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CROP BREEDING AND
APPLIED BIOTECHNOLOGY

Multiple Centroid Methodology to analyze genotype adaptability

Moysés Nascimento[1*], Adésio Ferreira[2], Ana Carolina Mota Campana[1], Caio Césio Salgado[3], and Cosme Damião Cruz[3]

ABSTRACT – *This study was carried out to propose a modification of the centroid method. The method is modified in the choice of the ideotype, to be defined according to the researcher's interest, using the bi-segmented regression model, rather than based on experimental data, which limit the comparison of genotypes. To illustrate the method, one trait was simulated in 25 genotypes in 8 environments. For the simulations and statistical analyses the software package GENES was used. The multiple centroid is more flexible than the original centroid method, since it can be used according to the researcher's objective and the desired recommendation strategy. It is also readily interpretable for recommendation and unambiguous. Besides, the number of genotypes of interest can be compared, making a detailed study possible, by separating the genotypes into recommendation classes.*

Key words: Principal components, genotype-environment interaction, statistical analysis.

INTRODUCTION

Knowledge on the components of the genotype-environment (GxE) interaction is of great importance for genetic breeding but provides no detailed information on the performance of each cultivar under varying environmental conditions (Cruz et al. 2004). The analyses of adaptability and stability are therefore extremely important and necessary for the identification and recommendation of superior genotypes in different environments.

There are several methodologies of adaptability and stability analysis to study and quantify the GxE interaction. They differ from the widely used stability concepts and certain statistical principles (Cruz et al. 2004). As an example, it is possible to mention the methods based on: variance analysis: Plaisted and Peterson (1959) and Annicchiarico (1992); simple linear regression: Finlay and Wilkinson (1963) and Eberhart and Russell (1966); multiple linear regression: Verma et al. (1978) and Cruz et al. (1989); and nonparametric analyses: Lin and Binns (1988) and centroid analysis (Rocha et al. 2005).

An example of the use of these methodologies is the study of Silva and Duarte (2006), who used 11 different adaptability and stability methods to investigate the phenotypical adaptability in soybean. Mohebodoni et al. (2006) also used several methods in

[1] Universidade Federal de Viçosa, Departamento de Informática, Área de Estatística, Av. P. H. Rolfs, s/nº, 36571-000 Viçosa, MG, Brazil. *E-mail: moysesnascim@vicosa.ufv.br
[2] Universidade Federal de Viçosa, Campus Rio Paranaíba, Rodovia BR 254 – km 310, 38810-000, Rio Paranaíba, MG, Brazil
[3] Universidade Federal de Viçosa, Departamento de Biologia Geral, Av. P. H. Rolfs, s/nº, 36571-000 Viçosa, MG, Brazil

a detailed study on the GxE interaction in 11 lentil (*Lens culinaris* Medik) genotypes. Mahammed and Amri (2007) compared 20 parametric and non-parametric methods in the selection of durum wheat genotypes evaluated in different environments.

To facilitate the recommendation of a set of cultivars, the centroid method compares the Cartesian distance values between genotypes and four ideal references (ideotypes), which are created based on the experimental data. They represent genotypes with maximum general adaptability; with maximum specific adaptability to favorable or unfavorable environments; and with minimal adaptability (Rocha et al. 2005). However, there are certain restrictions to the use of this method in the evaluation of genotypes, since the ideotypes are created based on experimental data and the definition of the ideotypes may exclude essential comparisons of great interest for study purposes.

The centroid method is being used in studies of adaptability. For example, in the study of Rocha et al. (2005), focused on the environmental adaptability of *Eucalyptus grandis* clones, 74-month-old trees, representing 25 genotypes from clonal tests, were planted and evaluated in four environments. Ferreira et al. (2006) used the method of adaptability and stability analysis for common bean (*Phaseolus vulgaris*) under organic management. Altoé (2007) also evaluated the adaptability and stability of common bean under organic management in 20 environments, with different doses of organic fertilizers in different growing seasons. Besides the above-mentioned, an example of the use of this method is the study of Marchiori (2008), who evaluated the adaptability and stability of 20 soybean genotypes.

This study has the purpose of proposing a modification to the centroid method, by including the choice of the ideotype (ideal references), defined according to the researcher's interest, based on the bi-segmented regression model instead of experimental data. This makes the method more flexible and suitable, since researchers can make direct comparisons with respect to the focus of the study of interest.

