Multiple centroid method to evaluate the adaptability of alfalfa genotypes

Moysés Nascimento[*1], *Adésio Ferreira*[2], *Ana Carolina Campana Nascimento*[3], *Fabyano Fonseca e Silva*[4], *Reinaldo de Paula Ferreira*[5], *Cosme Damião Cruz*[6]

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ABSTRACT

This study aimed to evaluate the efficiency of multiple centroids to study the adaptability of alfalfa genotypes (*Medicago sativa* L.). In this method, the genotypes are compared with ideotypes defined by the bissegmented regression model, according to the researcher's interest. Thus, genotype classification is carried out as determined by the objective of the researcher and the proposed recommendation strategy. Despite the great potential of the method, it needs to be evaluated under the biological context (with real data). In this context, we used data on the evaluation of dry matter production of 92 alfalfa cultivars, with 20 cuttings, from an experiment in randomized blocks with two repetitions carried out from November 2004 to June 2006. The multiple centroid method proved efficient for classifying alfalfa genotypes. Moreover, it showed no unambiguous indications and provided that ideotypes were defined according to the researcher's interest, facilitating data interpretation.

Key words: principal components, genotype x environment interaction, *Medicago sativa* L.

RESUMO

Metodologia dos centroides múltiplos para avaliação da adaptabilidade em genótipos de alfafa

Este trabalho teve como objetivo avaliar a eficiência do método dos centroides múltiplos em um estudo de adaptabilidade de genótipos de alfafa (*Medicago sativa L.*). Neste método os genótipos são comparados a ideótipos definidos de acordo com o interesse do pesquisador por meio do modelo de regressão bissegmentada. Desta forma a classificação dos genótipos é realizada conforme objetivo do pesquisador e a estratégia de recomendação desejada. Apesar do grande potencial do método há a necessidade que o mesmo seja avaliado sob o aspecto biológico (com dados reais). Assim, diante deste contexto foram utilizados dados provenientes de um experimento em blocos casualizados com 2 repetições, que constituiu-se da avaliação da produção de

matéria seca de 92 cultivares de alfafa em 20 cortes, realizados no período de novembro de 2004 a junho de 2006. A metodologia dos Centroides Múltiplos mostrou-se eficiente na classificação de genótipos de alfafa. Além de não apresentar duplicidade de indicações e proporcionar que ideótipos fossem definidos de acordo com o interesse do pesquisador facilitando a interpretação dos dados.

Palavras-chave: componentes principais, interação genótipos x ambientes, *Medicago sativa L.*

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[1] Universidade Federal de Viçosa, Departamento de Estatística, Viçosa, Minas Gerais, Brasil. moysesnascim@ufv.br
[2] Universidade Federal do Espírito Santo, Centro de Ciências Agrárias, Alegre, Espírito Santo, Brasil. adesioferreira@gmail.com
[3] Universidade Federal de Viçosa, Departamento de Estatística, Viçosa, Minas Gerais, Brasil. ana.campana@ufv.br
[4] Universidade Federal de Viçosa, Departamento de Estatística, Viçosa, Minas Gerais, Brasil. fabyano.fonseca@ufv.br
[5] Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brasil. reinaldo@cppse.embrapa.br
[6] Universidade Federal de Viçosa, Departamento de Biologia Geral, Viçosa, Minas Gerais, Brasil. cdcruz@ufv.br
*Corresponding author: moysesnascim@ufv.br

INTRODUCTION

In the presence of genotype by environment interaction, it is necessary to obtain detailed information on the performance of each cultivar across environmental variations (Cruz *et al*., 2004). Thus, the adaptability and stability analyses become extremely important and necessary in order to identify and recommend superior genotypes for different environments.

A number of methods of adaptability and stability analyses are described in the literature, including the Eberhart & Russell (1966) and the Bayesian method proposed by Nascimento *et al*. (2011) that use the simple regression analysis as statistical principle. Non-parametric methods, such as those developed by Lin & Binns (1988), Nascimento *et al*. (2010), centroid (Rocha *et al*., 2005) and its subsequent developments the multiple centroid (Nascimento *et al*., 2009a) and the extended centroid (Nascimento *et al*., 2009b) are also reported.

