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EVALUATION OF PHENOTYPIC STABILITY IN BREAD WHEAT ACCESSIONS USING PARAMETRIC AND NON-PARAMETRIC METHODS

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ABSTRACT

In order to compare parametric and nonparametric stability measures, and to identify high-yield and stable bread wheat genotypes, 20 accessions were grown in a randomized complete block design with 3 replications under 6 irrigated and dryland conditions. Combined analysis of variance (ANOVA) and Bredenkamp test indicated the presence of significant genotype ×environment interactions. Parametric and non- parametric stability statistics introduced genotypes: G13, G15 and G18 as the most stable. The statistics ∂_i^2, *bi*, $S_i^{(3)}$ and $S_i^{(6)}$ were desirable for the simultaneous selection of yield and stability. Spearman's rank correlation indicated that most non-parametric measures were significantly (P<0.01) inter-correlated with parametric measures and therefore can be used as alternatives.

Key words: Bread wheat, GE interaction, parametric and non-parametric measures, stability.

INTRODUCTION

Wheat (*Triticum aestivum* L.) has become an important stable food and is the major cereal crop in Iran. On which the food security rests. Improvement in its productivity has played a significant role in making the country self-sufficient in food production. Plant breeders generally agree on the importance of high yield stability, but there is less accord on the most appropriate definition of 'stability' and on methods to measure and to improve yield stability. The development of varieties which can be adapted to a wide range of environments is the crucial goal of plant breeders in a crop improvement program (Mohebodini *et al*., 2006). High yield stability usually refers to a genotype's ability to perform consistently, across a wide range of environments (Annicchiarico, 2002). Knowledge of genotype-by-environment interaction presenting valuable information in plant breeding studies can help plant breeders to reduce the cost of extensive genotype evaluation by eliminating unnecessary testing sites (Letta, 2009). Stability, adaptability and mean yield across all environments are more important than yield for specific environments; hence, cultivars are being selected for a large group of environments (Piepho, 1996). Multi environment yield trial can be analyzed to extract more information on stability, adaptability and yield performance using various statistical methods and software used by different investigators (Hussein *et al*., 2000; Gauch, 2006; Yan *et al.,* 2007). Plant breeders use different methods for analysis of GEI.

Parametric methods for estimating genotype × environment interactions and phenotypic stability are widely used in plant breeding and production. The proper use of these parametric measures requires some statistical assumptions, like showing the normal distribution of errors and interaction effects (Hussein *et al*., 2000).

Some stability parameters are: environmental variance (S_{xi}^2) (Becker and Leon, 1988), superiority index (PI) (Lin and Binns, 1988), Wricke's (1962) ecovalence (W_i^2), Shukla's (1972) stability variance (∂_i^2), Francis and Kanenberg's (1978) coefficient of variability (CVi), Freeman and Perkins (1971) stability method. All these methods are parametric. Nonparametric measures for stability based on ranks provide a viable alternative to existing parametric measures based on absolute data. For many applications, including selection in breeding and testing programs, the rank orders of the genotypes are the most essential information. Stability measures based on ranks require no statistical assumptions about underlying the distribution of the phenotypic values. They are easy to use and interpret and less sensitive to errors of measurement in comparison with parametric measures. Furthermore, addition and deletion of one or a few observations is not as likely to cause great variation in the estimates as many of the nonparametric methods have recently been compared by others (Sabaghnia *et al*., 2006; Mohammadi *et al*., 2007a, 2007b, Farshadfar *et al*., 2012).

The level of association among adaptability or stability estimates of different models is indicative of whether one or more estimates should be obtained for reliable prediction of cultivar behavior, and also helps the breeder to choose the best adjusted and most informative stability parameter(s) to fit his/her concept of stability (Duarte and Zimmermann, 1995).The main objectives of this study were to (i) compare parametric and nonparametric stability statistics of 25 bread wheat genotypes' (ii) determine promising genotypes with high yield and stability (iii) evaluate the level of associations among the parametric and nonparametric stability parameters.

MATERIALS AND METHODS

This study was carried out across six locations during the 2011-2013 growing seasons. 20 bread wheat genotypes were grown in College of Agriculture, Razi University, Kermanshah, Iran (47° 9′ N, 34° 21′ E and 1319 m above sea level).

