A SAS macro for computing statistical tests for two-way table and stability indices of nonparametric method from genotype-by-environment interaction

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ABSTRACT. Genotype-by-environment interaction refers to the differential response of different genotypes across different environments. This is a general phenomenon in all living organisms and always has been one of the main challenges for biologists and plant breeders. The nonparametric methods based on the rank of original data have been suggested as the alternative methods after parametric methods to analyze data without perquisite assumptions needed for common analysis of variance. But, the lack of statistical software or package, especially for analysis of two-way data, is one of the main reasons that plant breeders have not greatly used the nonparametric methods. Here, we have explained the nonparametric methods and presented a comprehensive two-parts SAS program for calculation of four nonparametric statistical tests (Bredenkamp, Hildebrand, Kubinger and van der Laan-de Kroon) and all of the valid stability statistics including Hühn’s parameters ($S_{(1)}$, $S_{(2)}$, $S_{(3)}$, $S_{(6)}$), Thennarasu’s parameters ($NP_{(1)}$, $NP_{(2)}$, $NP_{(3)}$, $NP_{(4)}$), Fox’s ranking technique and Kang’s rank-sum.

Keywords: rank, multi-environment trials, nonparametric tests, two-way data, SAS code.

Introduction

The genotype-by-environment interaction (GEI) is a universal issue that relates to all living organisms, from bacteria to plants to humans (Kang, 2004). This problem is important in agricultural, genetic, evolutionary and statistical research. The GEI is defined as a change in the relative performance of a trait of two or more genotypes measured in two or more diverse environments (Kang, 1988; 2004). Annually, around the world the output of breeding programs during consecutive years is evaluated in multi-environment trials (METs) to determine the adaptability of genotypes for later release and recommendation to farmers. Depending on the type of GEI (crossover or non-crossover), the type of interpretations and then recommendations of results will be different. If GEI is crossover type, then selection and recommendation of genotypes should be done with cautious, although in some cases introducing a genotype across all environments is not possible. Many statistic measurements, including parametric, nonparametric and multivariate, have been proposed for investigating GEI (Flores, Moreno, & Cubero, 1998). These statistics can be used for a range of different data with two-way classification and are not only suitable for plant breeding but also for crop production.
(Kang & Gauch, 1996). The computations and analyses of phenotypic yield stability have been extensively studied in METs for decades by investigators from the disciplines of plant and animal sciences and statistics (Kang, 1988). In METs, decisions are regularly based on two-way (genotype – environment) data. There are a few fields exist that two-way analyses such as GEI in plant breeding to be challengeable.

Because of the importance of GEI in the field of medicine and in plant breeding, many statistical tests have been proposed for testing two-way interaction effects (Azzalini & Cox, 1984; Baker, 1988; Gail & Simon, 1985). Most of the proposed statistical tests, for instance F-test, are based on some assumptions, such as normality of data, homogeneity of variances, and additivity (linearity) of effects (Hühn, 1996). In the real world, these assumptions may not always be met (Magari; Kang, 1993; Shah, Shah, Khan, Ahmed, & Hussain, 2009), especially, interpretation of multiple trait data using common stability methods is difficult since traits are frequently correlated and non-normally distributed (Eskridge, Peterson, & Grombacher, 1994; Lin, Binns, & Lefkovitch, 1986). Therefore, the nonparametric methods based on the ranks of original data have been suggested as alternative methods to analyzing the data without perquisite assumptions relative to ANOVA (Nassar & Hühn, 1987). Nonparametric methods have additional advantages, such as simple calculations and interpretations, reducing the bias caused by outliers; also addition or deletion of one or a few genotypes is not likely to cause large variations in the estimates (Nassar & Hühn, 1987). If the sample size in data is small or if assumptions for the corresponding parametric method (e.g. Normality of the data) hold,

Nonparametric methods may lack power as compared with more traditional approaches (Siegel & Castellan, 1988). Nonparametric methods are geared toward hypothesis testing rather than estimation of effects. It is often possible to obtain nonparametric estimates and associated confidence intervals, but this is not generally straightforward. Tied values can be problematic when these are common, and adjustments to the test statistic may be necessary (Whitley & Ball, 2002). However, SAS software can be considered High, Low and Mean rank for tied values of a right-continuous observation (Statistical Analysis Software [SAS], 2008).

The Use of nonparametric methods is not limited to recent years; they have traditionally been applied for analyzing data from one-way layouts from different fields and experimental designs (e.g. Wilcoxon Signed-Rank Test, Mann-Whitney Test and Kruskal-Wallis Test). The available nonparametric tests can be grouped into three broad categories based on how the data are organized; e.g., one-sample tests, related-samples tests, and independent-samples tests (Sheskin, 2003). These statistical tests are not used for two-way layouts, such as G×E data.