MATERIAL AND METHODS

To illustrate the proposed method, the values of 25 genotypes were simulated in eight environments, using the program Genes (Cruz 2006). This procedure simulates experimental data spreadsheets, which represent the evaluation of a set of genotypes arranged in a randomized block design. Eight variables were simulated and considered environments; the means, variation coefficients and heritabilities are presented in Table 1. The software is based on the assumption that the block mean is 1.5 times higher than the mean-square error.

This way, starting from the CV value provided by the user, the value of the block mean square (BMS) can be estimated. With these mean squares (BMS and MSE), the variability component, associated with the fixed block effect (ϕ_b) is estimated by

$$\phi_b = \frac{BMS - MSE}{g}$$

where g is the number of simulated genotypes.

Knowing that a set contains n values, in arithmetic progression, with ratio r and mean $\overline{X}$, where the first term is denoted by X_1 and the last by X_n, the variance is given by

$$S^2 = \frac{n(n+1)}{3(n-1)^2}(X_n - \overline{X})^2$$

To estimate the block effects, the existence of b fixed effects is therefore admitted. These values represent an arithmetic progression of ratio r, with the specific aspect that $B_1 = -B_b$ and $\overline{B} = 0$. Then B_b is estimated by the expression:

$$B_b = \frac{(n-1)\sqrt{3\phi_b}}{\sqrt{n(n-1)}}$$

and the other effects are established considering the arithmetic progression ratio given by:

$$r = \frac{B_b - B_1}{b-1}$$

To estimate the genotype effects, the genetic variance must be known, which is computed based on information on the heritability (H^2) and experimental variation coefficient (CVe). The environmental variation must therefore be determined as

$$\sigma^2 = \left(\frac{\mu CV_e}{100}\right)^2$$

Table 1. Mean, coefficient of variation (CV %) and heritability (H²) values used for the simulation of 25 genotypes in 8 environments, to illustrate the modified centroid method and analyses by the original centroid and Lin and Binns methods

Parameters	Environments							
	1	2	3	4	5	6	7	8
Mean	3100,000	3300,000	3500,000	3600,000	3650,000	3700,000	3680,000	3750,000
CV (%)	10	10	10	10	10	10	10	10
H²(%)	80	80	80	80	80	80	80	80

It is known that:

$$H^2 = \frac{100\sigma_g^2}{\sigma_g^2 + \frac{1}{b}\sigma^2}$$

Then

$$\sigma_g^2 = \frac{\sigma^2 H^2}{b(100 - H^2)}$$

Therefore, random genotype effects are established, considering that $G_i \sim NID(0,\sigma_g^2)$. Since the value of σ^2_g is known, the effects are estimated using the random function described above.

The random error effects, in turn, are obtained considering $G_i \sim NID(0,\sigma_g^2)$. The value of σ^2 is known, based on the value of the experimental variation coefficient and the trait mean provided by the user. This way, the random and independent errors are estimated using the random function described above.

Based on the mean value and the effects involved, the phenotypic values are determined according to the equation

$$Y_{ij} = \mu + G_i + B_j + \varepsilon_{ij}$$

The values established for the 25 genotypes in 8 environments were also analyzed by the methods of Lin and Binns (1988) and centroid (Rocha et al. 2005).

The original centroid method uses a comparison of the Cartesian distance values between the genotypes and four ideal references (ideotypes), created based on experimental data to represent genotypes with maximum general adaptability, with maximum specific adaptability to favorable or unfavorable environments and genotypes with minimum adaptability. The ideotype with maximum general adaptability represents the maximum values observed for all environments studied (ideotype I). The ideotypes with maximum specific adaptability are those with the maximum response in

favorable environments and minimum response to unfavorable environments (ideotype II) or maximum response in unfavorable environments and minimum in favorable environments (ideotype III). The ideotype with minimum adaptability represents the lowest values observed in all environments studied (ideotype IV).

To use the centroid method, the environments are classified into favorable and unfavorable, using the environmental index, as proposed by Finlay and Wilkinson (1963):

$$I_j = \frac{1}{g}\sum_i Y_{ij} - \frac{1}{ag}Y_{..}$$

where,

Y_{ij} - is the mean of genotype^i, in environment^j;
$Y_{..}$ - is the total of the observations;
a - is the number of environments;
g - is the number of genotypes.