Adaptability and stability analyses have been used to identify genotypes of interest in various crops. Lédo *et al*. (2005) conducted studies to select alfalfa genotypes with improved adaptability and stability for dry matter production. Mohebodini *et al*. (2006) used several methods to study in detail the genotype x environment interaction of 11 lentil genotypes (*Lens culinaris* M.). Pelúzio *et al*. (2008) evaluated the performance, adaptability and stability of soybean genotypes in four sowing dates, in the municipality of Gurupi, Tocantins. Mahammed & Amri (2008) compared and evaluated the results of 20 parametric and non-parametric methods for selection of *Triticum durum* genotypes. In addition to these, Barreto *et al*. (2011) estimated the adaptability and stability of sweet potato genotypes in three environments in the South Central region of the State of Tocantins.

Among the cited methodologies, the multiple centroid method stands out in the literature as having a great potential for genotype recommendation, since the choice of the ideotypes (ideal references) is defined according to the researcher's interest using the bissegmented regression model. The method provides the researcher with a greater flexibility and genotype classification is carried out as determined by the objective of the researcher and the proposed recommendation strategy. However, despite the great potential of the method, it has not been used on actual data, hence the need to evaluate its applicability in the biological context (with real data).

Thus, this study aimed to evaluate the efficiency of the multiple centroid method in an adaptability study using real data from alfalfa genotypes (*Medicago sativa* L.).

MATERIAL AND METHODS

The multiple centroid method requires that the environments are classified into favorable and unfavorable using the environmental index proposed by Finlay & Wilkinson (1963):

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

where: Y_{ij} is the average of the genotype *i* in the environment j; $Y_{..}$ is the total number of observations; a is the number of environments; and g is the number of genotypes.

After the classification of the environments, the ideal hypothetical cultivar (ideotype), or reference, is defined by the bissegmented regression model with the following parameters: mean β_{0i} and the linear response to unfavorable environments β_{1i} and favorable environments $(\beta_{1i} + \beta_{2i})$ (Cruz *et al*., 1989).

Using the model $Y_{ij} = \beta_{0i} + \beta_{1i} I_j + \beta_{2i} T(I_j)$,

where: Y_{ij} is the ideal response of the hypothetical genotype in the environment j; β_{0i} is the value provided so that the ideal response is maximum in all sites; I_j is the environment coded index; $T(I_j) = 0$ if $I_j < 0$; and $T(I_j) = I_j - \bar{I}_+$ if $I_j > 0$, with the mean of the positive indices .

After establishing the centroids based on the researcher's interest, the principal component analysis is applied to obtain scores for plotting the graphs. Genotypes are classified by their position on the graphs in relation to the centroids and the Cartesian distances between points (genotypes) and each of the centroids defined by the researcher. As in the centroid method and its subsequent developments, a measure of spatial probability is calculated, which is defined as the inverse of the distance between a treatment and the ideotype defined by the researcher:

$$P_{d(i,k)} = \frac{\left(\frac{1}{d_{ik}}\right)}{\sum_i \frac{1}{d_{ik}}},$$

where: $P_{d(i,k)}$ is the probability of showing a pattern of stability similar to the *k*th centroid; and d_{ik} is the distance from the *i*th genotype to *k*th centroid.

Dry matter production data used in this study were obtained from an alfalfa evaluation experiment carried out by Southeast-Embrapa Livestock Research Center to develop alfalfa genotypes adapted to the different

Brazilian ecosystems. The experiment evaluated the dry matter production of 92 alfalfa genotypes in 20 cuttings in a randomized block design with two replications. The cuttings were considered to be representative of different environmental conditions because they were performed at different times during the period from November 2004 to June 2006.

