

Multi-information analysis to recommend alfalfa cultivars for adaptability and phenotypic stability

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Abstract: *This study provided a comprehensive overview of the behavior of alfalfa genotypes in response to environmental variations. We utilized established methods from literature and examined the unique aspects of each approach to collectively create a criterion for recommending cultivars. To this end, seventy-seven genotypes were cultivated with 24 consecutive cuts (months), during two years. Adaptability and stability analyses were conducted using multiple information estimates. The results indicated no significant effect of the genotypes, but there were significant effects from the environment and the genotype-by-environment (GxE) interaction. Through multi-information analysis, genotype 21 was identified as the most promising due to its superior dry matter yield, predictable performance, and responsiveness to environmental changes across various cuts. Combining data and thoroughly describing the behavior of alfalfa genotypes has proven to be an effective method for studying their adaptability and stability.*

Keywords: *Medicago sativa L., GE interaction, plant breeding*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a key forage legume in temperate countries (Annicchiarico et al. 2015) with growing use in Brazil, mainly due to the quality of its forage, given its excellent traits and high protein content (Ferreira and Vilela 2015). Embrapa Pecuária Sudeste maintains a collection of 77 alfalfa accessions originating from temperate regions. The organization regularly evaluates this germplasm to develop synthetic populations that are well-suited to Brazilian environments. The process of plant breeding is costly, demanding significant time, effort, and investment.

Phenotypic expression is the result of the genotype and environment effects. When genotypes are assessed across multiple environments, the observed variation includes not only the individual effects of genotype and environment but also the genotype-by-environment (GE) interaction (Silva Júnior et al., 2021). The GE interaction remains a significant challenge in breeding programs for any species, affecting both the selection and cultivar recommendation phases. When such interactions occur, a genotype may exhibit varying behaviors across different

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cuts due to the gene expression in response to environmental factors (Cruz, 2014). Thus, comprehensive studies on genotype behavior across different environments (cuts) are crucial for recommending cultivars suited to specific cropping systems. These studies focus on the behavior of genotypes, detailing their adaptability and stability (Maia et al., 2013). This approach is commonly applied to annual crops, where environments are considered distinct cultivation regions for the species. Adaptability refers to the capacity of genotypes to respond beneficially to environmental stimuli, while stability denotes the ability of genotypes to demonstrate highly predictable behavior in response to these stimuli (Cruz 2014, Silva Junior et al. 2021), which can be applied to both annual crops and perennial crops, even under conditions in which environments can be represented by periods of cutting, in successive times, of the genotypes, as occurs for alfalfa.

In this context, a variety of statistical methods can be used to assess adaptability and stability, each with unique statistical principles, biometric techniques, and ways of interpreting results (Euwijk et al., 2016). According to Cargnelutti Filho et al. (2007), these methods can be categorized into several groups, such as those based on Variance Analysis (Yates and Cochran, 1938; Plaisted and Peterson, 1959; Wricke, 1965), Linear Regression (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Tai, 1971), Bi-segmented Regression (Verma et al., 1978; Silva Barreto, 1985; Cruz et al., 1989), Non-parametric Methods (Lin and Binns, 1988; Huehn, 1990; Annicchiarico, 1992; Rocha et al., 2005; Nascimento et al., 2015), Quantile Regression (Barroso et al., 2015), Bayesian Inference (Couto et al., 2015), and Artificial Intelligence (Carneiro et al., 2018; 2019; Silva Júnior et al., 2021; 2023).

It is possible to find works that compare some of these methodologies in different crops, such as maize (*Zea mays*) (Oliveira et al. 2017), sugarcane sugar (*Saccharum officinaru*) (Paula et al. 2014), soybean (*Glycine max*) (Woyann et al. 2018), wheat (*Triticum spp*) (Rootaei et al. 2014), blackeyed cowpea (*Vigna unguiculata*) (Nunes et al. 2014) and rice (*Oryza sativa*) (Silva Junior et al. 2020). However, comparing these methodologies is not pertinent, as each one is designed to address different questions, even if some of them produce similar estimates. Despite the availability of numerous methods for studying adaptability and stability, the development of new methodologies suggests that existing approaches, while highly beneficial for breeders, remain inadequate for comprehensively analyzing such a complex phenomenon. However, employing multiple methodologies simultaneously in a multi-information analysis for cultivar recommendation can uncover insights that would not be apparent when using each methodology individually.