There is limited literature available on the rank statistics that address the valid statistical tests for two-way data. Hühn and Léon (1995) and Truber and Hühn (2000) have reviewed four nonparametric methods, including (Bredenkamp, 1974), (Hildebrand, 1980), (Kubinger, 1986) and (De Kroon & Van Der Laan, 1981) tests for many data sets obtained from METs from German official registration trials for faba bean, fodder beet, oat, sugar beet, and winter oilseed rape from 1985 to 1989. They suggested that if the assumption for parametric methods cannot be accepted, some of these tests are robust to be recommended for data with crossover interaction. On the other hand, several heuristic rank-based methods have been proposed for phenotypic stability of cultivars performance (Fox, Skovmand, Thompson, & Braun, 1990; Hühn, 1979; Kang, 1988; Nassar; Hühn, 1987; Thennarasu, 1995). These methods have been used to identify superior genotype(s) in METs data (Segherloo, Sabaghpour, & Dehghani, 2008).

The nonparametric tests can be used by researchers in different disciplines, but the lack of statistical software or package, especially for two-way data, is one of the main reasons that these tests are not used by scientists, particularly plant breeders. A literature review indicated that there was no comprehensive macro, code, software or package for aforementioned nonparametric tests. However, a basic computer program has been written for computing Kang’s yield-stability statistic (YS) (Kang & Magari, 1995); a SAS program exists for computing two of Hühn’s nonparametric measures (S(80) and S(0)) (Lu, 1995). Also, Hussein, Bjornstad and Aastveit (2000) developed SAS codes for various parametric stability statistics, but those are not calculated all of nonparametric statistics. In this paper, we present a comprehensive macro written in SAS (SAS, 2008) and consisting of two different parts which is given in the Appendix A. The first part applies to calculation of the four nonparametric interaction tests and the second part aims to assess all of nonparametric measurements of stability. The yield trials were used as an example (Appendix B) here had twenty promising barely varieties grown in fourteen environments for two years during the 2006-2008. All of the research stations are located in the cold region of Iran under the management of the seed and plant improvement (SPII). institute, Karaj, Iran. To get more details regarding data see Akbarpour, Dehghani, Sorkhi and Gauch (2014)
Statistical description

Statistical nonparametric tests on the basis of rank are the alternative approach instead of parametric methods such as analysis of variance when the analysis of data strongly depends on several assumptions, including normal distribution, independence, and variance homogeneity of METs data (Hühn & Léon, 1995). The tests of Bredenkamp (1974), Hildebrand (1980), and Kubinger (1986) are based on the usual linear model for noncrossover interactions; interactions are defined as deviations from additivity of main effects. The method of De Kroon and Van Der Laan (1981) defines interactions according to the crossover interaction model (Hühn & Léon, 1995; Truberg & Hühn, 2000). In this paper, the computational formulas are presented as described by Hühn and Léon (1995) and Hühn (1996). For all methods, the value of genotype $i$ in environment $j$ and replication $k$ is denoted by $X_{ijk}$ ($i = 1, 2, \ldots, l; j = 1, 2, \ldots, m; k = 1, 2, \ldots, n$). The first letter for each method is considered for parameters index.

Bredenkamp method

All $X_{ijk}$ are transformed into $R_{ijk}$ based on one single rank order. The test statistics for G, E and $G \times E$ differences (designated as $G_B$, $E_B$ and $E_{GB}$, respectively) are calculated as Equations 1, 2 and 3:

$$G_B = \frac{12L}{S(S+1)} \sum_{j=1}^{l} R_{ij}^2 - 3(S+1)$$

$$E_B = \frac{12M}{S(S+1)} \sum_{j=1}^{m} R_{ij}^2 - 3(S+1)$$

$$EG_B = \frac{12LM}{S(S+1)} \sum_{i=1}^{l} \sum_{j=1}^{m} (R_{ij}^2 - \frac{1}{m^2} R_{j}^2 - \frac{1}{l} R_{i}^2) + 3(S+1)$$

where:

$S$ is equal to $l \times m \times n$ and $G_{B}$, $E_{B}$ and $EG_{B}$ are approximately distributed as $\chi^2$, with $(l-1)$, $(m-1)$ and $(l-1) \times (m-1)$ degrees of freedom, respectively.

Hildebrand method

To use the Hildebrand method, the original data (X$_{ijk}$) are corrected for G, E and G × E as Equations 4, 5 and 6:

$$X_{ijk}^* = (X_{ijk} - \bar{X}_j + \bar{X}_.)$$

$$X_{ijk}^* = (X_{ijk} - \bar{X}_i + \bar{X}_.)$$

$$X_{ijk}^* = (X_{ijk} - \bar{X}_i - \bar{X}_j + 2\bar{X}_.)$$

The corrected data ($X_{ijk}^*$) are transformed into $R_{ijk}$ based on one single rank order. The statistics $G_{H}$, $E_{H}$ and $E_{GH}$ are as calculated the Equations 7, 8 and 9:

$$G_{H} = \frac{12}{l(s+1)} \sum_{i=1}^{l} (R_{i.} - \bar{R}_{.})^2$$

$$E_{H} = \frac{12}{m(s+1)} \sum_{j=1}^{m} (R_{.j} - \bar{R}_{.})^2$$

$$EG_{H} = \frac{12}{lm(s+1)} \sum_{i=1}^{l} \sum_{j=1}^{m} (R_{ij} - \bar{R}_{i.} - \bar{R}_{.j} + \bar{R}_{.})^2$$