All genotypes were selected from international nurseries provided by the Seed and Plant Improvement Institute (SPII) of Karaj, Iran.

The experimental layout was a randomized complete block design, with tree replications for bread wheat, in each environment. Sowing was done with an experimental drill in 2 m × 2 m plots, consisting of four rows spaced 20 cm apart. The seeding density was 400 seeds for each location.

Statistical Analysis: Mean grain yield data per plot was converted to gr and subjected to combined analysis of variance in order to partition sum of squares to genotype, environment and genotype-environment interaction effect using Statistical Analysis Software (SAS, V9).

Then eight parametric stability parameters were performed inaccordance with Eberhart and Russel's (1966) the regression coefficient (*bi*) and deviation from regression (s^2_{di}), Pinthus's (1973) coefficients of determination (R^2), Wricks's (1962) ecovalance (w^2_i), Shukla's (1972) stability variance (∂^2_i), Francis and Kannenberg's (1978) coefficient of variability (CVi) and genotypic variance (s^2_i); Lin and Binn's (1988) superiority index (Pi) which the genotypes of greatest interest would be those with the lowest Pi values.

Four nonparametric statistical methods of Kubinger (1986), Hildebrand (1980), Bredenkamp (1974) and De Kroon and Van der Laan (1981) were used to test the significance of GEI (Huehn and Leon, 1995). The methods of Kubinger and Hildebrand are applied on the basis of the usual linear model of interaction (deviation from additively of main effects for genotypes and environments). The method of De Kroon and Van der Laan (1981) defines interaction based on the crossover interaction model. The method of Bredenkamp (1974) employed the usual model for interactions defined as deviations from the additively of main effects. The test statistics for GEI are approximately χ^2 distributed with (p– 1) (q – 1) degrees of freedom, where p = the number of genotypes, and q = the number of environments. The nonparametric statistical procedures used for stability analysis of genotypes were proposed by Nassar and Huehn (1987), Kang (1988), Fox *et al*. (1990), and Thennarasu (1995). For a two-way data set with l genotypes and m environments, we denote as the rank of genotype i in the environment j, and $_{i.}$as the mean rank across all environment for genotype i. The genotype with the highest yield was given a rank of 1 (1= number of genotypes). The Huehn' s (1979) and Nassar and Huehn' s (1987) stability measures based on yield ranks of genotypes in each environment are expressed as follows:

$$S_i^{(1)} = 2\sum_{j}^{m-1}\sum_{j=j+1}^{m} |rij\text{-}rij| \,/\, [m(m-1)]$$

$$S_i^{(2)} = \sum_{j=1}^{m}\left(rij-\bar{r}i.\right)^2 / \left(m-1\right)$$

$$S_i^{(3)} = \sum_{j=1}^{m}\left(rij-\bar{r}i0\right)^2 / \bar{r}i0$$

$$S_i^{(6)} = \sum_{j=1}^{m} |r_{ij} \quad \bar{r}_{i0}| / \bar{r}_{i0}$$

Rank-sum, proposed by Kang (1988), is another nonparametric stability procedure in which both yield and Shukla's (1972) stability variance are employed as selection criteria. This index assigns a weight of 1 to both yield and stability statistics in order to identify high-yielding and stable genotypes. The genotype with the highest yield was given a rank of one and the genotype with the lowest stability variance was assigned the rank of one. In this manner, ranks of yield and stability variance are summed for each genotype. The genotype with the lowest rank-sum was the most desirable. Fox *et al*. (1990) suggested a nonparametric superiority measure for general adaptability using stratified ranking of cultivars. A genotype that occurred mostly in the top third (high TOP- value) was considered a widely adapted cultivar. Thennarasu's (1995) nonparametric stability analysis considers adjusted ranks of genotypes within each test environment. The nonparametric stability measures can be seen in Thennarasu (1995).