The aim of this study was to identify superior alfalfa genotypes and highlight the importance of comprehensively describing genotype behavior in response to environmental variations (successive cuts). Established methods from the literature were utilized, with a focus on the unique characteristics of each technique, to collectively form multi-information criteria for recommending cultivars.

MATERIAL AND METHODS

Field experiments

Data from 24 alfalfa cuttings including 77 genotypes from Embrapa Pecuaria Sudeste were used. The experiment followed a randomized complete block design with three replications. Each experimental unit comprised four rows, each 4.0 meters long and spaced 0.2 meters apart. The usable area included the two central rows, excluding 0.5 meters from each end. Accessions were irrigated by a central pivot, except for cuts eight, nine, 10, 19, 20, and 21, in which irrigation was suspended. Cultivation practices were performed as recommended for the crop. The dry matter yield (DMY) in kilograms per hectare was assessed at each cut when the accessions reached 10% flowering. The data from each cut underwent individual variance analysis.

Recommendations derived from multi-information analysis

Individual variance analyses were conducted, followed by a joint ANOVA based on the statistical model outlined in the equation:

$$Y_{ijk} = \mu + G_i + C_j + B_k + GC_{ij} + \frac{B}{C_{jk}} + \varepsilon_{ijk}$$

where: Y_{ijk} is the observation on the k^{th} block ($k= 1, 2$ and 3), evaluated on the i^{th} genotype ($i= 1, 2, \dots, 77$) and j^{th} environment ($j= 1, 2, \dots, 24$ cuts); μ is the overall mean of the experiments; G_i is the effect of the i^{th} genotype considered to be fixed; C_j is the effect of the j^{th} environment considered to be random; GC_{ij} is the random effect of the interaction

between genotype i and environment j ; $\frac{B}{C_{jk}}$ is the effect of block k within environment j ; and ϵ_{ijk} is the random error associated with observation Y_{ijk} .

The Scott-Knott clustering test was used at a 5% probability level to identify homogeneous groups based on their mean potential. Adaptability and stability analysis was performed using multi-information estimates, considering the following parameters:

Average value

The average value for each genotype was determined using the equation: $m_i = \frac{Y_i}{E}$ where Y_i is the grain yield of the i^{th} genotype across all environments (cut) and “ E ”, is the number of cuts.

Mean performance across various environmental conditions and plasticity

The average potential indicates the genotype’s production capacity under varying environmental conditions, categorized as general, favorable, or unfavorable. Favorable environments are characterized by optimal soil and climate conditions for the crop, while unfavorable environments are associated with adverse weather, poor soil quality, or low technological input. The general environment encompasses both favorable and unfavorable conditions. Genotype plasticity can be measured by analyzing combined experimental data and breaking down the sum of squares for environmental effects and genotype-environment interactions.

Influence on interaction

This metric assesses how much a particular genotype influences GE interaction. The contribution to the interaction’s total sum of squares can be described as per Wricke (1965) to the interaction’s total component $\hat{\sigma}_{ge}^2$, as per Plaisted and Peterson (1959).

Recommendation index linked to the i^{th} genotype

A high-performing genotype is characterized by its maximum average production potential and minimal environmental variability. The recommendation index is estimated using Annicchiarico’s (1992) methodology. Initially, the averages of each cultivar in each environment are converted into percentages of the environment’s average. Then, the standard deviation and the mean of these percentages for each cultivar are calculated.

Genotype i^{th} ability to adapt

It measures how effectively a genotype can respond to environmental improvements. The adaptability estimate is obtained by regression coefficients (β_{vi}), which is the linear response of genotype i to environmental changes, based on the models proposed by Finlay and Wilkinson (1963) and Eberhart and Russell (1966).