Kubinger method

In this method, the original data (X$_{ijk}$) are transformed into $R_{ijk}$ based on one single rank order, and then the ranked data are adjusted ($R_{ijk}$ → $R_{ijk}'$) and ranked again ($R_{ijk}'$ → $R_{ijk}^*$). For G, E and $G \times E$, $R_{ijk}^*$ are calculated, respectively, as Equations 10, 11 and 12:

$$R_{ijk}' = (R_{ijk} - \bar{R}_i + \bar{R}_.)$$

$$R_{ijk}' = (R_{ijk} - \bar{R}_j + \bar{R}_.)$$

$$R_{ijk}' = (R_{ijk} - \bar{R}_i - \bar{R}_j + \bar{R}_.)$$

Van Der Laan-De Kroon method

In this method, the original data (X$_{ijk}$) are transformed into ranks $R_{ijk}$ for each environment separately. The $G_{K}$, $E_{K}$ an $E_{GK}$ are calculated via as Equations 13, 14 and 15:

$$G_{K} = \frac{12}{l(s+1)} \sum_{i=1}^{l} (\bar{R}_{i.} - \bar{R}_{.})^2$$

$$E_{K} = \frac{12}{m(s+1)} \sum_{j=1}^{m} (\bar{R}_{.j} - \bar{R}_{.})^2$$

$$E_{GK} = \frac{12}{lm(s+1)} \sum_{i=1}^{l} \sum_{j=1}^{m} (\bar{R}_{ij} - \bar{R}_{i.} - \bar{R}_{.j} + \bar{R}_{.})^2$$

where:

The $G_{K}$, $E_{K}$ and $E_{GK}$ are approximately distributed as $\chi^2$, with $(l-1)$, $(m-1)$ and $(l-1) \times (m-1)$ degrees of freedom, respectively.
where:

The $G_v$, $E_v$ and $EG_v$ are approximately distributed as $\chi^2$ with $(l-1)$, $(m-1)$ and $(l-1) \times (m-1)$ degrees of freedom, respectively.

**Statistical description for phenotypic stability**

Consider a two-way table with $l$ rows (genotypes) and $m$ columns (environments). Phenotypic values $x_{ij}$ ($i = 1, 2, \ldots, l; j = 1, 2, \ldots, m$) for some statistics are corrected (Nassar & Hühn, 1987); the rank of 1 is given to the genotype with the highest value and the rank of $l$ is given for the lowest value. Let $r_{ij}$ be the rank of genotype $i$ in environment $j$. We provide below the formulas used for computing the nonparametric stability statistics. The formulas for the four rank-stability measures of Hühn (1979) and Nassar and Hühn (1987) are as Equations 19, 20, 21 and 22:

$$S_{i(1)} = 2 \sum_{j=1}^{m-1} \sum_{k=j+1}^{m} \frac{r_{ij}^* - r_{ik}^*}{[M(M-1)]}$$

$$S_{i(2)} = \frac{\sum_{j=1}^{m} (r_{ij}^* - \bar{r}_i^*)^2}{\bar{r}_i}$$

$$S_{i(3)} = \frac{\sum_{j=1}^{m} (r_{ij} - \bar{r}_i)^2}{\bar{r}_i}$$

$$S_{i(6)} = \frac{\sum_{j=1}^{m} |r_{ij} - \bar{r}_i|}{\bar{r}_i}$$

where:

$r_{ij}$ and $r_{ij}^*$ denote the rank of uncorrected (Table 2) and corrected (Table 3) values of the $i_{th}$ genotype in the $j_{th}$ environment, respectively ($\bar{r}_i$ and $\bar{r}_i^*$ as the mean ranks of corrected and uncorrected values across all environments for the $i_{th}$ genotype).

Significance tests for $S_{i(1)}$ and $S_{i(2)}$ were conducted according to Nassar and Hühn (1987). First, the null hypothesis that all genotypes have similar stability (e.g., equal $S_i^t$ values) was tested by summation of the standardized $S_i^t$ values across genotypes as Equation 23 ($\sum_{i=1}^{l} Z_i^t$, where $t = 1, 2$ and):

$$Z_i^t = \frac{[S_i^t - E(S_i^t)]^2}{V(S_i^t)}.$$

This test statistic has an approximate $\chi^2$ distribution with degrees of freedom (df) equal to the number of genotypes (Hühn, 1996). The rejection of the null hypothesis provides evidence for the existence of differential stability among genotypes. The alternative hypothesis tests for the stability of individual genotypes examined by testing the $Z_i^t$ statistic, which also has an appropriate $\chi^2$ distribution with 1 df under the null hypothesis. Nassar and Hühn (1987) defined the null hypothesis $Z_i^t = 0$ versus the alternative hypothesis for stability of genotype $i$ as stable and unstable, respectively. The alternative hypothesis may be restated, such that a genotype with significantly large $S_i^t$ (relative to the null hypothesis expectation) is unstable relative to others; a significantly small $S_i^t$ indicates that the genotype is more stable relative to others. Nassar and Hühn (1987), however, warned against overestimation of significant effects for specific genotypes, or committing a Type I error with higher probability than the usual $\alpha = 0.05$. They indicated that the expected comparison wise error rate would approximately equal $(l)(0.05)(0.95)^{l-1}$, which is much greater than the chosen significance level of 0.05. In general, Nassar and Hühn (1987) have suggested a significance level of $\sim 0.05 l^{-1}$. The null hypothesis is rejected if $Z_i^t > \chi^2_{(0.05, 1)}$ (Krenzer, Thompson, & Carver, 1992). Nassar and Hühn (1987) also stated that the global test is more reliable than specific test for handling of significant genotypic differences in stability.