The ideotypes for the method were defined according to Pereira & Ferreira (2008), considering that when the interest is the genetic improvement, one selects alfalfa genotypes in which a good performance for dry matter production is coupled with a high response to environment improvement and highly predictable behavior. Thus, using the bissegmented regression model, the following references of interest were created: ideotype I - mean higher than the overall mean of the assessed alfalfa genotypes and with general adaptability as ($\beta_{0i} > X_G; \beta_{1i} = 1$); ideotype II - mean higher than the overall mean and responsive to environmental changes ($\beta_0 > \overline{X}_G; \beta_{1i} + \beta_{2i} = 1{,}5$); and for discard: ideotype III - general adaptability with mean lower than the general mean ($\beta_{0i} < \overline{X}_G; \beta_{1i} = 1$), mathematically:

I. $Y_{ij} = (> \overline{X}_G) + I_j$;

II. $Y_{ij} = (> \overline{X}_G) + 0.5I_j + 1T(I_j)$;

III. $Y_{ij} = (< \overline{X}_G) + I_j$.

The results obtained from the multiple centroid method were also compared with the non-parametric methodology by Lin & Binns (1988).

The statistical analyses were carried out using the GENES software (Cruz, 2006) available at http://www.ufv.br/dbg/genes/gdown.htm.

RESULTS AND DISCUSSION

There was significant difference among dry matter production means of the alfalfa cultivars and significant cultivar (Cv) x cutting (Ct) interaction, at 5 and 1% probability levels, respectively (Table 1). The significance of the cultivar x cutting interaction shows that the cultivars had different performances in the various environmental conditions. Therefore, this interaction was studied in more detail using the analyses of adaptability and stability.

The ideotypes based on the work of Pereira & Ferreira (2008) and defined by the bissegmented regression model were characterized as follows:

I Y_{ij} = 1.250 + $1I_j$;

II Y_{ij} = 1.300 + 1Ij + $0{,}5I_j$;

III Y_{ij} = 1.100 + $1I_j$.

Ideotype I is a genotype with general adaptability with mean higher than the overall mean, which is of interest for breeding programs with a wide range of environments. Ideotype II is responsive to environment improvement and of great interest for alfalfa breeding (Pereira & Ferreira, 2008). Ideotype III has lower mean than the overall mean and can be discarded.

In the Principal Components Analysis, the cumulative percentage of variance in the first three components explained 75.44% of the variability in the data (Table 2), which, according to Johnson & Wichern (1992) and Melém Júnio. *et al*. (2008), is sufficient to a satisfactory interpretation of the results.

The genotypes were classified according to the multiple centroid method (Table 3). Of the 92 alfalfa

Table 1. Summary of the analysis of variance for the trait dry matter production of 92 alfalfa cultivars in 20 environments (cuttigs), in the municipality of São Carlos / SP, from November 2004 to June 2006

Sources of variation	DF	Mean Squares
Blocks	1	2002415.43
Cultivar (Cv)	91	1384475.75*
Error a	91	574269.72
Cutting (Ct)	19	62331022.56*
Error b	19	946917.67
Interaction (Cv x Ct)	1729	60682.46**
Error c	1729	55851.26
Mean	1176.80	

NS non-significant; * and ** - significant at 5 and 1% probability levels, respectively, by the F test

Table 2. Variance estimates (eigenvalues) of principal components and cumulative percent of variance explained by the components

Root	Root (%)	% Accumulated
12.210	61.050	61.050
1.596	7.980	69.030
1.282	6.412	75.443
0.811	4.057	79.500
0.699	3.494	82.994
0.539	2.696	85.690
0.459	2.294	87.985
0.401	2.004	89.989
0.360	1.801	91.790
0.320	1.599	93.389
0.234	1.170	94.560
0.210	1.052	95.612
0.176	0.879	96.491
0.156	0.781	97.271
0.143	0.717	97.989
0.124	0.621	98.610
0.085	0.425	99.036
0.074	0.369	99.404
0.065	0.326	99.731
0.054	0.269	100.000

Table 3. Classification and probability associated with the genotypes in each of the three groups characterized by the ideotypes defined by Multiple Centroid Method (MCM) and estimates of stability and adaptability by the Lin & Binns (1988) method for the trait dry matter production

