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# Outlying Observation in Stability Analysis of Genotype: AMMI vs Huehn Method

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**Abstract.** Producing a quality crop that has superior characteristics is one of the important things in agronomy. An assessment is needed to see if the superiority is stable in various environmental conditions or various locations. The most favorite and powerful assessment is the parametric approaches using the AMMI model which generates Biplots to visualize stability and adaptability. However, this approach requires assumptions, namely normality, and homogeneity of variance. The Huehn method as a non-parametric approach based on genotype ranking does not depend on those assumptions. Evaluating the performance of the two approaches is very important to characterize their statistical properties. By a particular scheme of simulation, it can be evaluated the resistance of AMMI approaches to the presence of outlying observation. This study added 2%, 5%, and 10% outliers for genotypes across environments to rice and soybean data sets. Outliers were given by adding 3 times the standard deviation to the largest value in the randomly selected column/environment. It was found that the AMMI was sensitive to the presence of outliers even in the low number of outliers. The Huehn method is robust to the presence of outliers, but it tends to infer genotypes as stable by the conservative chi-square test. We propose to see the stability of each genotype relative to the others, using the rank of  $Z^{(1)}$  and  $Z^{(2)}$  indexes. Some genotypes which are relatively the most stable compared to others both based on  $Z^{(1)}$  and  $Z^{(2)}$  are similar.

## BACKGROUND

Producing a variety that has superior properties is an important goal in agronomy. Breeders carry out a series of plant breeding programs to achieve these goals. In many cases, the superior properties of a plant are desired to be stable in various environmental conditions. So that an assessment of whether it is stable or not needs to be done, especially if the plant variety is still new [1].

Several stability statistics have been proposed to investigate G (Genotype), E (Environment), and GEI (Genotype  $\times$  Environment interaction) effects occurring in Multi-Environments Trials (MET) [2], [3]. The methods that can be used to determine genotype stability are parametric and non-parametric analysis approaches. The AMMI (Additive Main effect and Multiplicative Interaction) method is one of the most widely used. However, the AMMI method as a parametric analysis requires the fulfillment of certain assumptions, namely a normal distribution and homogeneous variance [4]–[6]. Theoretically, if there are outliers in the data, i.e. objects that are unusual or different from the majority of the data, it can cause the data to deviate from the assumption of normality so that the use of AMMI is not appropriate. Whereas in agriculture it is very possible for outliers [7], [8]. Some researchers overcome this by removing the outlier data. Whereas to get superior varieties, outliers become something valuable. Other researchers still use the parametric method without taking into account the state of the data whether the assumptions are met or not.

Meanwhile, there is another way of stability analysis using the Huehn non-parametric method, briefly written as the Huehn method. The Huehn method, like other non-parametric methods, is based on ranking, so it is a distribution-free test because they don't assume that the data follow a specific distribution [1], [2], [9]. According to Huehn [10], there are a few benefits of nonparametric stability analysis contrasted with parametric measures: (i) decrease the bias

caused by an outlier, (ii) no distribution assumption needed about phenotypic data, (iii) it is easy to use and interpret stability parameters based on ranks. Despite all these advantages, when the nonparametric method works under a few assumptions, it is considered less potent than the parametric approach. Parametric tests are in general more powerful and require a smaller sample size than nonparametric tests.

The objectives of this study were focused to: (i) identify the statistical properties of the AMMI and Huehn methods to assess stability, (ii) identify the sensitivity of AMMI to the outliers based on repeatability of stability classification before and after the addition of outliers, (iii) apply the Huehn method to the phenotypic data from MET, (iv) identify the most stable genotype based on two indexes,  $Z^{(1)}$  and  $Z^{(2)}$ , proposed by Huehn.

## METHODS

The main problem that has been studied in this research starts from the idea that theoretically if the data conditions have outliers and are not normally distributed, the analysis using AMMI is invalid. On the one hand, there is a non-parametric method that is based on rank, not affected by the presence of outliers, and no certain assumption is underlying. One of which is the Huehn method.

### AMMI Model: Parametric Analysis of Plant Genotype Stability

The AMMI model is a method that combines analysis of variance (ANOVA) on additive effects and Principal Component Analysis (PCA) on multiplicative effects [11], [12]. The multiplicative effect was obtained through the Singular Value Decomposition (SVD) procedure from the interaction matrix of environmental and genotype effect and extracted into several Interaction Principal Component (IPC) [13], [14].