Under the null hypothesis that all genotypes are equally stable, the expected value of means $E(S_i^t)$ and variances $V(S_i^t)$ can be computed from the discrete uniform distribution (1, 2, ..., $l$) as equations 24, 25, 26 and 27 (Nassar & Hühn, 1987):

$$E(S_i^{(1)}) = \frac{l^2 - 1}{3l}$$

$$E(S_i^{(2)}) = \frac{l^2 - 1}{12}$$

$$V(S_i^{(1)}) = \frac{(l^2 - 1)[(l^2 - 4)(m + 3) + 30]}{45lm(l - 1)}$$

$$V(S_i^{(2)}) = \frac{(l^2 - 1)[2(l^2 - 4)(m - 1) + 5(l^2 - 1)]}{360m(m - 1)}$$

**References**

Thennarasu (1995) proposed the following four nonparametric stability measures using the adjusted rank values as Equations 28, 29, 30 and 31:

\[
N_{Pi}^{(1)} = \frac{1}{m} \sum_{j=1}^{m} |r_{ij}^* - Md_i^*| / Mdi^* \\
N_{Pi}^{(2)} = \frac{1}{m} \sum_{j=1}^{m} |r_{ij}^* - Md_i^*| / Mdi \\
N_{Pi}^{(3)} = \sqrt{\frac{\sum_{i=1}^{m} (r_{ij}^* - \bar{r}_i^*)^2}{m}} \\
N_{Pi}^{(4)} = \frac{2}{m(m-1)} \sum_{j=1}^{m} \sum_{j=j+1}^{m} |r_{ij}^* - r_{ij}^*| / r_i \\
\]

where:

- \( r_{ij}^* \) is the rank of \( x_{ij}^* \);
- \( Mdi^* \) are the mean and median ranks for adjusted values, respectively; whereas \( Mdi \) are the same statistics computed from the original (unadjusted) values. \( N_{Pi}(1) \) means absolute deviation and is calculated in a similar manner as variance of ranks, except here the median is subtracted from each data point, producing deviations from the median. The genotype with the lowest \( N_{Pi}(1) \) is regarded as the most stable. The genotypes with the lowest \( N_{Pi}(2), N_{Pi}(3), N_{Pi}(4) \) and highest average yields are regarded as most desirable.

Other nonparametric techniques, including rank-sum (Kang, 1988) and Fox’s method (Fox, Skovmand, Thompson, & Braun, 1990) can be used for determining stability of genotypes. Kang’s rank-sum (Kang, 1988) uses ranks of mean performance of genotypes across environments plus the rank of Shukla (1972) stability variance, in which the genotype with the highest mean yield and the lowest stability variance receives the rank 1 plus 1. Upon summation of the two ranks for each genotype (rank-sum), a genotype with the lowest rank-sum is regarded as most desirable. The computation of the Fox’s statistic (Fox, Skovmand, Thompson, & Braun, 1990) is based on scoring of genotypes as ‘Top’, ‘Mid’ or ‘Low’ within each environment. The genotypes that are frequently occurred in the ‘Top’ third are considered to be stable.

In general, to realize the obvious effect of GEI, the METs are conducted around the world, annually. Because of the natural heterogeneity of these experiments, some assumptions regarding parametric analysis of the data may not be fulfilled. So, the alternative approaches, such as nonparametric analysis, can be used by researchers. Recently, the uses of nonparametric methods have been increased in plant breeding, but the most of calculations are done manually or performed by uncertain softwares. The presented SAS macro in the Appendix A simplifies the calculation of important nonparametric statistics relative to GEI and can be especially utilized by plant breeders. Users only need to know how to open the program file, provide the data, introduce the data to SAS, invoke the macro and print the output. This paper is intended for all levels of SAS users.

**Result and discussion**

The results of statistical tests for data are shown in Table 1. The simple effect of genotype by one of the methods (Hildebrand), environment by all of the methods and interaction effect (G×E) by all of methods except for Kubinger method were significant at 0.01 probability level. These results demonstrated that the responses of genotypes across environments are complex and the selection of a stable genotype needs to more precision and attention (Mut, Aydin, Bayramoğlu, & Özcan, 2009). Truberg and Hühn (2000) applied these statistical tests for their data and showed that the Hildebrand, Kubinger methods are approximately equivalent to analysis of variance (ANOVA). Because the assumptions for the parametric methods may be not valid, the nonparametric test can be applied for analyzing METs data (Hühn & Léon, 1995; Mut, Aydin, Bayramoğlu, & Özcan, 2009).