Genotype		MCM		Lin & Binns (1988)					
	Mean	Clas.	Prob.	Clas.	P_i	Clas.	P_i fav.	Clas.	P_i unfav.
Winter	1002.851	III	0.499	45	235472.34	41	37180.94	45	223575.45
SPS 6550	1133.702	III	0.473	41	262956.04	37	60064.99	66	415696.31
Primavera	1052.277	III	0.439	17	292152.13	17	78710.83	41	447681.11
LE N 1	1184.557	I	0.464	32	306196.73	32	84947.67	17	466785.92
Trindade 87	1467.199	I	0.383	63	329223.96	63	95972.39	32	487218.69
LE N 2	1352.261	I	0.442	5	337543.51	76	104554.55	18	503535.04
LE Semit 711	1140.254	III	0.419	33	339018.09	33	107414.39	9	510865.03
Topper	1144.191	III	0.394	66	340669.40	38	111939.38	63	520066.15
LE N 3	1394.53	I	0.364	76	350884.22	5	112580.47	5	521604.17
Sequel HR	1306.674	I	0.486	31	356239.01	10	126967.34	33	528512.02
LE N 4	1382.655	I	0.376	9	357513.50	36	129803.04	31	541067.87
Victoria INTA	1213.572	I	0.471	18	358002.24	65	129983.92	76	552426.67
Monarca INTA	1189.032	I	0.489	38	359788.25	31	130337.06	38	562573.68
Bárbara INTA	1315.94	I	0.425	37	369277.02	11	133563.51	11	577633.17
Primavera 1	1196.396	I	0.442	11	377801.82	6	135995.01	6	580475.23
Aca 900	1273.347	I	0.456	6	380459.13	43	138401.46	36	590067.59
5 939	1574.601	II	0.393	36	382948.54	30	145691.65	61	590308.28
WL 612	1399.081	I	0.383	59	395931.76	59	155565.54	59	592595.03
Medina	1251.655	I	0.425	65	398478.66	58	160601.77	14	593962.81
N 910	1183.788	I	0.411	61	399834.18	61	167032.49	78	598936.43
Coronado	793.913	III	0.43	14	414808.50	9	170083.85	19	601920.19
Eterna	835.141	III	0.445	10	425684.84	75	174267.94	16	610069.93
DK 193	915.606	III	0.429	43	426010.42	40	176812.57	65	618156.18
Candombe	651.019	III	0.397	78	431208.54	77	178856.38	60	618907.58
WL 414	830.552	III	0.43	58	434807.11	34	179851.14	37	622268.69
Crioula	1033.33	III	0.53	60	437716.42	18	180128.82	49	625826.26
LE Semit 711 1	991.079	III	0.492	16	437758.62	42	186655.01	29	629687.61
DK 181	1053.433	III	0.501	34	437994.65	83	188201.72	52	633972.21
5 929	1264.671	I	0.484	80	439337.39	35	189398.13	80	642935.34
Activa	1257.95	I	0.405	77	439439.26	80	190495.44	39	642976.43
Sequel 2	1411.095	I	0.388	35	443438.82	14	195842.11	34	649202.98
Califónia 60	1525.046	II	0.366	39	443590.45	39	199896.47	35	651290.30
Cuf 101	1437.202	I	0.394	75	444914.75	57	200187.51	77	652643.42
58 N 58	1263.816	I	0.485	19	444991.67	64	202143.70	58	659156.93
Diamind	1277.022	I	0.445	52	446558.49	13	210902.64	43	661326.84
Aurora	1336.743	I	0.413	29	448317.80	4	211621.99	12	662027.33
Sundor	1428.926	I	0.392	49	449907.69	55	216249.71	75	666353.04
Springfield	1383.944	I	0.419	30	464164.23	60	216260.57	10	670090.07
Sutter	1211.478	I	0.462	12	477254.08	52	217497.27	79	672229.49
Hunterfield	1189.427	I	0.424	62	479016.65	7	222260.92	62	672375.93
P 105	1622.763	II	0.362	79	481318.79	44	222944.05	50	682165.17
Prointa Patricia	1184.066	I	0.449	50	482536.00	78	226207.79	20	687557.17
Flórida 77	1288.054	I	0.452	15	491489.51	29	226643.59	8	690074.52
Siriver 2	1185.93	I	0.503	42	491885.34	16	227155.91	15	705311.63
WL 516	1334.343	II	0.338	40	492310.72	15	230151.35	74	717152.06
Tahoe	1041.086	III	0.513	44	498105.18	86	230334.96	81	719196.74
Esmeralda	1081.97	III	0.516	83	502460.53	82	231524.21	71	720367.64
DK 167	1122.714	III	0.475	4	503848.43	49	234896.10	44	723237.02
DK 177	1274.64	I	0.437	20	505046.01	50	238544.80	30	724732.69
5 683	1205.993	I	0.486	13	508807.97	62	242688.65	56	725279.11
WL 414 1	1010.343	III	0.501	82	513204.27	79	247983.49	90	727153.04

