al. 2013).

Eight elephant grass accessions donated by the germplasm bank of the State University of North Rio de Janeiro were used in the experiment. The female parental plants were IJ7139, CPAC, and IAC-Campinas, while male parental ones were Cameroon, Cubano Pinda, BAG-86, Capim cana D´Africa, and Vrukwona. These accessions were directly crossed among each other for promising hybrid combinations, selecting parents for late flowering, high dry matter production, and thick stems (Lima et al. 2011).

Crossings were performed following the method described by Silva et al. (2011). It consisted of collecting pollen grains from elephant grass genotypes (male parents), in paper bags, and then taking them to the female flowers, when inflorescence stigmas (protected by a paper bag) were receptive. There was no need for emasculation because of flower protogyny (Passos et al. 2005). The crossings were carried out between 8 and 10 in the morning, in 2013. Afterwards, seeds were sown in 128-cell trays filled with forest substrate. Field transplanting was held when seedlings reached 20 cm in height, nearly 40 days after germination, in December 2013.

The experimental design was a randomized block design with three replications; each block consisted of eight full-sib families. Only 15 seeds were obtained for each family due to a loss of seed viability. Each plot was composed of five plants spaced in 1 m between and within planting rows, according to Silva et al. (2017). The evaluations were carried out in two periods (cuts), the first made after 12 months of sowing (2014), and the second 8 months after the first (2015). For Rengsirikul et al. (2011), elephant grass dry matter production decreases after a 12-month cutting interval.

The assessed traits were correlated to the dry matter production (Menezes et al. 2014): number of tillers - NT (of every individual prior to harvest), stem diameter - SD (in cm, using three plants from each individual clump, at 10 cm above the soil with a digital caliper), plant height - PH (in m, measuring three random plants from each individual clump with a graduated ruler). The biomass quality traits were assessed at the Laboratory of Animal Science, Darcy Ribeiro State University of North Rio de Janeiro (LZO/UENF). DMP was estimated using the percentage of dry matter and the weight of tillers from individual plants, as described by Daher et al. (2014). Yet the percentage of neutral detergent fiber (%NDF) was determined as described by Mertens (2002), using a filter bag Technique (Ankom™).

These data underwent individual analysis of variance, based on the following random model: $Y_{ijk} = m + G_i + B_j + D_{ij} + E_{ijk}$; wherein: Y_{ijk}: observation in the k^{th} individual of the i^{th} full-sib family (FSF) assessed in the j^{th} block; m: overall average; G_i: random effect of the i^{th} FSF; B_j: effect of the j^{th} block; D_{ij}: random effect of the variation between plots; E_{ijk}: random effect of the variation between plants within the plot.

A combined analysis of variance was performed according to a statistical model considering each cut and each genotype as a random effect, as follows: $Y_{iklm} = m + b_{\frac{j}{k}} + G_i + C_k + (GC)_{ik} + e_{ijk} + d_{ijkl}$, wherein, m: general mean; G_i: effect of the j^{-th} FSF; C_k: effect of the k^{-th} cut; $(GC)_{ik}$: interaction effect of the j^{-th} FSF with the k^{-th} cut; e_{ijk}: effect of experimental error of the plots, d_{ijkl}: deviation inherent to plant l of FSF i, in replication j at cut k. Considering the model for combined analysis of randomized blocks, the expected mean squares [E (MS)] were based on Cruz et al. (2014).

To select the traits to be used in the selection index, a multicollinearity diagnosis was performed among them. For this, a matrix correlation method was used, resulting in a weak collinearity. All assessed traits had a condition number of 7 (CN - the ratio between the largest and smallest eigenvalue of the correlation matrix). According to Cruz et al. (2014), collinearity is classified as weak when CN < 100.

