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Evaluating leaf rust reactions of pure bread wheat landrace lines using non-parametric statistics

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Abstract This research aims to assess genotype × environment interactions (G × E) of the leaf rust (*Puccinia triticina*) reactions of 29 Turkish landrace-derived pure lines over seven environments. Field experiments were conducted in Çanakkale, Edirne and Samsun (Turkey) in three consecutive growing seasons from 2011-2012 to 2013–2014 under natural conditions. Leaf rust reactions of genotypes were recorded in accordance with the Modified Cobb scale. Genotype stabilities assessed by non-parametric stability statistics. Furthermore, a Principal Component Analysis (PCA) biplot of non- parametric stability statistics were used to compare their capability for capturing both stability and leaf rust reactions of genotypes in the given dataset. PCA Biplot results showed that non-parametric parameters of Thennarasu and Percentage Availability (PA) showed a positive association with mean disease severity while Rank Means (RM) were negatively associated. Non-parametric stability analyses revealed that Genotypes 29, 25 and 15 were the most stable pure lines when only 25 and 15 could be considered as resistant-stable. In order to determine both stability and leaf rust resistance in bread wheat, PA and RM are recommended.

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Introduction

Leaf rust (brown rust) is the most common fungal disease of wheat worldwide, caused by *Puccinia triticina*. The easy spread of leaf rust urediniospores by wind and its high potential for epidemics results in severe yield losses and decreases in grain quality (Bolton et al. 2008). The most effective way to control leaf rust is through the development of resistant genotypes; which requires searching for new gene sources to incorporate into resistance breeding programs with constant monitoring of virulence frequencies and current efficiencies of the resistance genes (Huerta-Espino et al. 2011). On the other hand, the stability of leaf rust reactions in those resistant genotypes is not guaranteed due to the mutable nature of the pathogen and other factors such as environments.

Genotype performances vary across environments, usually in a complex way that is difficult to evaluate without a statistical approach. Genotype environment interaction (G × E) is difficult to investigate because the concept of "environmental variation" is a combination of many different sources of variation. Even if the effects of some important environmental factors such as precipitation or temperature on genotypic variation can be evaluated, it would still be very difficult to explain different behaviors of a set of genotypes across a series of environments with precision. This is due to the complex relationships among a vast number of environmental factors; most of which remain unidentified. Therefore, in order to evaluate GxE interactions of a genotype collection, researchers usually choose to focus on varying genotype performances over environments rather than how and why environments differ from each other. Performances of *m* genotypes over *n* environments provides an $m \times n$ two-way multi-environmental data matrix where any given genotype may or may not show a clear pattern across environments. Therefore, multi-environmental data becomes more confusing as the number of genotypes and environments increases (Allard and Bradshaw 1964). There are many studies illustrating different approaches to overcome this confusion. In order to see if there is a significant G × E interaction effect, an analysis of variance is conventionally included in most research (Primomo et al. 2002; Zewdie and Bosland 2000), often used along with another method to explore G x E interaction further or assess genotype stability. Several multivariate methods offer simplicity for genotype selection, including joint-regression (Yates and Cochran 1938), Principal Component Analysis (PCA) (Yan 2014), PCA with biplot (Sabaghnia 2016) or Additive Main Effects and Multiplicative Interaction (AMMI) (Adugna and Labuschagne 2002; Mohammadi and Amri 2008; Purchase et al. 2000).

Another approach to assess genotype stability is by using non-parametric methods which may be most appropriate when basic assumptions required for parametric methods are violated, such as a non-normal distribution or presence of outliers. Non-parametric methods compare two populations in terms of their central tendencies (typically medians) and can be applied in many data sets because they have fewer assumptions than parametric methods. Various research comparing parametric and non-parametric methods concluded that some frequently used non-parametric methods were useful to assess genotype stabilities (Akcura et al. 2009; Kilic et al. 2010; Yue et al. 1997).

In this study, 17 non-parametric methods were applied to evaluate leaf rust reactions across seven environments of 29 pure lines derived from bread wheat landraces delivered from Adiyaman and Kahramanmaraş provinces in Turkey. In addition, we used PCA biplot analysis to investigate interrelations among non-parametric measures.

Materials and methods

Test material and disease tests

Twenty-nine Turkish pure bread wheat lines previously derived from landraces of Adıyaman and Kahramanmaraş locations were used as test materials. Experiments were conducted throughout three consecutive growing seasons (from 2011–2012 to 2013–2014) at Çanakkale, Edirne and Samsun provinces. Çanakkale and Edirne are located in the Marmara Region. Long term annual temperature averages and precipitations of Çanakkale and Edirne are 15.0 °C and 13.8 °C with 616.3 mm and 605.0 mm of precipitation, respectively. Samsun, on the other hand, is located in the Black Sea Region, receiving 686.3 mm precipitation annually with an annual temperature average of 14.1 °C (Anonymous 2018). According to Köppen-Geiger classification, climates of Çanakkale and Edirne are classified as Csa (Mediterranean climate with dry and hot summers), where Samsun is classified as Cfa (humid subtropical) (Peel et al. 2007; Ozturk et al. 2016).