After the environment classification, referential points representative of the ideotypes are created with differentiated responses to favorable and unfavorable environments, aiming at the classification of other points of teh diagram considering the Cartesian distance values between the points of each one of the four ideotypes. It is possible to calculate a measure of spatial probability, using the inverted distance from a treatment to the four ideotypes:

$$P_{d(i,j)} = \frac{\left(\frac{1}{d_i}\right)}{\sum_i \frac{1}{d_i}}$$

where,

$P_{d(i,j)}$ - is the probability that a stability pattern is similar to the th centroid and;
d_i - is the distance from the th point to the th centroid.

The methodology proposed here modifies the original centroid method so that the cultivars are compared with ideotypes of real interest for the researcher, rather than ideotypes generated from the experimental data, which also allows the researcher to choose the desired concept. The methodology of the original centroid differs from the traditional concept, which considers a genotype with high yield capacity, low response to unfavorable environments and responsive to favorable environments the ideal. It is therefore suggested that the hypothetical ideal or referential cultivar be defined based on the bi-segmented regression model, whose parameters are the mean (β_{0i}) and the linear response to the unfavorable (β_{1i}) and favorable environments ($\beta_{1i} + \beta_{2i}$) (Cruz et al. 1989).

Using the model:

where,

$Y_{ij} : \beta_{0i} + \beta_{1i}I_j + \beta_{2i}T(I_j)$

Y_{ij} : ideal response of the hypothetical genotype in environment;

β_{0i} : value established so that the ideal response be maximum for all locations;

I_j : index of codified environment;

$T(I_j) = 0$ if $I_j < 0$; and

$T(I_j) = I_j - \bar{I}_+$ if, $I_j > 0$, being the $\bar{I}_+$ mean of the positive (I_j) indices.

The parameter values of the regression model are defined based on the researchers' interest. If the interest is to find the ideal genotype described in the traditional concept, it is recommended to establish the values as $I_j (\beta_{1i} < 1)$ and $(\beta_{1i} + \beta_{2i} > 1)$, indicating low response to unfavorable environments and high response to favorable environments, respectively. It would be advisable to attribute values to β_{0i} that lie between the maximum and the minimum of the variable throughout the experiment, making it possible to study with mean extracts. For example, if the interest is focused on the least values of the variable analyzed β_{0i}, is given the lowest value of the variable in the experiment and the regression coefficients of the unfavorable and favorable environments is given value zero. When the interest is focused on a fixed value, as in the case of plant height, this value is given to β_{0i}. Again, the zero value will be assigned to regression coefficients in the unfavorable and favorable environments.

After the creation of the ideotypes of interest, the analysis is performed, as in the original centroid method.

The simulations and statistical analyses were carried out using the GENES program (2006).

RESULTS AND DISCUSSION

The variation coefficient observed for the simulated variable was 9.94. The joint variance analysis indicated the existence of significant genetic variance among the genotypes, significant difference among the environments evaluated and a significant genotype-environment interaction. In all cases, the significance was verified by the F test at 1% probability for the simulated variable (Table 2).

The presence of significant genotype-environment interaction demonstrates a different performance of the genotypes in the environments studied, justifying the need for the use of adaptability and stability techniques.

To exemplify the use of the multiple centroid method, nine ideotypes were created based on the bi-segmented regression model, which were:

I. $Y_{ij} = 300;$
II. $Y_{ij} = 3000 + I_j;$
III. $Y_{ij} = 3000 + 0.5I_j + T(I_j);$
IV. $Y_{ij} = 3500;$
V. $Y_{ij} = 3500 + I_j;$
VI. $Y_{ij} = 3500 + 0.5I_j + T(I_j);$
VII. $Y_{ij} = 4000;$
VIII. $Y_{ij} = 4000 + I_j;$
IX. $Y_{ij} = 3500 + 0.5I_j + T(I_j);$

The ideotypes I, IV and VII can be seen as genotypes that do not vary according to the improvement of the environments; they also form three distinct "classes" within the set of genotypes, which are groups with low, medium and high means, respectively. It was observed that, if the study focuses on genotypes with low means, it would be indicated to select the ones classified as I, if the interest is a stable genotype with high mean also, the ones classified as VII would be indicated.

Table 2. Variance analysis of a factorial experiment in eight environments for the simulated variable

Sources of Variation	DF	MSE
Blocks/Environments	24	173948.060
Genotype	27	3290066.740**
Environments	7	5108570.300**
Genotype-environment	168	470063.440**
Residue	576	128196.250
Mean		3535.000
CV		10.130

Where: ** significant at 1%.