Stability or predictability

This metric evaluates the consistency of genotype i ’s behavior in response to environmental changes, using the linear regression model described by Eberhart and Russell (1966). The stability parameter (σ_{di}^2) is calculated using the analysis of variance method, based on the mean square of the regression deviation for each genotype and the mean square of the residual.

Another method to assess predictability is by using the coefficient of determination, which quantifies the proportion of total variation accounted for by the genotype’s linear behavior.

Response pattern of genotype j and optimal pattern

This trait is beneficial for a genotype, as it demonstrates the ability to maintain high yields under adverse conditions while being responsive in favorable environments. The models proposed by Finlay and Wilkinson (1963) and Eberhart and Russell (1966) are insufficient for detecting this capability, as they only consider a single regression coefficient. In contrast, bi-segmented regression models, as suggested by Cruz et al. (1989), can effectively quantify this genotypic trait. In this context, an ideal genotype would outperform all others across all environments. Although this ideal genotype

typically doesn't exist or isn't included in the experiment, it's possible to measure how far the evaluated genotypes are from this hypothetical 'champion' standard. This information can be derived for all environments or specifically for favorable or unfavorable ones, following the methodology proposed by Lin and Binns (1988).

Recommendation index based on the centroid method

This metric describes each genotype by its closeness to hypothetical standard genotypes, extending beyond those recommended by Lin and Binns (1988) and other relevant standards. The centroid method, introduced by Rocha et al. (2005), involves comparing Cartesian distance values between genotypes and four pre-established reference points (ideotypes) based on experimental data. Once each of the parameters described above was obtained, they were compiled into a table that includes diverse information from various adaptability and stability study proposals. This thorough method highlights key characteristics of each cultivar for recommendation, allowing for a concurrent evaluation of the indices that define the multi-information analysis. The analyses were carried out using GENES software (Cruz, 2016).

RESULTS AND DISCUSSION

Table 1 presents the result of the combined analysis of variance (ANOVA) for dry matter yield (kg ha^{-1}) across 77 alfalfa genotypes over 24 harvests. Each harvest was individually analyzed, revealing significant effects for all genotypes. The joint ANOVA revealed significant effects for genotypes, harvests, and their interaction. The significant GC interaction indicates that the relative behavior of the genotypes was not the same in all cuts, since the most productive genotypes in a given cut may not have performed better at another time (Costa et al. 2021). Thus, when we release such a cultivar on the market, we will be helping the producer to use a smaller amount of bulky supplementation from another source (silage, hay, among others) to maintain milk production per animal. Thus, in intensive milk production systems, we will be helping to avoid an increase in the cost of production.

We found that the genotypes' behavior was affected by environmental conditions, specifically cutting in this instance, supporting the application of methodologies to categorize genotypes according to their adaptability and stability. The calculated coefficient of variation was 20.80%, aligning with values reported in the literature for alfalfa cultivation.

Table 2 demonstrates the strategy of the multi-information technique, which integrates diverse data from various studies on adaptability and stability. These combined analyses identify key characteristics of each cultivar for recommendation. For instance, genotype 77 (Crioula cultivar) is recognized for its high quality, erect growth habit, and limited tolerance to severe defoliation, making it suitable for global cultivation.

For each parameter in the multi-information sheet, the minimum (Min) and maximum (Max) reference estimates were presented. To get an idea of the positioning of the particularized genotype 77 (crioula cultivar) we must compare it with the reference estimates. It is important to highlight that in the case of the average potential parameter, the reference value for the average of DMY is also presented for quantitative comparisons. To facilitate the interpretation of the multi-information technique, consider a qualitative position that can be obtained through rank (Table 2). A rank with a value of 1 represents the best scenario for all criteria. Thus, if the statistic is the mean, rank 1 signifies that the genotype achieved the highest average. However, if the observed parameter is the champion pattern given by the value of P_i (Lin and Binns 1988), rank 1 signifies that the genotype exhibits a lower value, thereby approximating the hypothetical genotype with optimal performance across all harvests. Additionally, the stability parameter (%) presented represents the coefficient of determination, accompanied by the significance level associated with the hypothesis that the regression model deviation is null.