AMMI models are equipped with the use of a biplot. The AMMI biplot is the main advantage of the AMMI model which has made a major contribution to breeding research. The first step to start AMMI analysis is to look at the additive effect of genotype and environment using ANOVA and the decomposition of the GEI was obtained from the PCA. The first two principal components are used to define the region of stability in the AMMI Biplot [2], [4], [15], [16].

The interaction effect and mean error  $(\tau\delta)_{ij} + \bar{\epsilon}_{ij}$  can be disparted into  $S$  main components and then modeled as follows (Eq.1):

$$\bar{y}_{ij} = \mu + \tau_i + \delta_j + \sum_{k=1}^K l_k a_{ik} t_{jk} + \theta_{ij} \quad (1)$$

Where  $l_k$  is the singular value for the  $k$ -th principal component axis,  $a_{ik}$  is the genotype feature vector for the  $k$ -th axis, and  $t_{jk}$  is the environmental feature vector. The residual  $\theta_{ij}$  appears if not all axes are used. The maximum number of axes used is  $(g - 1, e - 1)$  axes. Depending on how many axes are used, the AMMI model can be expressed as AMMI-0, AMMI-1, ..., AMMI- $K$  models. Where AMMI-0 if no axes are used, while AMMI- $K$  if all axes are used.

To see stable and specific genotypes in a particular environment, AMMI-2 biplot was used [17], [18]. The AMMI-2 biplot is a plot between component 1 and component 2. From the AMMI-2 biplot, it can be seen which genotype interacted with a specific environment or location. The closer the location is to the genotype, or the smaller the angle between them, the stronger the interaction [16].

The AMMI model requires the assumption of normality and homogeneity of variance. In case the data is not normally distributed, the feasibility of the AMMI model is not met. If the data matrix is independent, normally distributed with constant variance, the maximum probability estimator is reduced to SVD. When the distribution is not normal, this is no longer true. Data that are non-normal distributed tend to have heterogeneous variance, and when modeled with AMMI is biased, and the estimated scale may also be unsatisfactory. Both of these phenomena may require the inclusion of additional interaction terms [19].

### Huehn Method as Non-Parametric Stability Analysis

The non-parametric method can be used in determining stability based on genotype ranking in addition to the parametric method which is based on absolute data and has been widely known [20]. Stability measurement based on ranking does not require certain assumptions so that it is easier to use and interpret and is less sensitive to measurement error than parametric. The addition or subtraction of one or more observational data does not cause large variations in the estimation results when compared to the effect on parametric methods [21].

Huehn Method [10], uses two indexes,  $S_i^{(1)}$  and  $S_i^{(2)}$  to test overall genotype. The formulas are shown in Eq. 2 and Eq. 3 below:

$$S_i^{(1)} = \frac{\sum_{j < j'} |r_{ij} - r_{ij'}|}{e(e-1)/2} \quad (2)$$

$$S_i^{(2)} = \frac{\sum_{j=1}^e (r_{ij} - \bar{r}_i)^2}{e-1} \quad (3)$$

$S_i^{(1)}$  is the mean of the absolute value of the difference in the ranking of  $i$ -th genotype for all  $e$  locations.  $S_i^{(2)}$  is the variance of the rank,  $\bar{r}_i = \sum_{j=1}^e r_{ij}/e$ ;  $\bar{r}_i$  interpreted as the expected value of  $r_{ij}$  in the maximum stability.  $r_{ij}$  = rank of  $i$ -th genotype in  $j$ -th environment. The  $i$ -th genotype may be considered to be stable over environments if its ranks are similar over environments (maximum stability = equal ranks over environments). Under the null hypothesis, there is no significant difference stability between the genotypes, the test is conducted using  $\chi^2$  distribution with  $g$  degrees of freedom ( $g$  is the number of genotypes); The test statistic is  $Z(m) = \sum_{i=1}^g Z_i^m$ . If the  $Z(m) > \chi^2_{(\alpha; g)}$  then  $H_0$  rejected. The formulas needed to calculate  $Z_i^m$  were in Eq. 4 - Eq. 6.