**Table 1.** Result of statistics tests for significant of effects.

<table>
<thead>
<tr>
<th>Prob Genotype × Environment</th>
<th>Genotype × Environment</th>
<th>Prob Environment</th>
<th>Environment</th>
<th>Prob Genotype</th>
<th>Genotype</th>
<th>Test</th>
</tr>
</thead>
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<td>5424.24</td>
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<td>575.20</td>
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<td>12.09</td>
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<td>0.00</td>
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<td>267.09</td>
<td>0.00</td>
<td>307.84</td>
<td>0.27</td>
<td>22.34</td>
<td>Kubinger</td>
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<td>399.86</td>
<td>0.00</td>
<td>2612.09</td>
<td>0.42</td>
<td>19.66</td>
<td>Van der Ianh de Kroon</td>
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Table 2. Uncorrected yield ranks for each environment.

<table>
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<th>Genotype</th>
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Table 3. Corrected yield ranks for each environment.

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</table>

Table 4. Nonparametric measurements of (Nassar, & Hühn, 1987), (Thennarasu, 1995) and Rank-Sum (Kang, 1988). The results of statistical tests showed the crossover and noncrossover interactions were existed for the data, so the stability of the genotypes was evaluated based on nonparametric measurements. The statistics of Z1 and Z2 for each individual genotype are shown in Table 4. To avoid overestimation of the specific stability of genotypes, the expected comparisonwise error for Z1 and Z2 and also $\chi^2$ were calculated (Table 4) and the critical value for $\chi^2$ with one degree of freedom was 9.14. Comparison of Z1 and Z2 values with the critical $\chi^2$ value showed that all genotypes were not significant for specific stability at the given probability level. Also the sums of Z1 and Z2 over all genotypes (24.64 and 23.65) were less than 26 for Chi-Square statistic for sum of Z1, Z2 (31.4) (Table 4). So neither specific stability nor general stability were not significant at given probability level (Table 4). However, by changing the level of probability to 0.1 or 0.2, the specific and general stabilities may be significant, and subsequently the parameters of $S_i^1$ and $S_i^2$ would be interpreted. But the lack of significant of those parameters showed that variation of $S_i^1$ and $S_i^2$ are not interpreted.

The result of $S_i^3$, $S_i^4$, $NP_i^1$, $NP_i^2$, $NP_i^3$ and $NP_i^4$ are given in Table 4. The minimum value for each of these statistics indicates maximum stability for each genotype. Based on both parameters, G16 and G17 had lowest and highest values and correspondingly those detected as stable and unstable genotypes. $S_i^1$ and $S_i^2$ had a closely results and the rank of genotypes based on these parameters were almost similar. Sabaghnia, Dehghani and Sabaghpour (2006) resulted a highly correlated rank for $S_i^1$, $NP_i^1$, $NP_i^2$, $NP_i^3$ and $NP_i^4$ parameters.
The result of rank-sum of (Kang, 1988) in Table 4 showed that G4 with lowest value and G15 with highest value were the desirable and undesirable genotypes, respectively. By the Fox, Skovmand, Thompson, and Braun, (1990)’s method, G18 with 7.14% and G10 with 57.14% frequently occurred in the Low third category were the best and undesirable genotype (Table 5). In addition, G18 and G16 with 71.43 and 64.29% as well as G14, G17, G9, G13, G5 and G10 with 21.43% frequently occurred in the Mid third category were the stable and unstable genotypes, respectively. Finally, G14 and G16 were the best and worst genotypes with 57.14 and 7.14% respectively. In summary, by all of the parameters, G16 was detected as stable genotype across environment except by Top parameter. Due to the no significantly general and specific Z1 and Z2 for genotypes, the $S_1$ and $S_2$ parameters were not interpreted. Also, the presented macro program in SAS (2008) is calculated the statistical tests for genotype, environment G×E interaction and given stability parameters based on rank of data and simplified the use of nonparametrical methods in plant breeding.

### Conclusion

The use of nonparametric methods are preferred for analyzing METs data when some of assumptions such as normality are violated. Our study were founded in two parts by rank approach; the first part was related to theoretical explanation of two-way data such as G × E structure as well as focusing on the stability of METs data; the second part presented a macro SAS program for calculating nonparametric statistical tests and also stability indices in plant breeding. The results showed significant G×E based on all testes except for Kubinger method. Moreover, G16 had a maximum stability across environments using the presented nonparametric stability indices.

### References


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**Table 5.** The ranking technique of (Fox, Skovmand, Thompson, & Braun, 1990) parameters.

<table>
<thead>
<tr>
<th>Fox Parameters</th>
<th>Genotype</th>
<th>Top</th>
<th>Mid</th>
<th>Low</th>
<th>Total</th>
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<tr>
<td>35.71</td>
<td>G14</td>
<td>42.86</td>
<td>21.43</td>
<td>1</td>
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<tr>
<td>14.29</td>
<td>G15</td>
<td>42.86</td>
<td>42.86</td>
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<tr>
<td>21.43</td>
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<td>50.00</td>
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<td>G17</td>
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<td>42.86</td>
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<td>50.00</td>
<td>5</td>
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</tr>
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<td>6</td>
<td></td>
</tr>
<tr>
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<td>G10</td>
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<td>G12</td>
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<td>28.57</td>
<td>8</td>
<td></td>
</tr>
<tr>
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<td>35.71</td>
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<td>G9</td>
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<td>57.14</td>
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<td>14</td>
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<td>35.71</td>
<td>19</td>
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</tr>
<tr>
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<td>28.57</td>
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</tr>
<tr>
<td>85</td>
<td>G15</td>
<td>98</td>
<td>97</td>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>


Received on January 25, 2015.
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Appendix A. Nonparametric program (%NPSTAB).