Ideotypes were also defined, which represent the ideal genotype as described by Eberhart and Russell (1966). In other words, genotypes responsive to environmental variations, or mathematically, and, which are represented by the ideotypes II, V and VIII. Besides the above, there is also the concept of the ideal genotype as proposed by Verma et al. (1978) and Cruz et al. (1989), which is little responsive to unfavorable environments and responsive to favorable environments, namely, and, defined by III, VI and IX. These genotypes, as well as their "classes" are shown in Figure 1.
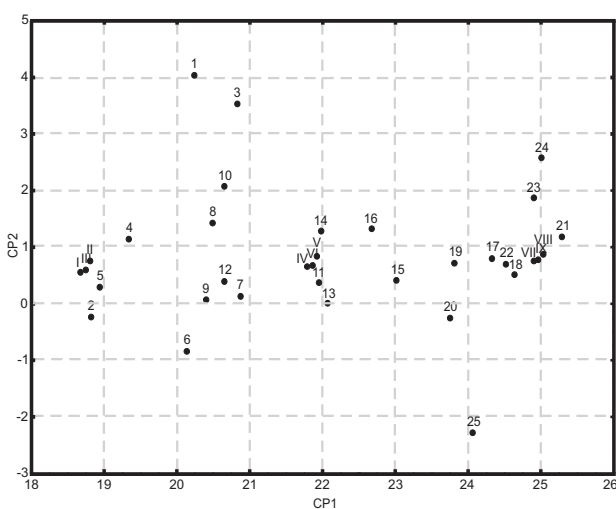
Figure 1. Dispersion diagram of the first two main components of 25 genotypes of the simulated variable response in eight environments. The nine points with Roman numbers represent the centroids

A visual analysis of the diagram of the main components shows the existence of three different classes, as described, and the ideotypes of interest are compared within these "classes". The classification of the genotypes was also compared with the defined centroids (Table 3).

Analogously to the original method, the method os adaptability of genotypes is readily analyzed and interpreted. It can be seen that within the first "class", genotypes 2 and 4 were classified as little adapted, namely, stable and with low mean; genotype 9 was classified as ideal genotype, as characterized by Eberhart and Russell (1966), and genotypes 5 and 6 were classified as ideal by Verma et al. (1978) and Cruz et al. (1989) (Figure 1 and Table 3). However, if breeders are interested in high means for a certain trait, these genotypes can be discarded. In the second "class", genotype 1 was little adapted, and the genotypes 3, 8, 10, 11, 12, 13, 14, 15 and 16 may be considered ideal, according to Eberhart and Russell (1966) and 7 is ideal by Verma et al. (1978) and Cruz et al. (1989). In the third "class" no genotype was classified as invariant with high mean; the genotypes 17, 18, 19, 20, 21, 22, 23 and 24 were defined as ideal by Eberhart and Russell (1966) and genotype 25 as ideal by Verma et al. (1978) and Cruz et al. (1989). By this method, a direct classification is possible, easy interpretable, according to the study interest.

The genotypes were classified according to the original centroid method (Table 4). The classification by the original centroid method is much more restricted than the method proposed, since only four centroids are defined based on the experimental data. A comparison with the genotype of real interest in the study is therefore not possible. The ciompáriason also showed that the original centroid method allocates 10 genotypes as non-adapted, causing the elimination of genotypes that could be of interest for the study purpose Another advantage over the original method

Table 3. Classification of the genotypes in one of the nine groups characterized by the centroids, by the bi-segmented regression model and probability associated to its classification