The adjusted rank, r^*_{ij} (Thennarasu 1995) is determined on the basis of the adjusted values ($X^*_{ij} = X_{ij0} - X_{i0} + X_{00}$), where X_{i0} is the mean performance of genotype i, X_{ij} is the performance of genotype i in environment j and X_{00} is the overall mean across environments. The ranks, obtained from these adjusted values (X^*_{ij}), depend only on GE interaction and error effects.

$$NP_i^{(1)} = \frac{1}{m}\sum_{j=1}^{m} |r^*_{ij} - M^*_{di}|$$

$$NP_i^{(2)} = \frac{1}{m}\left(\sum_{j=1}^{m} \left| r_{ij}^{*} - M_{di}^{*} \right| / M_{di}\right)$$

$$NP_i^{(3)} = \frac{\sqrt{\sum \left(r_{ij}^{*} - \bar{r}_{i0}^{*} \right)^2 / m}}{\bar{r}_{i0}}$$

$$NP_i^{(4)} = \frac{2}{m(m-1)}\left[\sum_{j=1}^{m-1} \sum_{j'=j+1}^{m} \left| r_{ij}^{*} - r_{ij'}^{*} \right| / \bar{r}_{i0}\right]$$

In the above formulas, r_{ij}^{*} is the rank of X_{ij}^{*} and $\bar{r}_{i0}^{*}$ and M_{di}^{*} are the mean and median ranks for adjusted values, where $\bar{r}_{i0}$ and M_{di} are the same parameters computed from the original (unadjusted) data.

The non- parametric statistics measures were derived from the grain yield data and Spearman's rank correlation between parametric and nonparametric methods was estimated to assess the interrelationship and similarity among them. All analysis was performed using SAS and SPSS software (SAS Institute, 1999).

RESULTS AND DISCUSSION

The genotype × environment (GE) interaction is a major challenge to plant breeders. Many stability parameters for genotypes grown in different environments were developed for this purpose and each has its advantages and limitations. In various methods, GE interactions are used to characterize the response of genotypes to changing environments along with mean grain yields. Accordingly, genotypes with a minimal variance for yield across environments are considered stable (Mohammadi, 2012).

The values of the test statistics for the used statistical procedures are presented in Table 2. F and χ^2 values were respectively used for ANOVA and non-parametric methods with the aim of test the effects of G, E and GE interaction. Genotypes and environments main effects and GEI were significant at ANOVA and Bredenkamp method (Ayalneh *et al*., 2013). But, another procedure was not significant. GEI effect suggest that there are significant differences in responses of genotypes to environments, and hence sensitivity and instability (Ajcura *et al*., 2009, Mohammadi *et al*., 2009 and Kilic, 2012).

Parametric measures: The results of eight parametric stability parameters and mean grain yield are presented in Table 2. The measures of adaptability and stability are necessary for its suggestion to target environments for preferring genotypes. According to Eberhart and Russell (1966) model, a stable genotype has high mean yield, bi= 1 and S_{di}^2 = 0.Genotypes have general adaptability when associated with high mean yield while genotypes are poorly adapted to environments when associated with low mean yield. Regression coefficient (bi) values above1.0 define genotypes with higher sensitivity to environmental alterations. Regression coefficients decreasing below 1.0 ensure a measure of greater resistance to environmental variation, and hence, increasing specificity of adaptability to low yielding environments. Genotypes G9, G12, G16 and G19 had higher grain yields and a coefficient values greater than 1.0 (Table 3). These genotypes are sensitive to environmental variations and would be suggested for cultivation under suitable conditions, whereas G6, G8 and G11 with *bi*<1 and lowest average yields were poorly adapted across environments and might have specific adaptation to harsh conditions. On the contrary,G2, G13 and G20 had higher grain yields and a coefficient values near 1.0. These genotypes showed average stability. Among these cultivars, G18 was the most appropriate one, because it had higher yield value than the mean, *bi* values near 1.0 and low S_{di}^2 (i.e. regression coefficient not significantly different from 1.0 with grain yields above grand mean).According to Francis and Kannenberg's (1978) coefficient of variation stability parameter (*CVi*), genotypes G5, G15 and G19 were considered to be stable although they had low performance except G5,and the genotypes G3, G7, G9 and G16 with the highest yield performance were considered unstable.