$$Z_i^m = \frac{[S_i^m - E(S_i^m)]^2}{Var(S_i^m)}, m = 1, 2 \quad (4)$$

$$E(S_i^{(1)}) = \frac{g^2-1}{3g}, Var(S_i^{(1)}) = \frac{(g^2-1)[(g^2-4)(e+3)+30]}{45g^2e(e-1)} \quad (5)$$

$$E(S_i^{(2)}) = \frac{g^2-1}{12}, Var(S_i^{(2)}) = \frac{(g^2-1)[2(g^2-4)(e-1)+5(g^2-1)]}{360e(e-1)} \quad (6)$$

The individual test for each of the  $i$ -th genotype performed on  $S_i^{(1)}$  and  $S_i^{(2)}$  index using null hypothesis that the  $i$ -th genotype is stable and the test statistic is  $Z_i^{(m)}$ ;  $m = 1, 2$ ;  $i=1, \dots, g$ .  $H_0$  is rejected if  $Z_i^{(m)} > \chi^2_{(\alpha; 1)}$ .

Genotype classification using the Huehn method was determined based on  $Z_i^{(m)}$ . The smaller  $Z_i^{(m)}$  the more stable the genotype. A simple way that can be used to study relative stability among the tested genotypes is by ranking the genotypes based on the values of  $Z_i^{(1)}$  and  $Z_i^{(2)}$ .

## The Scheme of Data Simulation

This study used two secondary data sets from the multi-environments trials. The first was obtained from the Indonesian Center of Rice Research (ICRR) in a series of functional rice development through the Fe biofortification program in collaboration with IRRI. This experiment was conducted at 8 locations, involving 8 genotypes and 2 comparing varieties. The second data set is the result of a MET of soybean from the Indonesian Legume and Tuber Crop Research Institute (ILETRI), involving 15 soybean genotypes at 8 locations.

The simulation was conducted by adding outliers in the row or column in the GEI matrix of size  $g \times e$  because the addition of outliers in the replication did not affect the stability analysis as reported by [22]. Each cell of the GEI matrix was the mean of all replications of a certain genotype in a certain environment. The scenario of outliers addition was adding outlier as much as 2%, 5%, and 10% of the number of cells of the GEI matrix, without replacement, but the maximum number of outliers added was  $e$  (number of column of the GEI matrix). This was done with the consideration that what was being studied was the stability of genotypes across environments. So that the implementation on the column, adjusted to the position of the GEI matrix where the columns are the environment, and the rows are genotypes. The outliers given are extreme values in the right/upper direction, extreme left values are considered the same because the normal distribution is symmetrical. They were given by adding 3 times the standard deviation to the largest value in the randomly selected column.

After outliers addition, the stability analysis was carried out using AMMI to classify genotype as stable or not. Then, study the consequences of outlier observations on the results of the analysis of genotype stability of original data (without outlier), by looking at shifts and changes in classifying the genotype. The application of the Huehn method on both data set is also conducted. Then assess the stability of the genotype relative to others.

## RESULT AND DISCUSSION

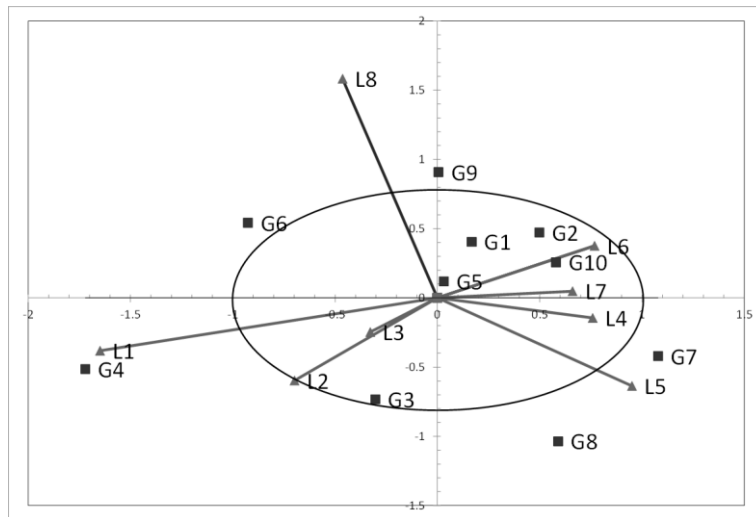
### Effect of Outliers on Genotype Stability Analysis of AMMI