Gen.	Mean	Classif.	Probability								
			(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)
1	3303.542	IV	0.125	0.111	0.118	0.132	0.116	0.124	0.094	0.088	0.091
2	3048.160	I	0.184	0.142	0.163	0.114	0.101	0.108	0.064	0.061	0.063
3	3321.560	V	0.110	0.126	0.116	0.118	0.139	0.126	0.085	0.092	0.088
4	3105.049	I	0.206	0.150	0.176	0.109	0.098	0.104	0.053	0.052	0.053
5	3030.411	III	0.190	0.221	0.239	0.076	0.078	0.079	0.039	0.040	0.040
6	3230.654	III	0.138	0.133	0.141	0.130	0.127	0.133	0.066	0.066	0.067
7	3325.071	VI	0.110	0.114	0.119	0.145	0.154	0.167	0.063	0.064	0.065
8	3278.558	V	0.121	0.136	0.127	0.135	0.157	0.143	0.060	0.061	0.060
9	3235.931	II	0.122	0.155	0.144	0.117	0.145	0.136	0.059	0.061	0.061
10	3298.295	V	0.107	0.136	0.123	0.121	0.168	0.144	0.064	0.069	0.067
11	3502.483	V	0.063	0.068	0.067	0.139	0.252	0.214	0.063	0.068	0.067
12	3291.184	V	0.110	0.139	0.123	0.121	0.164	0.140	0.065	0.070	0.068
13	3523.946	V	0.072	0.078	0.077	0.138	0.205	0.188	0.077	0.084	0.083
14	3504.393	V	0.065	0.073	0.069	0.127	0.282	0.176	0.065	0.074	0.070
15	3671.949	V	0.056	0.059	0.058	0.127	0.206	0.170	0.095	0.118	0.110
16	3604.413	V	0.073	0.083	0.078	0.121	0.190	0.147	0.092	0.114	0.103
17	3891.496	VIII	0.041	0.042	0.042	0.083	0.094	0.092	0.145	0.245	0.216
18	3922.173	VIII	0.046	0.048	0.047	0.084	0.099	0.094	0.135	0.254	0.193
19	3798.135	VIII	0.055	0.058	0.057	0.107	0.139	0.125	0.124	0.183	0.154
20	3778.588	VIII	0.061	0.062	0.062	0.120	0.135	0.132	0.130	0.151	0.147
21	4029.039	VIII	0.043	0.044	0.044	0.076	0.083	0.080	0.151	0.278	0.201
22	3920.850	VIII	0.053	0.055	0.055	0.094	0.106	0.106	0.142	0.197	0.192
23	3979.603	VIII	0.046	0.047	0.046	0.083	0.091	0.088	0.157	0.245	0.198
24	3963.833	VIII	0.069	0.073	0.071	0.103	0.121	0.112	0.129	0.173	0.150
25	3815.680	IX	0.078	0.079	0.080	0.118	0.123	0.124	0.128	0.134	0.136

Gen. – Genotypes; Classif. – Classification

Table 4. Classification of the genotypes in one of the four groups characterized by the centroids, defined by the experimental data based on the original centroid method, and the probability associated to the classification

Genotypes	Mean	Classification	Prob(I)	Prob(II)	Prob(III)	Prob(IV)
1	3303.542	III	0.196	0.170	0.381	0.254
2	3048.160	IV	0.150	0.153	0.331	0.367
3	3321.560	IV	0.222	0.260	0.236	0.283
4	3105.049	IV	0.166	0.176	0.294	0.364
5	3030.4101	IV	0.149	0.175	0.237	0.440
6	3230.654	IV	0.191	0.204	0.279	0.326
7	3325.071	IV	0.217	0.232	0.262	0.289
8	3278.558	IV	0.201	0.220	0.264	0.315
9	3235.931	IV	0.196	0.230	0.246	0.328
10	3298.295	IV	0.208	0.247	0.240	0.305
11	3502.483	I	0.260	0.251	0.248	0.241
12	3291.184	IV	0.207	0.261	0.227	0.305
13	3523.946	II	0.267	0.280	0.223	0.231
14	3504.393	II	0.262	0.268	0.233	0.237
15	3671.949	I	0.324	0.281	0.203	0.192
16	3604.413	II	0.285	0.308	0.200	0.207
17	3891.496	I	0.458	0.235	0.165	0.141
18	3922.173	I	0.465	0.248	0.152	0.135
19	3798.135	I	0.382	0.265	0.187	0.167
20	3778.588	I	0.363	0.270	0.193	0.174
21	4029.039	I	0.540	0.204	0.140	0.118
22	3920.850	I	0.4503	0.233	0.172	0.145
23	3979.603	I	0.508	0.215	0.151	0.127
24	3963.833	I	0.397	0.314	0.147	0.142
25	3815.680	I	0.334	0.273	0.205	0.188

Class I – General Adaptability (++); Class II - Specific adaptability to favorable environments (+-); Class III - Specific adaptability to unfavorable environments (-+); Class IV – Little adapted (--)

is that, with the possibility of defining ideotypes (centroids) in "classes", the discrimination is more detailed, and genotypes with different performances within the defined "classes" can be developed. Together with the ease of analysis and interpretation of the adaptability of genotypes of the original method, with the wide possibilities of desirable comparisons by the proposed modification, the method seems to be highly promising for researchers of this area and may become one of the methods recommended for adaptability studies.

The data were also analyzed by the method proposed by Lin and Binns (1988) (Table 5). In comparison it is easier to analyze and interpret the genotype adaptability by the modified centroid method than by the methodology of Lin and Binns (1988). This ease in the interpretation is due to the absence of ambiguous indications, which occur in the method of Lin and Binns (1988). In this study, for example, genotype 21 has the best performance when all environments are considered and general adaptability. It is also the best in

unfavorable environments and second best, when favorable environments are considered.