Pinthus's (1973) stability parameter or coefficient of determination (R_{di}^2) values which are the predictability of response estimates (R_{di}^2 =1), ranged from 0.1 to 0.9, in which a variation of mean grain yield was accounted for by genotype response across environments. None of values of coefficient of determinations was significantly different from 1.0. In terms of this parameter, all of genotype could be considered stable for grain yield (Table 3).

An unbiased estimate using stability variance (∂_i^2) of genotypes was determined according to Shukla (1972). The stability variance (∂_i^2) revealed that the genotypes G6, G8 and G11 had the smallest variance across the environments, while the genotypes G7, G9, G12 and G16 had the largest ∂_i^2 . Hence, the genotypes G6, G8 and G11 were stable while the genotypes G7, G9, G12 and G16 were unstable.

Wricke (1962) suggested using ecovalance (W_i^2) as a stability parameter. According to this stability parameter, genotypes with the smallest ecovalance (W_i^2) values are consider ed stable. The W_i^2 was lowest for genotypes G4, G13, G18 and highest for G6, G9, G19 and G20 (Table 3). The good correlation between W_i^2

and S_i^2 (r = 0.85**) showed that these two measures led to similar results. According to the environmental variance (S_i^2), G18 followed by G4 and G13 had the lowest variation across environments and G6 followed by G6, G9 and G20 showed the largest variation (Tables 3).

Non-parametric measures: The most severe limitation of the regression approach is the poor repeatability of both *bi* and S_{di}^2 (Jalaluddin and Harrison, 1993); its usefulness in measuring genotype adaptability depends largely on the assumption that genotypes respond linearly to the environments. In such cases, the results of nonparametric estimation and testing procedures, which are based on ranks, can be more reliable (Mut *et al*., 2009). Several multivariate methods proposed allow a more detailed analysis of GEI, but the complexity of these methods, sometimes regarded as their main advantage, paradoxically is the main obstacle to their widely usage in plant breeding (Flores *et al*., 1998 and Soltankohi *et al*., 2015).The results of the significance test for GEI using different nonparametric statistical procedures are presented in Table3. The Kubinger, Hildebrand, and De Kroon/Van der Laan methods were not significant but Bredenkamp procedure was significant. Bredenkampresult was in agreement with the result of ANOVA (Table 2).

The result of 10 different nonparametric stability statistics and genotype mean yields are presented in Table 4. The tests of significance of $S_i^{(1)}$ and $S_i^{(2)}$ were derived from Nassar and Huehn (1987). For each genotype, Z_1 and Z_2 values based on the ranks of adjusted and summed data across genotypes were used to obtain Z values (Table 4); Z_1 sum = 235.8 and Z_2 sum = 30.68. As both of these statistics were more than the critical value, $X^2_{0.05, df = 19}$ =30.1, there were significant differences in rank stability between the 20 genotypes grown in the 6 environments. Upon in section of the individual Z values it was observed that most of the genotypes were significantly unstable relative to each other, because they had small Z values in comparison with the critical value, X^2=3.84. Two rank stability measures ($S_i^{(1)}$ and $S_i^{(2)}$) from Nassar and Huehn (1987) were based on the ranks of genotypes across environments, and they gave equal weight to each environment. For a genotype with maximum stability ($S_i^{(1)} = 0$) $S_i^{(2)}$ gives the variance among the ranks across environments. Zero variance is an indication of maximum stability. Accordingly, $S_i^{(1)}$ and $S_i^{(2)}$ of the tested genotypes showed that genotypes G3, G13, G18, and G19 had the lowest values; therefore, these genotypes were regarded as the most stable genotypes according to $S_i^{(1)}$ and $S_i^{(2)}$. On the other hand, G6, G9, and G16 had the highest $S_i^{(1)}$ and $S_i^{(2)}$ values; therefore, they were determined to be unstable (Tables 4).