Stability analysis through the AMMI model was carried out by using the concept of an ellipse as a normal bi-variate confidence area as used by many authors [1], [18], [23]. The Analysis of variance (ANOVA) of the AMMI model for rice data is presented in Table 1, while the AMMI Biplot and its genotype stability ellipse are shown in Fig. 1. Figure 1 shows that G4, G6, G7, G8, and G9 are outside the ellipse, the confidence interval to determine genotype stability. This provides information that those 5 genotypes were unstable. In the case with an additional 2% outlier, 2 genotypes changed their status, both of which changed from unstable to stable (Table 2). Meanwhile, in the additional 5% of outliers, 4 genotypes changed their status, one of which changed from stable to unstable. An additional 10% outlier resulted in the same genotypic stability as for an additional 5%. With additional 2%, 5%, and 10% outliers between genotypes, some genotypes changed their stability status, but those changes were identical for 5% and 10% outlier. These results indicate that the AMMI model tends to be susceptible to outlier provide genotype stability results.

**TABLE 1.** AMMI analysis for rice data

Source of Variation	Degrees of Freedom	Sum of Square	Mean Square	F-Value	Sig.
Genotype	9	19.92	2.214		
Environment	7	79.19	11.313		
Interaction (GEI)	63	59.35	0.942		
IPC 1	15	36.41	2.427	8.7617	2.8756
IPC 2	13	13.23	1.018	3.6751	2.9827
Residual	35	9.71	0.277		
Total	79				

Note: IPC= Interaction Principal Component



**FIGURE 1.** AMMI-2 biplot and ellipse plot for genotype stability of rice data without outliers

**TABLE 2.** Genotype stability classification of the rice using AMMI due to outliers addition

Genotype	Original Data	2% Outliers	5% Outliers	10% Outliers
G1	S	S	NS	NS
G2	S	S	S	S
G3	S	S	S	S
G4	NS	NS	NS	NS
G5	S	S	S	S
G6	NS	<b>S</b>	<b>S</b>	<b>S</b>
G7	NS	<b>S</b>	<b>S</b>	<b>S</b>
G8	NS	NS	NS	NS
G9	NS	NS	<b>S</b>	<b>S</b>
G10	S	S	S	S

Note: S=stable; NS=Not stable; bold=shifted stability compared to the original data

**TABLE 3.** Changes in the genotype stability of soybean using AMMI due to outliers addition

Genotype	Original Data	2% Outliers	5% Outliers	10% Outliers
G1	S	S	S	S
G2	S	S	S	S
G3	NS	NS	<b>S</b>	<b>S</b>
G4	NS	<b>S</b>	<b>S</b>	<b>S</b>
G5	S	S	NS	S
G6	NS	NS	<b>S</b>	NS
G7	NS	NS	NS	NS
G8	NS	NS	<b>S</b>	NS
G9	NS	NS	<b>S</b>	<b>S</b>
G10	S	<b>NS</b>	S	S
G11	NS	<b>S</b>	<b>S</b>	NS
G12	S	S	S	S
G13	S	S	S	S
G14	S	S	NS	S
G15	S	S	NS	S

Note: S=stable; NS=Not stable; bold=shifted stability compared to the original data

The use of AMMI to analyze the second data set, soybean seed weight per plant, is as follows. Based on the ellipse confident interval, G3, G4, G6, G7, G8, G9, and G11 are determined as unstable (ellipse not shown). The stability classification on the original data and the data with outliers using the same scenario is shown in Table 3. An additional 2% of outliers, 3 genotypes changed their stability status, namely G4, G10, G11. With the addition of 5% outliers, the number of changes increased threefold, from 3 to 9 genotypes that changed stability. It appears that the number of changed genotypes increased by the increasing number of outliers.

However, for an additional 10% of outliers, the number of changes is not as much as 5% of outliers. This is caused by the fact that in one-dimensional data, outliers are always found at a low percentage. Outliers with a greater frequency are no longer detected as outliers but will shift the overall center of the distribution, and the mean. Moreover, in a multidimensional GEI matrix, outliers in the column direction will simultaneously affect the rows. This will cause the slightest outlier to be detected in both row and column directions.