Table 1. Summary of the combined variance analysis for dry matter production (kg ha^{-1}) across 24 cuts of 77 alfalfa genotypes

Sources of variation	df	Fcal	p-value
Genotypes (G)	76	11.11	0.0 ***
Cut (C)	23	418.20	0.0 ***
G x C	1748	2.09	0.0 ***
Residual	3694		
Average	1881		
CV (%)	20.8		

***: Significant at 0.1% probability by F test. SV: Source of Variation; DF: Degrees of freedom; CV: Coefficient of variation in %.

Table 2. Genotypic recommendation for genotype 77 (Crioula) based on multi-information analysis of about dry matter production (kg ha⁻¹) in alfalfa

Parameter		Genotype 77			Reference	
		Value	Rank	Min	Max	Average
Average potential	General Environment (%)	2218	3	1171	2275	1881
	Favorable Environment (%)	2597	10	1452	2717	2285
	Unfavorable Environment (%)	1839	2	889	1840	1477
Plasticity QMG/A		817663	14	529982	1626487	-
Interaction Contribution	S ² GxC (%)	0.63	2	0.60	3.09	-
	SQGxA (%)	0.61	2	0.57	3.16	-
Recommendation Index	General Environment (%)	103	2	35.12	105.23	-
	Favorable Environment (%)	99.93	4	36.71	106.05	-
	Unfavorable Environment (%)	109.09	1	30.46	109.09	-
Adaptability (%)		0.97 ^{ns}	-	109.09	1.32	-
Stability (%)		83.86 ^{ns}	-	47.44	90.95	-
Response pattern j	Adaptability β_1	0.97 ^{ns}	-	0.70	1.33	-
	Adaptability $\beta_1 + \beta_2$	1.04 ^{ns}	-	-0.26	2.51	-
	Stability (%)	83.91 ^{ns}	-	49.88	91.06	-
Champion pattern	General Environment	193061.51	4	157770	1393667	-
	Favorable Environment	319657	7	219945	1924476	-
	Unfavorable Environment	66466	1	66466	863406	-
Recommendation Index	4 Centroid			I		
	7 Centroid			VII		

** , * and ns: significant at 1%, 5% and not significant by the F test, respectively; ⁽¹⁾ Reference: Minimum (Min), Maximum (Max), and average grain yield, respectively; I: High overall adaptability (maximum production in favorable and unfavorable environments); VII: Medium specific adaptability to unfavorable environments (medium production in favorable environments and maximum in unfavorable environments).

Based on the Annicchiarico recommendation index for favorable environments and the champion pattern for unfavorable environments, the Crioula cultivar achieved rank 1, positioning it as the top genotype among the others for these criteria (Table 2). This cultivar was assigned rank 2 for the parameters of average potential in unfavorable environments, interaction contribution, and the Annicchiarico recommendation index for the general environment. The response pattern J for adaptability and stability was not significant, which indicates that the cultivar Crioula stands out as the one whose average behavior is more predictable. According to the centroid recommendation index from the four- and seven-centroid methodologies, this genotype was classified as having general adaptability and moderate specific adaptability to unfavorable environments.

To facilitate the comparison of the other selected genotypes, we represent them in a single multi-information analysis table (Table 3). Note that through rank, genotype 21, excelled in terms of average potential for both general and favorable environments. However, for this parameter for the unfavorable environment, genotype 61 was in the first position. Nonetheless, note that, even though genotype 21 is not ranked first, it is very well ranked third.

It is worth noting that, if we had to select between genotypes 21 and 61 for the standard response parameter j, we would make the decision not to select genotype 61, as it had regression deviation statistically different from zero and its level of unpredictability may affect the recommendation of the cultivar since its R^2 is below 80% (Cruz et al. 2012). Note that for the Annicchiarico recommendation index parameter, the best ranking was also for genotype 21, since it was awarded the ranked first for general and unfavorable environments, and second for unfavorable environments compared to other selected genotypes (Table 3).