Two other nonparametric statistics ($S_i^{(3)}$ and $S_i^{(6)}$) combine yield and stability based on yield ranks of genotypes in each environment (Nassar and Huehn, 1987). $S_i^{(3)}$ and $S_i^{(6)}$ ranged from 7.37 to 28.21 and 1.25 to 4.27,respectively. Genotypes G3, G4, G11 and G13 had the lowest $S_i^{(3)}$ and $S_i^{(6)}$ values; hence, these genotypes were characterized as the most stable genotypes, as well as with regard to $S_i^{(1)}$ and $S_i^{(2)}$ statistics (Table 4). Nonetheless, while the mean yields of G13was high, the mean yield of G3, G4 and G11 were found lower than total mean. While genotypes G2 and G12 were the 2 highest mean yield in genotypes, they were characterized as unstable genotypes according to $S_i^{(2)}$, $S_i^{(3)}$, and $S_i^{(6)}$ parameters (Tables 4).

According to rank-sum (RS) statistics (Kang, 1988), genotypes with a low rank-sum are regarded as the most desirable. This parameter revealed that genotypes G1,G7, G15 and G17 had the lowest values, and were stable genotypes, whereas genotypes G12, G13, and G19, which had the highest values, were undesirable (Tables 4).

Using the stability statistics $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ genotypes with minimum low values are considered more stable (Thennarasu, 1995). According to $NP_i^{(1)}$, genotypes G9, G12, G16 and G18 were considered stable in comparison to the other genotypes, because these genotypes had lower values (Table 4).

On the other hand, genotypes G4, G6, and G8 were unstable according to $NP_i^{(1)}$. Genotypes 3,17and 20 had the lowest $NP_i^{(2)}$ value (it was stable). Although genotypes G12 and G19 had the highest mean yields, their stability was low because of their high $NP_i^{(2)}$ values (Tables 4).

Genotypes G3, G11, G17 and G18 had the lowest $NP_i^{(3)}$ values and, therefore, they were the most stable genotypes. Nonetheless, these genotypes had lower mean yields than the grand mean yield. The genotypes that were unstable based on $NP_i^{(3)}$ were G12, G16, and G19, which had the highest mean yields (Table 4).

According to the $NP_i^{(4)}$ stability parameter, G18 had the minimum value (indicating it was the most stable genotype), followed by G4, G11 and G13. Genotypes G3 and G13 were also identified as stable based on $S_i^{(2)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$ rank-sum (RS). On the other hand, genotypes G9, G12, G16 and G19 had the highest $NP_i^{(4)}$ values and, therefore, were unstable genotypes (Tables 4).

Interrelationship among parametric and nonparametric measures: Rank correlation is an important useful tool in order to reveal the statistical relations between the non-parametric and parametric methods for finding the method used as an alternative to the other methods and eliminating similar parameters. The use of the rank correlation allowed a study of the relationship among the parameters and the similarity of non-parametric and parametric methods. According to the matrix correlation analysis, parametric and non-parametric measures evaluated in this study revealed that these parameters can be used for evaluating the responses of bread wheat genotypes to changing environments. The rank correlations between grain yield and stability measures are given in Table 5.Grain yield is significantly correlated with *bi*, ∂_i^2 (P<0.01) and with the measures of $S_i^{(3)}$ and $S_i^{(6)}$ (P<0.05) and significantly negative association was found with $NP_i^{(1)}$ (P<0.01). In the simultaneous selection for high yield and stability, only the ∂_i^2, *bi*, $S_i^{(3)}$ and $S_i^{(6)}$ were used. Coefficient of regression (*bi*) had negative and significant correlations with $NP_i^{(1)}$ (P<0.01) and showed a significant correlation with S_i^2, *CVi* (P<0.01) and $S_i^{(3)}$ (P<0.05). Deviation from Regression (S_{di}^2) is significantly correlated with S_i^2, W_i^2 (P<0.01) and with the measures of *pi* (P<0.05) and had negative and significant correlation with $S_i^{(6)}$ (P<0.01). Environmental variance (S_i^2), was significantly correlated with *pi*, $S_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$ (P<0.01) and with measures of $S_i^{(1)}$, $S_i^{(3)}$ (P<0.05). W_i^2 was positively and significantly associated with *pi*, S_i^2, $S_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$ (P<0.01) and $S_i^{(1)}$, $S_i^{(3)}$ (P<0.05). Stability variance (∂_i^2) had positive and significant correlations with $S_i^{(3)}$, *CVi* (P<0.01) and negative and significant correlations with $NP_i^{(1)}$ (P<0.01). The superiority index (*Pi*) is significantly correlated with $S_i^{(2)}$, $S_i^{(3)}$ (P<0.05) and $NP_i^{(3)}$, $NP_i^{(4)}$ (P<0.01).