The number of outliers is very relative, depending on whether they are in the same row or column. If the addition of 2% or as many as 3 outliers in different rows will change 3 genotypes, then 6 outliers (5%) change 9 genotypes. An additional 10% of outliers in the GEI matrix of soybean means that there are an additional 12 outliers. They will

be spread across 15 rows across 8 columns so that the probability that they will be in the same column will be even greater. If they are on the same column, it is most likely not detected as two outliers on that row but have changed/or shifted the overall distribution.

### The Robustness of Huehn's Non-Parametric Genotype Stability Analysis Against Outliers

Stability analysis using the Huehn method on soybean seeds weight per plant was shown in Table 4. For testing the overall genotype, the test statistics  $Z(1) = \sum_{i=1}^{15} Z_i^{(1)} = 13.5076$ , and  $Z(2) = \sum_{i=1}^{15} Z_i^{(2)} = 15.694$ . The critical value was  $\chi^2_{(0.05; 15)} = 24.996$ ; The test statistic  $Z(m) = \sum_{i=1}^g Z_i^m$  showed insignificant results at  $\alpha = 0.05$ . The test statistics for both were less than the critical value, which means that there is no difference in ranking variance between the 15 soybean genotypes or it can be stated that in general there was no difference in the stability of the 15 soybean genotypes. The individual tests on  $S_i^{(1)}$  and  $S_i^{(2)}$ , test statistic  $Z_i^m$ ,  $m=1,2$  of each of  $g$  genotype is less than the critical value  $\chi^2_{(0.05; 1)} = 3.841$  means that all 15 genotypes were stable at  $\alpha = 0.05$ .

Genotype classification by the Huehn method is determined based on the  $Z_i^{(m)}$  index. The index can be used to obtain relative stability among the tested genotypes. The smaller the  $Z_i^{(m)}$  the more stable the genotype. It is simply obtained by ranking the  $Z_i^{(1)}$  and  $Z_i^{(2)}$  index as shown in Table 4.

TABLE 4. Stability analysis of soybean genotype using Huehn method

Genotype	$S_i^{(1)}$	$S_i^{(2)}$	$Z_i^{(1)}$	$Z_i^{(2)}$	Rank $Z_i^{(1)}$	Rank $Z_i^{(2)}$
G1	6.1786	26.8393	1.4831	1.4265	12	11
G2	5.8929	24.2679	0.8613	0.6701	9.5	8
G3	5.6429	24.5000	0.4550	0.7267	8	10
G4	5.8929	24.4107	0.8613	0.7047	9.5	9
G5	6.6071	30.6964	2.7306	3.0907	14	15
G6	3.8214	10.2679	1.3753	1.5065	11	12
G7	3.2857	7.7143	2.9448	2.5619	15	13
G8	4.8214	15.6964	0.0251	0.1884	1.5	3
G9	4.3571	13.3571	0.3962	0.6021	6.5	6
G10	5.2500	19.1250	0.0762	0.0045	4	1
G11	4.7143	15.1429	0.0714	0.2652	3	5
G12	4.6071	15.4107	0.1413	0.2264	5	4
G13	4.3571	13.1429	0.3962	0.6517	6.5	7
G14	6.2500	30.4107	1.6648	2.9457	13	14
G15	4.8214	16.2679	0.0251	0.1229	1.5	2
Total			13.5076	15.6940		

**TABLE 5.** Stability analysis of rice genotype using Huehn method

Genotype	$S_i^{(1)}$	$S_i^{(2)}$	$Z_i^{(1)}$	$Z_i^{(2)}$	Rank $Z_i^{(1)}$	Rank $Z_i^{(2)}$
G1	1.6429	1.9286	6.4366*	4.4249*	8	8
G2	2.7857	6.2143	0.6199	0.4589	3	3
G3	2.2143	4.0000	2.7629	2.0001	5	5.5
G4	4.7857	17.6429	5.1738*	9.7694*	7	10
G5	1.5000	1.7143	7.5942*	4.7300*	10	9
G6	3.3214	7.6964	0.0011	0.0339	1	2
G7	4.0714	11.7143	1.3948	1.3289	4	4
G8	2.9286	7.7143	0.3234	0.0318	2	1
G9	2.0714	4.0000	3.5378	2.0001	6	5.5
G10	1.5357	2.2679	7.2958*	3.9627*	9	7
Total			13.5076	15.6940		

Note: \*= significant = not stable

It can be seen that according to the rank of  $Z_i^{(1)}$ , the most stable genotypes relative to others were G8, G15, G11, G10, G12, respectively. The G8 and G15 relatively have similar stability, indicated by their similar ranking. While based on  $Z_i^{(2)}$ , the most stable were G10, G15, G8, G12, G11, respectively. From both indexes, the most stable genotypes were similar, although in a different order.