According to the centroid recommendation index for four centroids, all selected genotypes were classified as having high general adaptability. With seven centroids, we observed general average adaptability for genotypes 21, 40, 64, and 66, and average adaptability to unfavorable environments was observed only for genotype 61 (Table 3).

To justify the selection of genotype 21, we can take into account the interaction contribution parameter, as it is estimated by the relative contribution of each genotype to the genotype-environment interaction is assessed, identifying

Table 3. Recommendation based on multi-information analysis of genotypes with superior average performance in relation to the dry matter production (kg ha⁻¹) of alfalfa genotypes evaluated in 24 cuts

Parameter	Genotype 21		Genotype 40		Genotype 61		Genotype 64		Genotype 66		Reference		
	Value	Rank	Min	Max	Average								
Average potential	2275	1	2186	6	2229	2	2210	4	2189	5	1171	2275	1881
Favorable Environment	2717	1	2578	13	2618	6	2633	5	2679	3	1452	2717	2285
Unfavorable Environment	1832	3	1795	4	1840	1	1787	5	1700	8	889	1840	1477
Plasticity QMG/A	1012030	45	1167294	60	958930	35	1185353	63	1220820	66	529982	1626487	-
Interaction Contribution	0.70	6	1.85	64	1.11	39	1.85	65	1.41	53	0.60	3.09	-
S ² GxC (%)	0.68	6	1.86	64	1.10	39	1.87	65	1.42	53	0.57	3.16	-
General Environment (%)	105.23	1	92.19	12	96.6	3	92.64	9	93.39	5	35.12	105.23	-
Favorable Environment (%)	106.05	1	87.3	29	98.19	5	92.94	12	94.14	11	36.71	106.05	-
Unfavorable Environment (%)	105.86	2	97.97	6	97.35	8	92.09	13	91.63	14	30.46	109.09	-
Adaptability (%)	1.10 ^{ns}	-	1.03 ^{ns}	-	1.0 ^{ns}	-	1.04 ^{ns}	-	1.13 ^{ns}	-	0.71	1.32	-
Stability (%)	85.96 ^{ns}	-	65.31 ^{**}	-	74.96 [*]	-	65.58 ^{**}	-	75.66 ^{**}	-	47.44	90.95	-
Adaptability β_1	1.04 ^{ns}	-	0.95 ^{ns}	-	0.99 ^{ns}	-	1.05 ^{ns}	-	1.12 ^{ns}	-	0.70	1.33	-
Adaptability $\beta_1 + \beta_2$	1.62 [*]	-	1.70 [*]	-	1.09 ^{ns}	-	0.89 ^{ns}	-	1.20 ^{ns}	-	-0.26	2.51	-
Stability (%)	88.12 ^{ns}	-	68.38 ^{**}	-	72.02 ^{**}	-	65.63 ^{**}	-	75.70 ^{**}	-	49.88	91.06	-
General Environment	157770	1	248149	7	174950	2	221845	6	186659	3	157770	1393667	-
Favorable Environment	219945.44	1	398594.1	16	254335.22	3	331342.41	12	236993.63	2	219945	1924476	-
Unfavorable Environment	95594.81	3	97704.852	4	95564.486	2	112347.15	5	136324.78	8	66466	863406	-
Recommendation Index	4 Centroid	I	I	I	I	VII	I	I	I	I	-	-	-
	7 Centroid	V	V	V	V	V	V	V	V	V	-	-	-

** * and ns: significant at 1%, 5% and not significant by the F test, respectively; (1) Reference: Minimum (Min), Maximum (Max), and average grain yield, respectively; I: High overall adaptability (maximum production in favorable and unfavorable environments); V: Average overall adaptability (average production in favorable and unfavorable environments); VII: Medium specific adaptability to unfavorable environments (medium production in favorable environments and maximum in unfavorable environments).

those with greater stability. In this respect, by comparing genotype 21 with genotype 61, we can conclude that genotype 61 is the one that provides the greatest contribution to interaction, in addition to producing 2% less DMY on average. Its contribution to interaction is a consequence of its invariance, that is, the genotype does not respond to the improvement of the environment but is affected by adverse environmental conditions. This fact caused the 61 genotypes to be ranked in the 39th position for this parameter. Thus, genotype 61 should not be selected if the breeder must choose only the genotype based on DMY.