According to Cruz et al. (2014), gain estimates are seen in a direct selection based on the estimator: $GS_x = DS_x h^2_x$; wherein: GS_x is the direct gain predicted in the variable X; DS_x is the selection variable differential X, and h^2_x is the heritability coefficient of the variable X, in a broad sense. Selection gain rates were determined by the following expression: $[(GS)]_{\downarrow}x\% = [(GS)]_{\downarrow}x*100)/X_{\downarrow}0$.

The gain estimates obtained by indirect selection were calculated according to the following estimator: $GS_{y(x)} = DS_{y(x)} h^e_y$; wherein: $GS_{y(x)}$ is the selection gain in Y by selecting the variable X; $DS_{y(x)}$ is the differential of indirect selection, in which the average of selected ones is obtained as a function of the progenies, which show superiority for the auxiliary variable X, and h^e_y is the heritability coefficient of the main variable Y. The rate of selection gain was estimated according to the following expression: $GS_{y(x)}\% = \frac{GS_{x(y)}*100}{\square} X_0$.

We used the classical index proposed by Smith (1936) and Hazel (1943). In these indexes both the selection index (I) and the genotypic aggregate (H) are described by: $I = b_1x_1 + b_2x_2 + ... + b_nx_n = \sum_{i=1}^{n} b_ix_i = b'x$ and $H = a_1g_1 + a_2g_2 + ... + a_ng_n = \sum_{i=1}^{n} a_ig_i = a'g$; wherein: n is the number of evaluated traits; b' is the vector (1 x n) of the weighting coefficients of the selection index to be estimated; x is the matrix (n x p) of trait means; a', is the vector (1 x n) of previously established economic weights; and g is the matrix (n x p) of unknown genetic values of the n traits considered. The indexes were established by the following equation system: $Pb = Ga$; wherein: P is the matrix of phenotypical covariance; G is the matrix of phenotypical covariance; a is the vector of economic weights, and b is the vector of coefficients of the selection index.

When using a classical index, the genetic variation coefficient (CVg), genetic standard deviation (SD) and random weights of each trait were considered as economic weights. For both cuts, the random weights were 100, 90, 90, 80, and 100 for the variables DMP, NT, PH, SD, and NDF, respectively. The selection intensity applicable among individuals was 15%, corresponding to 18 selected individuals. All statistical analyses were performed using the Genes software (Cruz et al. 2013).

RESULTS AND DISCUSSION

While DMP and NT presented significant differences among families in both cuts, SD and NDF showed significant differences only in the second cut (Table 1). Such difference points out to a genetic variability among FSFs, besides a potential for selection. This fact confirms this species diversity for biomass production. Likewise, Daher et al. (2014) had observed variability among 16 hybrids concerning the same agronomic traits related to energy purposes.

At the family level, the heritability coefficient exceeded those obtained at the individual level within a family for all measured traits (Table 1). The highest estimates were observed for traits in the second cut. These results can be explained by a great unevenness in the first cut evaluations, which decreases with the course of crop establishment in the field. NT and NDF reached estimates of 0.95 and 0.86, respectively, which indicates high perspectives of genetic gain by selection. However, the estimates for heritability within families ranged from 19 to 74%.

Rosado et al. (2009) claimed that, when considering the same selection intensity, averages of families should be more efficient than the within-family ones. In this case, both among and within selections can be combined to explore variability properly, raising the total genetic gain. It is noteworthy that all negative heritability was regarded as null (zero). PH showed a heritability equal to zero, which, as stated by Jung et al. (2008), features a low genetic variance (Table 1).

The joint analysis of variance showed significant differences among families for all traits (Table 2). These variations indicate good prospects for selections among families and for continuity in the elephant grass genetic breeding program. The effects of family x cut interactions and of the cut itself were significant for all traits except for DMP and SD, and DMP, respectively. Conversely, significant interactions indicate that the families showed distinct responses in the different sections. Such a result can be explained by the long periods through which evaluations were carried out; thus, the families were longer exposed to edaphic and climatic variations, especially irregular precipitation. Nevertheless, there were no significant differences for dry matter production, which had a greater participation in the genotype variance than did the environmental range.