************************************************************************;
***********************Nonparametric Tests*****************************;
/*This program has been designed for doing the nonparametric tests of two-way data table such as multi-
environment trials data. This kind of data is related to the characteristics of many genotypes which are
evaluated in many environments. The newly developed macro are called %NPSTAB. This macro contains
two computational parts. One part is covered the computational steps for statistical assumptions rank-based
including Bredenkamp (1974), Hildebrand (1980), Kubinger (1986) and de Kroon and Van Der Laan
(1981) tests which each of those are used for testing the genotype, environment and genotype × environment.
The second part is calculated the parameters of genotypic stability including S1, S2, S3, S6, Np1, NP2, Np3,
Np4, Fox's ranking technique of Fox, Skovmand, Thompson, & Braun, (1990) - and Kang’s rank-sum (Kang,
1988) for each genotype.
To run the SAS macro program, data should be structured in a two way table format with L columns
(genotypes) and M × N rows (environments × replication) as shown here. If the data has a different format,
to correctly read the data by the data step, should be changed according to the objective. The external file is
fed into SAS with an INFILE statement. The provided data must be introduced to SAS by a readable format
such as *.TXT or *.DAT format.
************************************************************************/
option nodate nonumber;
%Macro NPSTAB(L,M,N, ALPHA);
/*******************************************************************************
L= Number of Genotypes
M= Number of Environments
N= Number of Replications
Alpha= Probability level for testing the Chi-square statistics of Z1 and Z2 in nonparametric stability
measurements. This macro variable is not used for tow-way tests.
*******************************************************************************/
DATA ORI;
INFILE 'E:\path of data\filename.TXT';
****** The path of data file must be entered************************************/
DO ENV=1 TO &M;
DO REP=1 TO &N;
DO Genotype=1 TO &L;
INPUT Y @@;
OUTPUT;
END;
END;
END;
RUN;
/*******************************************************************************
ASEMB macro is calculated the sum and mean of each introduced variable to macro program by each factor
such as Genotype, ENV, combination of Genotype × Environment and also total sum and mean and put all
of generated data with their variables in a final data.
DATA= name of data that is used for averaging and summing
Var= The variable that is used from the DATA
suffix = The letter or word for different variable which is generated by each run to prevent the replacement of
previous variables
*******************************************************************************
%MACRO ASEMB(DATA,VAR,suffix);
PROC SORT DATA=&DATA;
BY ENV Genotype;
PROC MEANS DATA=&DATA NOPRINT;
BY ENV Genotype;
VAR &VAR;
OUTPUT OUT=GE_&DATA MEAN=YM_GE&suffix SUM=YS_GE&suffix;
PROC MEANS DATA=&DATA NOPRINT;
BY ENV;
VAR &VAR;
OUTPUT OUT=E_&DATA MEAN=YM_E&suffix SUM=YS_E&suffix;
PROC MEANS DATA=&DATA NOPRINT;
VAR &VAR;
OUTPUT OUT=T_&DATA MEAN=YM_T&suffix SUM=YS_T&suffix;
DATA &DATA;
IF _N_ = 1 THEN SET T_&DATA; MERGE E_&DATA GE_&DATA &DATA;
BY ENV;
PROC SORT DATA=&DATA;
BY Genotype ENV;
PROC MEANS DATA=&DATA NOPRINT;
BY Genotype;
VAR &VAR;
OUTPUT OUT=G_&DATA MEAN=YM_G&suffix SUM=YS_G&suffix;
DATA &DATA;
MERGE G_&DATA &DATA;
BY Genotype;
RUN;
%MEND;
%MACRO PROB;
   PROB_G=1-PROBCHI(G,(&L-1));
   PROB_E=1-PROBCHI(E,(&M-1));
   PROB_GE=1-PROBCHI(GE,(&L-1)*(&M-1));
%MEND;
%ASEMB(ORI,Y,F);
DATA ORI;
SET ORI;
   G_ADJ = Y - YM_GEF + YM_GF;
   E_ADJ = Y - YM_GEF + YM_EF;
   GE_ADJ = Y - YM_GF - YM_EF + (2 * YM_TF);
RUN;
PROC SORT DATA=ORI;
BY ENV Genotype;
RUN;
PROC RANK DATA=ORI OUT=R.ORI TIES=MEAN;
BY ENV;
VAR Y;
RANKS VD_Y;
RUN;
PROC RANK DATA=R.ORI OUT=R.ORI1 TIES=MEAN;
VAR Y G_ADJ E_ADJ GE_ADJ;
RANKS RH_G RH_E HG_GE;
%ASEMB(R.ORI1,RY,K);
DATA R.ORI1;
SET R.ORI1;
   K_G=RY - YM_GEK + YM_GK;
   K_E=RY - YM_GEK + YM_EK;
A SAS macro for computing statistical tests