Given the results presented, we highlight the great contribution of using the recommendation form through multi-information analysis for the recommendation of alfalfa cultivars. Through this form it was possible to consider several methodologies, whether they are based on the existence of the G x C interaction or distinguish them from the concepts of adaptability and stability, adopting different approaches. It was also possible to consider that some methods are alternative, while others are complementary, and can be used together in the breeder's decision-making. In this way, it was genotype 21 was identified as a promising candidate due to its superior dry matter yield (DMY), consistent performance, and adaptability to varying environmental conditions across different cuts., in different cuts.

One of the main challenges for breeders is determining which methodologies to use for assessing adaptability and stability to recommend a specific cultivar for either a particular region or a broader area. Consequently, numerous studies in the literature compare these methodologies across various crops, including maize (Oliveira et al., 2017), sugarcane (Paula et al., 2014), soybean (Woyann et al., 2018), wheat (Roostaei et al., 2014), pea (Fikere et al., 2014), blackeyed cowpea (Nunes et al., 2014), and rice (Silva Junior et al., 2020).

However, comparing these methodologies is not relevant, as each one is designed to answer different questions, even if some provide similar estimates.

For many decades, cultivar recommendations based on evaluations across various environments have been a significant area of interest. Currently, new methodologies continue to be proposed to aid breeders in this task. A thorough analysis highlights significant contributions to key concepts like production potential, relative superiority, ecovalence, invariance, predictability, plasticity, and responsiveness. Additionally, various statistical modeling strategies have been developed to succinctly capture these concepts for breeders' use. For instance, modern computational intelligence methodologies (Silva Junior et al., 2022) and fuzzy logic (Silva Junior et al., 2021) are noteworthy as they enable machine learning to provide less subjective interpretations of information or concepts initially presented by Eberhart and Russell (1966) or Lin and Binns (1988). Techniques like GGE biplot and AMMI leverage the GE or GC interaction phenomenon, allowing for a series of graphical analyses that simultaneously interpret environments and genotypes, visualizing invariance, responsiveness, and response pattern similarities.

The presence of multiple methodologies to address crop adaptability and stability, or to analyze the same dataset, suggests that the ideal method has yet to be determined. Consequently, modern and robust techniques like Bayesian inference (Couto et al., 2015) and quantile regression (Barroso et al., 2015) aim to encompass concepts that breeders already recognize and value. These methods do not necessarily introduce new concepts but offer more precise approaches considering experimental heterogeneities, failures, disruptions, and other factors. It is also assumed that incorporating all important concepts for evaluating an individual's superiority and recommendation into a single statistical model is unnecessary. Instead, these concepts should be easily accessible to support meta-analysis, enabling swift and effective decision-making. Consequently, it is recommended to generate information for well-established and readily available concepts, even if they are distinct, within a framework of proposed methodologies.

CONCLUSION

Combining information and providing a comprehensive overview of the behavior of alfalfa genotypes has proven to be an effective approach for studying adaptability and stability. The multi-information analysis identified genotype 21 as the most promising due to its relative superiority in dry matter yield (DMY), predictable behavior, and responsiveness to environmental variations across different harvests. Recommendations based on multi-information analysis help breeders develop new varieties that not only have high yield but are also resilient to climate variations. These analyses provide valuable information to farmers and agronomists about which cultivars are best suited for specific regions, optimizing resource use and increasing sustainability. In addition to helping farmers select cultivars with high adaptability and stability, they can help farmers minimize the risks associated with crop failures due to adverse weather conditions.

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DATA AVAILABILITY

The datasets generated and/or analyzed in this study, as well as the supplementary tables and figures, are available from the corresponding author upon reasonable request.

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