Rocha et al. (2005) calculated the Spearman correlation coefficient between the original centroid method and the methodology proposed by Lin and Binns (1988). The authors found that the concepts of the centroid (ideotype I) and the respective method of Lin and Binns were similar with respect to the study of adaptability. However, correlation values for the ideotypes II, III and IV were not high, since the concept of the centroid method is different from the traditional, once the ideotypes with the desired performance are those with a maximum response in one group of environments (favorable or unfavorable) and minimum in another group. In the methodology proposed here, depending on the interest of study, any concept can be used for adaptability studies, because the ideotypes (centroids) are defined by researchers, and consequently, a recommendation strategy that is most appropriate for the interests of producers and/or consumers will be adopted.

Table 5. Stability and adaptability estimates of 25 genotypes evaluated in 8 environments, by the methodology of Lin and Binns (1988)

Gen.	Mean	General Pi	Gen.	Fav. Pi	Gen.	Unf. Pi
21	4029.039	45507.438	24	15971.112	21	35362.290
23	3979.603	55989.512	21	51594.527	23	47675.252
18	3922.173	68928.278	23	60978.068	22	52933.822
17	3891.496	72914.350	18	61541.854	17	66734.406
22	3920.850	84732.690	17	76622.317	18	81238.985
24	3963.833	124366.687	22	103812.010	1	125628.817
19	3798.135	125439.681	19	124510.243	19	126988.744
20	3778.588	156350.849	20	158357.155	20	153007.004
15	3671.949	182702.410	15	176483.599	15	193067.094
16	3604.413	256653.437	16	205657.753	25	222275.995
25	3815.680	267848.240	13	278286.427	11	238291.768
13	3523.946	299381.992	25	295191.588	14	288168.628
14	3504.393	303463.563	14	312640.524	24	305025.978
11	3502.483	310688.174	11	354126.017	13	334541.267
7	3325.071	471967.499	12	453182.480	16	341646.244
10	3298.295	486526.550	10	478238.507	8	393134.107
12	3291.184	508051.349	7	508454.950	7	411155.081
8	3278.558	514113.245	3	516160.705	2	426406.648
9	3235.931	562013.973	8	586700.729	4	458054.508
3	3321.560	586920.460	9	591776.552	6	492267.586
6	3230.654	616439.380	6	690942.456	10	500339.955
4	3105.049	729137.455	5	858405.180	9	512409.676
1	3303.542	733073.611	4	891787.223	12	599499.463
5	3030.411	783775.821	1	1097540.487	5	659393.556
2	3048.160	869729.420	2	1135723.083	3	704853.384

Gen. – Genotypes; Pi General – Stability and adaptability estimate of the cultivar^i for all environments; Fav Pi - Stability and adaptability estimate of cultivar^i for favorable environments; Unf. Pi – Stability and adaptability estimate of cultivar^i for unfavorable environments

CONCLUSIONS

1. The modification proposed provided the original method with greater flexibility, particularly for the researchers' objectives and the desired recommendation concept.

2. Similar to the original, the modified method is readily interpretable and clear for recommendation, with no ambiguity in interpretation.

3. A detailed study is possible by dividing the genotypes into "classes", established according to the interest of the researcher.

Metodologia dos Centróides Múltiplos para fins de análise de adaptabilidade genotípica

RESUMO - *Este trabalho teve como objetivo propor alteração no método centróide. A alteração se dá na escolha do ideótipo, sendo este definido de acordo com o interesse do pesquisador, através do modelo de regressão bissegmentada, não mais com base nos dados experimentais, que trás limitações na comparação dos genótipos. Para ilustrar o método, foi simulada uma característica em 25 genótipos em oito ambientes. As simulações e as análises estatísticas foram realizadas com auxílio do programa GENES. A utilização do método dos Centróides Múltiplos proporciona ao pesquisador maior flexibilidade que o original, fazendo com que este seja utilizado conforme objetivo do pesquisador e estratégia de recomendação desejada.*

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Apresenta também grande facilidade de interpretação para a recomendação, além de não possuir efeito de duplicidade de interpretação. E, ainda, possibilita comparar quantos genótipos forem de interesse, sendo possível fazer um estudo pormenorizado dividindo os genótipos em "classes" de recomendação.

Palavras-chave: Componentes principais, interação genótipos x ambientes, análise estatística.

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