K_GE = RY - YM_GK - YM_EK;
RUN;
PROC RANK DATA=R_ORI1 TIES=MEAN;
VAR K_G K_E K_GE;
RANKS K_G K_E K_GE;
RUN;
DATA BERD;
MERGE G_R_ORI1 E_R_ORI1 GE_R_ORI1;
YS_GK = (YS_GK)**2;
YS_EK = (YS_EK)**2;
YS_GEK = (YS_GEK)**2;
PROC MEANS NOPRINT DATA=BERD;
VAR YS_GK YS_EK YS_GEK;
OUTPUT OUT=BER SUM=G_B E_B GE_B;
DATA BREDENKAMP;
SET BER;
TEST="BREDENKAMP"; /* calculation of statistics for Bredenkamp method */
DROP _FREQ_ _TYPE_;
G = (12*(M**2)*((L+N*M)**2)+(L+N*M)**2)/((L+N*M)**2);  /* calculation of statistics for Hildebrand method */
E = (12*(M**2)*((L+N*M)**2)+(L+N*M)**2)/((L+N*M)**2);  /* calculation of statistics for Kubinger method */
GE = (12*(M**2)*((L+N*M)**2)+(L+N*M)**2)/((L+N*M)**2);  /* calculation of statistics for Hildebrand method */
%PROB;
RUN;
%ASEMB(R_ORI1, H_G, HG);
%ASEMB(R_ORI1, H_E, HE);
%ASEMB(R_ORI1, H_GE, HGE);
%MACRO HK(DATA, V, METHOD);
DATA R_ORI1;
SET R_ORI1;
G_&DATA = (YM_G&V.G - YM_T&V.G)**2;
E_&DATA = (YM_E&V.E - YM_T&V.E)**2;
GE_&DATA = (&V._GE - YM_G&V.GE - YM_E&V.GE + YM_T&V.GE)**2;
RUN;
PROC MEANS DATA=R_ORI1 NOPRINT;
VAR G_&DATA E_&DATA GE_&DATA;
OUTPUT OUT=&DATA MEAN=G_&DATA E_&DATA GE_&DATA;
RUN;
DATA &DATA;
SET &DATA;
DROP _FREQ_ _TYPE_;
TEST="&METHOD";
G = (12/(M*N*L)**2)*G_&DATA;
E = (12/(M*N*L)**2)*E_&DATA;
GE = (12/(M*N*L)**2)*GE_&DATA;
%PROB;
%MEND;
%HK(HIL, H, HILDEBRAND); /* calculation of statistics for Hildebrand method */
%ASEMB(R_ORI1, K_G, KG);
%ASEMB(R_ORI1, K_E, KE);
%ASEMB(R_ORI1, K_GE, KGE);
%HK(KUB, K, KUBINGER); /* calculation of statistics for Kubinger method */
%ASEMB(R_ORI1, VD_Y, V);
DATA VAN_DE;

MERGE G_R.ORI1 E_R.ORI1 GE_R.ORI1;
YS_GV=YS_GV**2;
YS_EV=YS_EV**2;
YS_GEV=YS_GEV**2;
PROC MEANS NOPRINT DATA=VAN_DE;
VAR YS_GV YS_EV YS_GEV;
OUTPUT OUT=VAN_DER SUM=G_V E_V GE_V;
DATA VAN_DER;
SET VAN_DER;/*calculation of statistics for Van Der Laan-De Kroon method*/
DROP _FREQ_ _TYPE_; TEST="VAN DER LAAN-DE KROON";
G=((12/((&L*(&N**2))*((&L*&N)+1)))*(G_V - G_V/&)M));
G=((12/((&L*(&M*(&N**2))*((&L*(&N)+1)))*G_V)-(3*(&M*(((&L*&N)+1));
E=((12/((&L*(&M*(&N**2))*((&M*&N)+1)))*E_V)-(3*(&L*(((&M*&N)+1));
%PROB:
DATA STATISTIC;
SET BREDENKAMP HIL KUB VAN_DER;
KEEP TEST G E GE PROB_G PROB_E PROB_GE;
PROC PRINT DATA=STATISTIC label noobs;
TITLE "Result of Statistics Tests";
LABEL
G='Genotype'
E='Environment'
GE='Genotype × Environment'
PROB_G = 'prob Genotype'
PROB_E = 'prob Environment'
PROB_GE = 'prob Genotype × Environment';
var TEST G PROB_G E PROB_E GE PROB_GE;
footnote;
RUN;
/*Following codes calculate the parameters of nonparametric satiability*/
The measurements of stability are calculated based on means genotype × environment table over replications;
DATA NPSTAB;
SET GE.ORI;
RENAME YM_GEF=YIELD;
DROP _TYPE_ _FREQ_ YS_GEF;
RUN;
%ASEMB(NPSTAB,YIELD,);
%MACRO UNI2 (DATA,VAR);
PROC SORT DATA=&DATA;
BY &VAR;
%MEND;
RUN;
DATA LD;
Set NPSTAB;
Set NPSTAB;
GE=(Yield - YM_G - YM_E +YM_T);
LDEV=YM_G-YM_T;
CYLD=YIELD-LDEV;/*Calculation Corrected yield*/
%ASEMB(LD,CYLD,C);
%ASEMB(LD,GE,INT);
%UNI2 (LD,ENV);
PROC RANK DATA=G_NPSTAB DESCENDING OUT=RY;
VAR YM_G;
PROC RANK DATA=LD DESCENDING OUT=R;
BY ENV;
VAR CYLD YIELD;
RANKS RCYLD RUCY; /* Rank of yield and corrected yield*/
%UNI2 (LD,Genotype);
%UNI2 (R,Genotype);
PROC MEANS NOPRINT MEAN MEDIAN VAR DATA=R;
BY Genotype;
VAR RCYLD RUCY;
OUTPUT OUT=M_RANK MEAN=M_RCY M_RUCY MEDIAN=M_D_RCY M_D_RUCY VAR=SI2 VAR_RUCD;
DATA RANK;
MERGE LD R M_RANK RY;
BY Genotype;
DATA RANKS;
IF _N_=1 THEN SET T_LD;
DATA RANKS;
IF RUCY<T THEN FOX='Top';
IF T<=RUCY<L THEN FOX='Mid';
IF L<=RUCY THEN FOX='Low';
PROC MEANS DATA=RANKS MEAN SUM NOPRINT;
BY Genotype;
VAR SI2 SI3 SI6 NP1 NP2 NP3;
OUTPUT OUT=NONP MEAN=L.SIGMA SI2 SI3 SI6 NP1 NP2 NP3 SUM=S.SIGMA SI2 SI3 SI6 SNP1 SNP2 SNP3;
RUN;
%MACKRO RANK (D=, VAR=);
DATA &D;
ARRAY E{&M} ;
DO J=1 TO &M;
SET RANKS;
BY Genotype;
E{J}=&VAR;
END;
IF LAST.Genotype THEN RETURN;
%MEND;
%MACKRO RANK (D=DATA3, VAR=RUCY);
%MACKRO RANK (D=DATA1, VAR=RCYLD);
PROC PRINT DATA=DATA3 NOOBS;
VAR Genotype E1-E&M ;
TITLE 'Uncorrected Yield Ranks';
*The corrected data have employed for S1 and S2 and uncorrected data for S3 and S6 measures, because the first two methods are mostly associated with yield level and shown a little or no correlation with stability parameters, whereas in the latter two methods, both yield level and stability of performance are considered simultaneously (Hühn, 1996);