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Genotype		MCM		Lin & Binns (1988)					
	Mean	Clas.	Prob.	Clas.	P_i	Clas.	P_i fav.	Clas.	P_i unfav.
Express	1267.468	I	0.459	57	515157.44	66	248969.83	42	741619.25
F 708	1108.731	III	0.505	56	519602.71	45	250012.98	54	741980.19
Perla INTA	1129.403	III	0.495	90	520874.07	48	250084.75	4	742942.78
Prointa Lujan	1088.188	III	0.456	81	531261.57	12	251420.10	82	743669.78
DK 166	1150.3	I	0.413	54	535170.87	19	253190.14	40	750445.57
Platino	1179.549	I	0.44	2	547132.91	2	263962.50	13	752548.70
Maxidor	1274.525	I	0.475	71	549586.81	56	268220.46	87	752835.68
Amerigraze701	1355.009	I	0.424	86	554039.38	90	268755.32	88	757295.23
13 R Supreme	1270.49	I	0.486	8	554619.87	46	272116.87	83	759581.37
Pecos	1322.879	I	0.463	53	560568.51	53	274909.98	57	772860.10
Califórnia 50	1211.353	I	0.48	48	566289.10	20	281976.82	84	777777.82
Maricopa	1459.601	I	0.375	64	571848.02	54	282403.92	2	778817.79
Kern	1120.366	III	0.416	7	581177.86	28	295297.47	73	783982.29
Costera INTA	1357.178	I	0.422	84	582795.17	81	301563.02	3	791458.63
F 686	1271.352	I	0.395	55	584800.90	1	307733.23	53	794289.13
Monarca	1034.199	III	0.535	74	587982.62	47	319583.61	67	811667.00
Patrícia	971.805	III	0.486	47	598152.14	26	328898.10	86	818888.46
Tango	804.511	III	0.439	88	601359.98	51	333057.38	48	825001.75
Bárbara	872.003	III	0.437	73	605967.74	71	340854.69	47	826071.85
Rio Grande	1116.735	III	0.439	67	607833.35	84	344483.05	27	848775.18
Key II	904.77	III	0.436	28	611185.60	67	358703.35	89	854982.88
Gala	1041.232	III	0.519	46	621245.02	68	378522.17	85	867773.36
Lujan	1131.267	III	0.389	26	627654.51	73	388394.41	28	869639.53
Perla	1252.125	I	0.482	51	635286.40	8	389064.19	26	872091.56
5683 L	1404.397	I	0.397	87	636339.60	72	405143.81	64	874333.38
Victoria	1283.158	I	0.436	3	636876.92	88	410772.45	7	874837.17
DK 194	1300.291	I	0.426	1	657604.37	74	430108.85	51	882564.69
WL 442	1194.413	I	0.49	27	669589.00	3	447943.73	55	886342.78
P 30	1259.664	I	0.501	85	681077.22	91	447965.36	46	906895.33
P 5715	1141.713	III	0.447	68	686745.10	27	450583.67	91	915265.24
Alfa 200	1165.585	I	0.465	91	704980.30	85	452893.05	68	938927.49
Aca 901	1200.671	I	0.417	89	715246.96	87	493955.50	1	943862.57
Gapp 969	1093.061	III	0.512	72	761389.50	23	529952.30	25	957100.39
Rocio	974.527	III	0.485	23	776073.87	89	544458.62	70	970024.80
GT 13 R Plus	1143.141	III	0.433	70	792140.75	22	566244.10	23	977446.06
WL 525	1013.744	III	0.468	25	818314.66	70	574726.91	21	1028229.01
Sequel	1056.443	III	0.491	22	823283.57	92	619670.22	22	1033588.58
DK 187 R	934.305	III	0.426	69	857406.25	69	630696.17	69	1042896.33
Pinto	1148.741	III	0.427	21	868788.76	25	648687.66	72	1052863.23
Bacana	939.335	III	0.481	92	969241.67	21	673917.34	24	1109434.51
Siriver	718.896	III	0.418	24	1059743.34	24	999009.68	92	1255254.68