Huehn Stability analysis on Rice Fe content was shown in Table 5. Testing the overall genotype resulting in the test statistics  $Z(1) = 35.1402$ , and  $Z(2) = 28.7406$ . The critical value was  $\chi^2_{(0.05; 10)} = 18.3070$ ; The test statistic  $Z(m) = \sum_{i=1}^g Z_i^m$  showed significant results at  $\alpha = 0.05$ , which means that in general there was a significant difference in the stability of the 10 rice genotypes. The individual tests on  $Z_i^{(1)}$  and  $Z_i^{(2)}$ , showed that G1, G4, G5, and G10 were unstable genotypes at  $\alpha = 0.05$ , which were the test statistic  $Z_i^m$ ,  $m=1,2$  of that four genotypes is more than the critical value  $\chi^2_{(0.05; 1)} = 3.841$ .

According to the rank of  $Z_i^{(1)}$  (Table 5) the most stable genotypes relative to others were G6, G8, G2, G7, G3, respectively. While based on  $Z_i^{(2)}$ , the most stable were G8, G6, G2, G7, G3, and G9. Based on  $Z_i^{(2)}$ , the G3 and G9 relatively have similar stability, indicated by their similar ranking. From both  $Z_i^{(1)}$  and  $Z_i^{(2)}$  indexes, the most stable genotype were mostly similar, except for G9 that did not exist in  $Z_i^{(1)}$ ; G6 and G8 have a reverse ranking. Which index is better in analyzing stability is still unanswered. There is still no conclusive argument for the preference of one of the stability index  $S_i^{(1)}$  and  $S_i^{(2)}$  related to the 'classical' measures can be given [10].

The scenario of adding outliers by randomly selecting the environment and adding a determined value to the genotype with the highest value in the selected environment will not change the ranking of the genotype in the selected environment. This, of course, will not change the value of the Huehn index which is calculated based on the ranking of genotypes in each environment. Furthermore, it will also not affect the test statistic in the chi-square test to assess the stability of the genotype. So the analysis using the Huehn method on data that has been given additional outliers no longer needs to be done because the results will be the same as the results of the analysis on the original data without outliers.

Very different from what happened in the AMMI model, as has been shown previously. Stability analysis by the AMMI model is very susceptible to outliers. However, it should be noted that with the chi-square test, both individually and in total, in both soybean and rice data, there is an indication of the tendency of the Huehn method to declare a stable genotype as condition H0, and with a strict probability of error ( $\alpha$ ) to declare an unstable genotype. The ranking of the  $Z_i^{(1)}$  and  $Z_i^{(2)}$  seem to be an alternative way to express the stability of individual genotypes relative to each other. It is shown in the rice and soybean data, the ranking method based on  $Z_i^{(1)}$  and  $Z_i^{(2)}$  gives relatively the same results namely G8, G10, G11, G12, and G15 in soybean data, and G2, G3, G6, G7, and G8 in rice data.



## CONCLUSION

The genotype stability analysis obtained from the AMMI model tends to be sensitive to outliers in the interaction matrix, even in the low number of outliers. While the non-parametric Huehn method is robust to outliers. Classifying genotype using AMMI can be different because of the existence of outlier. Therefore, the use of AMMI needs to be aware of the existence of outliers that can change the results of genotype stability. On the other hand, Huehn's method is very prospective to overcome the problem of outliers.

It is necessary to study the development of the Huehn method regarding the tendency of this method to reveal a stable genotype. As an alternative way, the ranking of the square of standardized index can be used to express the stability of individual genotypes relative to others, where genotypes with smaller indexes are relatively more stable. The most stable genotype based on two Huehn's indexes reveals the same stable genotype, using either  $Z^{(1)}$  or  $Z^{(2)}$ . The G8, G10, G11, G12, and G15 in soybean data, and G2, G3, G6, G7, and G8 in rice data were determined as the most stable genotypes relative to others.

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