PROC RANK DATA=NONP OUT=R_SIGMA;
VAR L_SIGMA;
RANKS R_SIG; /*Rank for Shukla parameter, the second step of RS_KANG*/
RUN;
DATA DATA2;
ARRAY E{&M}; /*Array statement for calculation of S1 and NP4*/
SET DATA1;
BY Genotype;
SUMQ=0;
DO J=1 TO &M-1;
DO JJ=J+1 TO &M;
   AD=ABS(E{J}-E{JJ}); Kang, 2004);
   SUMQ+AD;
END;
END;
NN=&M*(&M-1)/2;
SI1=SUMQ/NN;
PRE=2/(&M*(&M-1));
KEEP Genotype PRE SUMQ SI1;
DATA STAT;
   K2=&L**2;
   ES1=(K2-1)/(3*(&L)); ES2=(K2-1)/12;
   VS1=(K2-1)*((K2-4)*(&M+3)+30)/(45*(K2)*(&M*(&M-1)));
   VS2=(K2-1)*((2*(K2-4)*(&M-1)+5*(K2-1))/(360*(&M*(&M-1)));
   ALPHAS=1-(&ALPHA/&l);/*The new level of probability is calculated to reduce the comparisonwise error rate in specific stability test for each genotype*/
   PCHIS=CINV (ALPHAS,1);
   PCHI=CINV (1-&ALPHA,&L);
   PROB=1-PROBCHI(PCHI,&L);
RUN;
/*Expected mean, variance, Chi-square for Z1 and Z2, sum of Chi-square for Z1 and Z2 and level of probability are estimated. The rank-corrected and rank-uncorrected data are applied together to estimate NP1, NP2, Np3 and NP4 parameters. Also the original data (uncorrected data) are used for calculation of Fox's rank and Kang's rank-sum. The Kang's rank-sum values shown in output are summation of ranks*/
DATA ALL;
IF _N_=1 THEN SET STAT;
SET DATA2;
SET NONP;
SET R_SIGMA;
SET M_RANK;
SET RY;
SET G_NPSTAB;
   SIG_Z1="ns";
   SIG_Z2="ns";
data npstab(20,14,3,0.05);
Appendix B. Example.

To illustrate the calculation of nonparametric measurements, we used the author’s data (Akbarpour, Dehghani, Sorkhi, & Gauch, 2014) from yield trials of 20 barley cultivars evaluated in 14 environments (2007–2008).

The macro %NPSTAB invoked is as follows:

\%
NPSTAB(L,M,N,Alpha);
\%
NPSTAB(20,14,3,05);

where:
L=Number of Genotypes,
M=Number of Environments,
N=Number of Replications and
Alpha = Probability level for testing the Chi-square statistics of \( Z_i^1 \) and \( Z_i^2 \) in nonparametric stability measurements. This macro variable is not used for tow-way tests.

The output of analysis is shown in table 2.