genotypes, 49 were classified as having high general adaptability with means higher than the overall mean of the experiment (ideotype I), four were classified as specific for favorable environments (ideotype II) and 39 as having general adaptability with means lower than the overall mean (ideotype III) (Table 3).

Among the 49 genotypes classified as ideotype I, ten showed greater probabilities, including Siriver 2 (0.503); P 30 (0.501); WL 442 (0.490); Monarca INTA (0.489); 5 683 (0.486); Sequel HR (0.486); 13 R Supreme (0.486); 58 N 58 (0.485); 5 929 (0.484); and Perla (0.485). These genotypes can be recommended to sites with large environmental variability. The genotypes classified as ideotype II of great interest for alfalfa breeding programs were: 5939 (0.393); Califonia 60 (0.366); P 105 (0.362); and WL 516 (0.338).

Among the 39 genotypes classified as ideotype III, which can be discarded, the ten with the largest probabilities were: Monarca (0.535); Crioula (0.530); Gala (0.520); Esmeralda (0.516); Tahoe (0.513); Gapp

969 (0.512); F 708 (0.505); DK 181 (0.501); WL 414 1 (0.501); and Winter (0.501).

The first three principal components scatterplot of the 92 genotypes in twenty environments (cuttings) showed a mass of genotypes around the ideotypes I and III, which confirms the results presented (Figure 1).

The genotypes were also analyzed by the method of Lin & Binns (1988) (Table 3). The results showed that the first five genotypes classified as of general adaptability were: WL 516; P 105; 5939; California 60; and Maricopa. The first five genotypes classified as adaptable to favorable environment were: P 107; Sundor. 5 939; California 60; and Maricopa. The genotypes WL 516, F 686, P 105, 5 939 and Maricopa were the first five classified as adaptable to unfavorable environments.

The four genotypes classified as adaptable to favorable environments by the multiple centroid method were also classified as of general adaptability by the method of Lin & Binns (1988). Besides, of these four genotypes, two, 5 939 and California 60, were also classified as of specific adaptability to favorable environments. Among those that can be discarded, which were classified as ideotype III by the multiple centroids and of specific adaptability to unfavorable environments by Lin & Binns (1988), none showed equivalent classification.

The results of this study corroborate the work of Nascimento *et al.* (2009a) and demonstrate the ease of analysis and interpretation of adaptability by the multiple centroid method compared to the method of Lin & Binns (1988). This easiness is due to the non-occurrence of possible ambiguous indications in the multiple centroid method, as it happens in the Lin & Binns (1988) method, as well as the direct comparison with the ideotype of interest.