Comparing Tables 3 and 4, we observed for most of the assessed traits that direct gains by selection among-and-within-family were greater than were the indirect ones. Direct selection among-and-within-family provided total gains as expected for the first cut concerning DMP (5.74), NT (5.27), and for the second cut for DMP (6.42) and NT (5.08). It is worthy highlighting that, in the present study, evaluations occurred at different plant ages; therefore, different sets of genes could be responsible for the phenotypic expression of genotype at each plant age.

Nevertheless, in certain cases, it must be highlighted here that indirect gains exceeded direct ones in both cuts, regarding SD (2.57 and 8.57) and NDF (11.7 and 15.5), as shown in Table 4. This finding becomes possible whether heritability of an auxiliary trait is greater than the target one (under selection), and when the genetic correlation between them is of major magnitude. In contrast, indirect gains for selection of PH were low, and for some other variables, were negative. Therefore, performing an indirect selection for one variable to obtain a gain in another one is unfeasible, since there might be a loss in the first one (Verardi et al. 2009).

Based on the classical index, predicted gains were maximized for all traits when it was used genetic variation coefficient, standard deviation, and heritability coefficient at the family level, as economic weights (Table 5). Teixeira et al. (2012) evaluated 25 progenies of açaí palm and observed small fruit production gains when the genetic variation

Table 1. Summary of the variance analysis of dry matter production (DMP), number of tillers (NT), stem diameter (SD), plant height (PH), and neutral detergent fiber (NDF) for full-sib families of elephant grass in two harvest cuts (C)

		DMP ($t\ ha^{-1}\ year^{-1}$)		**NT** (tillers)		**SD** (mm)		**PH** (m)		**NDF** (%)	
SV	**df**	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
Block	2	40.10	141.10	264.07	60.31	9.07	20.48	0.97	0.49	25.00	40.80
Families	7	1562.50*	1717.80**	1175.80**	1030.20*	3.28	55.70**	0.55	2.79	88.50	218.00**
Among	14	512.40	273.47	131.41	117.21	7.80	8.61	0.48	0.53	41.80	28.80
Within	96	355.90	370.11	138.55	125.55	4.39	2.16	0.22	0.17	29.60	28.30
Mean		59.20	57.07	31.79	26.52	14.99	12.35	3.03	3.49	68.40	77.00
CV (%)		17.08	12.95	16.12	11.58	8.33	10.62	10.20	9.36	2.42	3.12
h^2_m		0.67	0.84	0.88	0.95	0	0.84	0	0	0	0.86
h^2_d		0.19	0.26	0.50	0.52	0	0.74	0	0	0	0.52

** and * significant at 1 and 5% probability level by the F-test, respectively. Environmental variation coefficient (CV%), mean progeny heritability (h^2_m), heritability at plot level (among plants within families) (h^2_d).

Table 2. Summary of the variance analysis of dry matter production (DMP), number of tillers (NT), stem diameter (SD), plant height (PH), and neutral detergent fiber (NDF) for full-sib families of elephant grass in two harvest cuts (C)

		Mean Squares				
SV	**df**	**DMP** ($t\ ha^{-1}\ year^{-1}$)	**NT** (tillers)	**PH** (m)	**SD** (mm)	**NDF** (%)
Blocks	2	17.12	253.12	0.80	11.63	62.68
Families (F)	7	2958.10**	2119.24*	2.68*	40.80**	200.23*
Error A	14	642.12	136.68	0.83	6.85	51.20
Cut (C)	1	289.23	1664.37**	12.71 **	419.44**	4427.11**
Error B	16	146.35	45.61	0.25	10.62	17.53
F x C	7	322.24	86.86**	0.660*	18.26	107.09*
Residue Within	192	363.03	132.05	0.20	3.28	19.00