Non- parametric $S_i^{(1)}$, is significantly correlated with $S_i^{(2)}$, $S_i^{(3)}$ (P<0.01) and $NP_i^{(2)}$, $NP_i^{(3)}$ (P<0.05). $S_i^{(2)}$ had positive and significant correlations with $S_i^{(3)}$ and $NP_i^{(2)}$ (P<0.05). $S_i^{(3)}$ was positively associated with $NP_i^{(2)}$, $NP_i^{(3)}$ (P<0.01) and with $S_i^{(4)}$ (P<0.05). $S_i^{(4)}$ is significantly correlated with $NP_i^{(2)}$ (P<0.05) and $NP_i^{(3)}$, $NP_i^{(4)}$ (P<0.01). $NP_i^{(1)}$ had positive and significant correlations with $NP_i^{(2)}$ (P<0.05). $NP_i^{(3)}$ showed positive correlations with $NP_i^{(4)}$ (P<0.01). $NP_i^{(4)}$ was positively and significantly associated with W_i^2, S_i^2, *pi*, $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(3)}$ (P<0.01) and $S_i^{(1)}$, $S_i^{(2)}$ (P<0.05). Scapim *et al*. (2000) also observed significant and positive correlations between and $S_i^{(1)}$, $S_i^{(2)}$ and $S_i^{(3)}$ in maize. Kara (2000) and Mut (2009) also reported the same correlations in wheat. Sabaghnia *et al*. (2006), and Mohammadi and Amri (2008) reported high rank correlations between $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(4)}$ in lentil Interpreting Genotype × Environment Interaction in Bread Wheat (*Triticum aestivum* L.). Considering significant rank correlation of the Also, mean grain yield and coefficient of regression (*bi*) of genotypes and stability variance (∂_i^2) were significantly positively correlated to the non-parameters of $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$ used. Similarly, Akçura and Kaya(2008),Kılıç *et al*. (2010) and Kilic(2012) reported high rank correlations between grain yield and these measures of stability. Although they do not supply information about genotype adaptability, nonparametric stability measurements seem to be useful alternatives to parametric measurements (Yue *et al*., 1997).

To better understand the relationships between the nonparametric and parametric methods a principal component analysis (PCA) based on the rank correlation matrix was performed. When applying the PCA, the first 2 PCAs explained 60.6% (38.78% and 21.82% with PCA1 and PCA2, respectively) of the variance (Figure 1). The PCA1 axis mainly differentiated the methods of ∂_i^2, *bi, CVi* from the other methods. Mean yield also grouped near these statistics, which we referred to as group 1 (G1) stability measures. The second PC axis separated

$S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$, S_{di}^2, S_i^2, W_i^2, piand R_i^2 (group 2, [G2]) from $NP_i^{(1)}$ (group 3 [G3]) (Figure 1). These methods classify genotypes as stable or unstable in a similar manner. Consequently, only one of these parameters would be sufficient for selecting stable genotypes in a breeding program.

Though, different stability methods are indicative of high, intermediate or low stability performance, the stability values do not provide information for reaching definitive conclusions (Mohammadi and Amri, 2008). Many stability measures used in this study considered that stability of genotypes is related to yield and stability. Hence, it is essential that both yield and stability should be considered simultaneously in order to reveal the beneficial effects of GEI and to select the genotypes. The repeatability, reliability and suitability of parametric and non-parametric methods to select the best genotypes in different crops need to be further investigated. *bi*, ∂_i^2, $S_i^{(3)}$ and $S_i^{(6)}$ measures with mean grain yield were best methods for analyzing the yield stability of a genotype across environments.

According to both parametric and non-parametric stability parameters, bread wheat genotypes G13, G15, G18 and G20 were more stable varieties, which had 8, 3 and 9 stability parameters out of 17 stability statistics used, respectively.

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