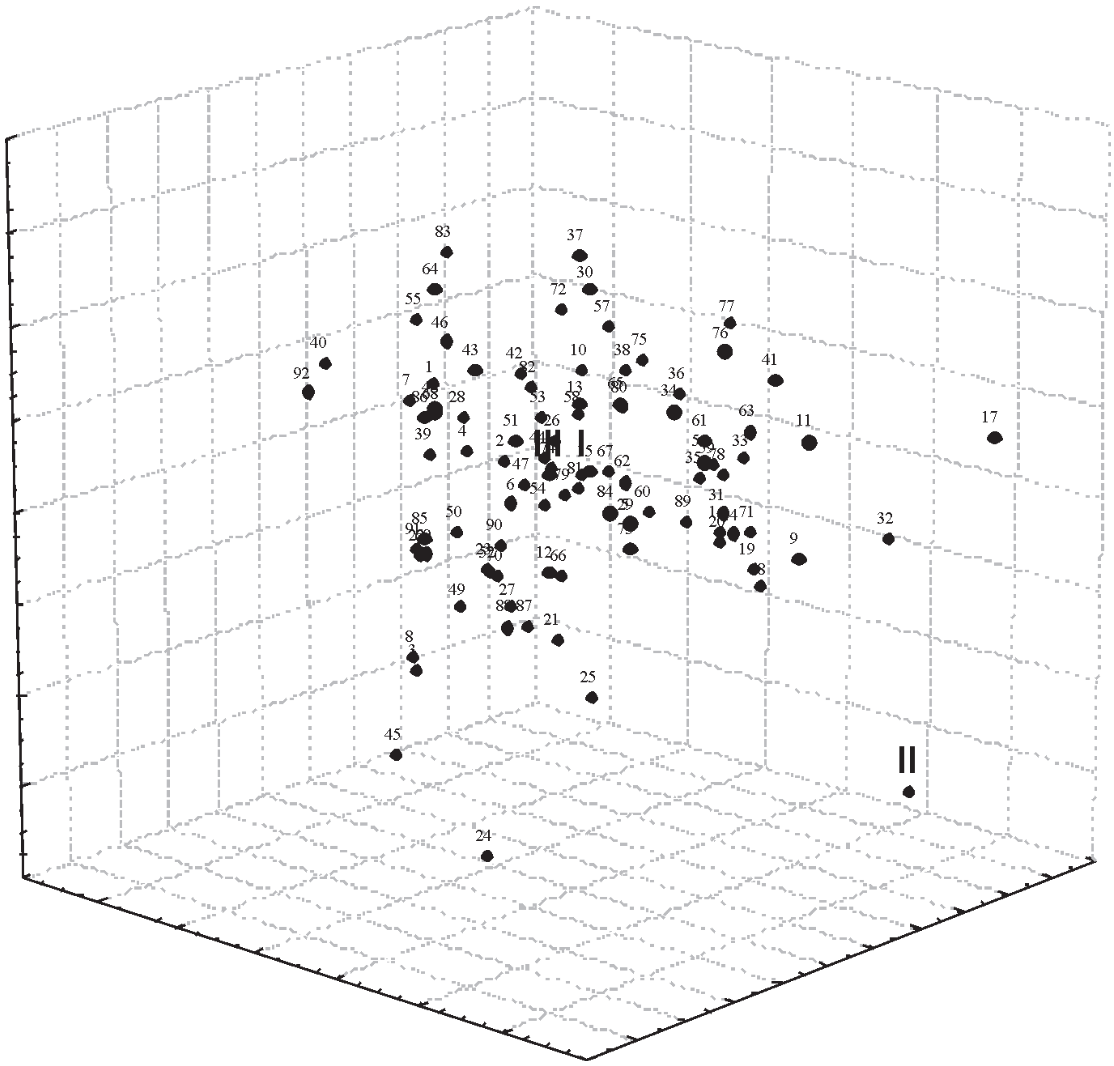

Figure 1. Scatterplot of the first three principal components of 92 genotypes for the response of dry matter production to twenty environments (cuttings). The three points numbered with Roman numerals represent the centroids.

CONCLUSIONS

The multiple centroid method was efficient to classify alfalfa genotypes without showing ambiguous information as it occurs with the Lin & Binns (1988) method. Furthermore, the definition of ideotypes according to the researcher's interest facilitated data interpretation.

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