** and * significant at 1 and 5% probability level by the F test, respectively.

Table 3. Predicted gains by direct selection for dry matter production (DMP), number of tillers (NT), stem diameter (SD), plant height (PH), and neutral detergent fiber (NDF) in full-sib families of elephant grass in two harvest cuts (C)

	DMP ($t\ ha^{-1}\ year^{-1}$)		**NT** (tillers)		**PH** (m)		**SD** (mm)		**NDF** (%)	
Estimates	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
X_o	59.2	57.07	31.79	26.52	3.03	3.49	14.49	12.35	68.42	77.01
X_s	67.8	64.71	37.73	31.85	3.17	3.74	15.02	12.95	69.6	78.81
h_2	67.2	84.08	88.82	95.41	12.42	80.82	-137.9	84.56	52.67	86.79
SG_t	5.74	6.42	5.27	5.08	0.01	0.2	-0.03	0.5	0.62	1.55
SG (%)	9.69	11.26	16.6	19.15	0.58	5.83	-0.25	4.08	0.91	2.02

X_o and X_s: original mean and mean of the selected individuals, heritability (h^2), selection gains (SG).

Table 4. Predicted gains (SG %) by among (SGa) and within (SGw) selection criterion, and total gains (SGt) by indirect selection of dry matter production (DMP), number of tillers (NT), stem diameter (SD), plant height (PH), and neutral detergent fiber (NDF) for full-sib families of elephant grass in two harvest cuts (C)

Trait		**DMP** ($t\ ha^{-1}\ year^{-1}$)		**NT** (tillers)		**PH** (m)		**SD** (mm)		**NDF** (%)	
	SG (%)	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
DMP	SG_a	-	-	4.73	4.39	0.11	0.26	0.11	0.86	0.86	2.09
($t\ ha^{-1} year^{-1}$)	SG_w	-	-	5.64	5.46	0.13	0.32	0.13	5.07	1.02	2.6
	SG_t	-	-	10.4	9.85	0.24	0.58	0.24	5.93	1.88	4.69
NT	SG_a	4.52	5.37	-	-	0.065	0.37	0.04	0.39	0.365	1.32
(tillers)	SG_w	5.92	9.6	-	-	0.086	0.2	0.58	0.7	0.478	2.37
	SG_t	10.4	15	-	-	0.151	0.57	0.62	1.09	0.843	3.69
PH	SG_a	5.92	5.34	3.49	3.5	-	-	-0.1	0.81	0.69	1.75
(m)	SG_w	0.96	5.62	0.56	3.68	-	-	0	0.85	0.11	1.84
	SG_t	6.88	11	4.05	7.18	-	-	-0.1	1.66	0.8	3.59
SD	SG_a	2.17	3.58	0.89	11.3	-0.05	0.16	-	-	-0.19	1.64
(mm)	SG_w	0.4	4.99	0.16	1.86	-0.01	0.22	-	-	-0.03	2.29
	SG_t	2.57	8.57	1.05	13.2	-0.06	0.38	-	-	-0.22	3.93
NDF	SG_a	5.74	4.75	4.73	2.27	0.11	0.19	0.11	0.9	-	-
(%)	SG_w	5.92	10.8	7.15	5.59	0.086	0.44	0.06	2.04	-	-
	SG_t	11.7	15.5	11.9	7.86	0.196	0.63	0.17	2.94	-	-

coefficient and average progeny heritability were used as economic weights. By using selection intensity indexes of 15% within FSF, direct gains were observed only for DMP and NT in both cuts (Table 5). This result was already expected since the selection was carried out giving priority to traits with high heritability coefficients and genetic variance, which are considered most important.

Taking into account the Smith and Hazel index, in which standard deviation (SD) was used as an economic weight, we observed higher gains for NT in the first and second cuts (47.66% and 76.99%) (Table 5), being higher than those of direct and indirect selection for the same trait. Likewise, the same result was reported when using families of other crops such as popcorn (Amaral Junior et al. 2010, Freitas et al. 2013) and wheat (Cargnin et al. 2007). Moreover, Costa et al. (2008) reported high genetic gains by selection index in 32 soybean families using SD as economic weight.

In both cuts, advantageous results were provided to the selection process when it was used random weights (RA) as economic weight, reaching positive gains for SD (0.21% and 0.32%), also bringing superior results for NDF (2.16% and 2.38%) and PH (5.75% and 6.19%). Furthermore, selection index promoted most satisfactory estimates and greater genetic gains for SD, NDF, and PH if compared to direct and indirect selections (Table 5). Costa et al. (2008), performing an among-and-within-FSF selection in soybeans, observed direct gains similar to those obtained by selection indexes, as observed in our study.

Although poorly differentiated, the results attained using different economic weights for Smith and Hazel index showed to be advantageous if compared to direct and indirect selections, since the predicted gains reached by those were higher

Table 5. Predicted selection gains (SG%) by the classical selection index of Smith & Hazel (SH) for dry matter production (DMP), number of tillers (NT), stem diameter (SD), plant height (PH), and neutral detergent fiber (NDF) in full-sib families of elephant grass in two harvest cuts (C)

	SG C_1			SG C_2		
Trait	**SD**	**CVg**	**RW**	**SD**	**CVg**	**RW**
DMP (T $ha^{-1}year^{-1}$)	25.38	25.09	24.78	35.53	30.71	31.9
NT (tillers)	47.66	49.95	45.85	76.99	71.43	68.12
PH (m)	4.33	5.23	5.75	5.07	5.52	6.19
SD (mm)	-0.52	-0.248	0.21	-0.89	-0.28	0.32
NDF (%)	1.71	1.69	2.16	2.04	1.76	2.38

Standard deviation (SD), genetic variation coefficient (CVg), and random weights (RW) as economic weight.

Table 6. Selection of families and individuals by direct and indirect selection methods and by Smith and Hazel index in two harvest cuts (C)

	Direct and indirect selections				Smith and Hazel index			
	C_1		C_2		C_1		C_2	
Order	**Family**	**Individual**	**Family**	**Individual**	**Family**	**Individual**	**Family**	**Individual**
1	1	4	1	4	1	4	1	4
2	1	5	1	5	1	6	1	5
3	1	6	1	6	1	7	1	6
4	1	7	1	7	1	11	1	7
5	1	9	1	9	1	13	1	12
6	1	12	1	11	1	14	2	29
7	1	14	1	14	1	15	4	46
8	2	23	1	15	3	45	4	51
9	3	36	2	18	4	49	4	53
10	3	39	3	32	4	51	4	55
11	3	40	3	34	4	52	5	62
12	3	45	3	44	4	55	6	84
13	4	52	3	45	6	81	6	86
14	4	55	4	48	6	87	7	91
15	4	59	4	49	7	93	7	93
16	6	81	4	51	7	94	7	94
17	6	82	4	52	7	95	7	100
18	6	83	4	53	7	97	7	101

than the latter for all assessed traits. The differences in all traits between cuttings can be explained by environmental changes from one year to the other, where the better conditions in the second year might have favored a larger tillering.

Among the evaluated families, family 1 (Table 6) stood out for its better genetic gains. Within this family, the individuals 4, 6, and 7 were considered the most thriving ones for both cuts. Hence, these individuals should be highlighted as promising in terms of biomass production for superior genotype selection.

CONCLUSIONS

The gains predicted by the classic index of Smith and Hazel were higher for all the assessed traits, especially for dry matter production.

Stem diameter showed major gains through indirect selection by means of dry matter production and the number of tillers.

Family 1 and its individuals 4, 6, and 7 were pointed out as the most promising ones for energy purposes when assessing selection among- and within-families using the Smith and Hazel index.

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