Landrace bread wheat pure line genotypes were sown by hand in the first quarters of November in each year and location. Experiments were laid out in a randomized complete block design with two replications. Plant rows were 1 m long and 20 cm apart from each other. Since the disease was developed naturally; susceptible control cultivars cv. Gün were sown once in every 6 rows when cv. Thatcher and cv. Morocco were sown once in every ten rows among plant material to ensure a uniform spread of leaf rust. In addition, an international differential set composed of 20 genotypes containing different leaf rust resistance genes were also sown to identify which resistance genes were virulent/avirulent to the leaf rust populations in our environments. The list of these virulent and avirulent Lr genes are declared in Akan and Akcura (2018).

The results of 2012–2013 and 2013–2014 growing seasons from the Samsun location were excluded due to lack of sufficient disease for proper evaluation. Hence, multi-environmental data used in our study consisted of seven environments: Çanakkale in 2011–2012, Edirne in 2011–2012, Samsun in 2011–2012, Çanakkale in 2012–2013, Edirne in 2012–2013, Çanakkale in 2013–2014 and Edirne in 2013–2014. Test materials were screened for leaf rust infections under natural conditions. Leaf rust infections were recorded using the Modified Cobb scale and disease reactions determined by coefficients of infections and reaction groups. Coefficients of infections (CI) varied between 0 and 100 according to disease spread on plant leaves within the plots, upon which the reaction of groups were assigned: R (resistant), very small uredia surrounded by necrotic tissues; MR (moderately resistant), small uredia surrounded by necrotic tissues; MS (moderately susceptible), moderate-sized uredia without necrotic tissues; S (susceptible), large uredia without necrotic tissues (Peterson et al. 1948; Akan and Akcura 2018). Susceptible control cultivars reached 80–100 *S* in all seven environments, indicating an adequate disease infection for further evaluation. After that point, leaf rust infections of landrace bread wheat pure lines were assessed three times in every ten days. The replication with the maximum score was taken as the basis of reaction at each location (environment). CI were grouped as follows: Immune (I): 0, Resistant (R): 0.1–5.0, Moderately Resistant (MR): 5.01–20.0, Moderately Susceptible (MS): 20.1–40.0, Susceptible (S): 40.1–100 (Akan and Akcura 2018). Additionally, a scatter plot of mean disease severity versus standard deviation (Fig. 1) were created in Minitab 17 (Minitab 17 Statistical Software 2010).

Statistical evaluation

S_1, S_2, S_3 and S_6 rank stability statistics from Huehn (1979) with the top, middle and lower third of entries (TOP, MIDDLE and LOWER) from Fox et al. (1990) were analyzed using the SAS 9.1 statistics software® (SAS Institute, Cary NC).

Additionally, Percent Adaptability (PA) from St.Pierre et al. (1967), Rank Sum (RS) from Kang (1988), reaction indexes R1i and R2i from Langer et al. (1979), Rank Means (RM) and Ranks Standard Deviation (RSD) from Ketata et al. (1989), $NP^1{}_i$, $NP^2{}_i$, $NP^3{}_i$ and $NP^4{}_i$ from Thennarasu (1995), NS_1 and NS_2 from Sabaghnia (2015) were analyzed with MS Excel.

Rankings of genotypes over environments (r_{ij}) were calculated from actual disease severity data (DS_{ij}, presented in Table 2) for calculating rank based non-parametric statistics, forming a matrix. This matrix is similar to disease severities except it consists of rankings of genotypes instead of disease scores (CI) ranging between 1 and 29 for each environment. Formulas applied to both matrices are shown in Table 1. For the calculations above, k = number of test genotypes, n = number of environments, DS_{ij} = disease severity of genotype *i* in environment *j*, r_{ij} = rank of genotype *i* in environment *j*, r_i = rank mean across environments for genotype *i*, M_{di} = rank median across environments for genotype *i*. In the ranking matrix, Q_1 and Q_3 are the first and third quartiles when D_1 and D_9 are the first and ninth deciles of the ranks of a given genotype across all environments, respectively (Sabaghnia 2015). In addition, r_i^*, r_{ij}^* and M_{di}^* are adjusted versions (adjusted phenotypic values) of r_i, r_{ij} and M_{di} computed according to $DS_{ij}^* = DS_{ij} - DS_i$ in order to eliminate the genotype effect as described by Thennarasu (1995).

Calculated non-parametric stability statistics were presented in Table 3. Since the selection criteria of non-parametric methods vary, results are arranged by MDS and presented as a heatmap for convenience. Background color of outputs for each non-parametric statistic is arranged between shades of green and red (white being neutral) depending on how well genotypes comply with their criteria: desired genotypes with high stability are expected to score lower for $NP^1{}_i$, $NP^2{}_i$, $NP^3{}_i$, $NP^4{}_i$, PA, RSD, S_1, S_2, S_3, S_6, TOP, MIDDLE, RS, NS_1, NS_2 and higher for RM, MR, LOWER or close to zero for R1i and R2i (Table 3). In addition to genotype selection, outputs of the non-parametric methods statistically evaluated with PCA biplot to reveal which statistics would be more useful to select for both stability and leaf rust resistance.

PCA Biplot analysis is a well-known multivariate method to evaluate interrelationships among genotypes and environments by reducing the dimensionality and plotting all in one biplot graphic (Akcura and Ceri 2011). PCA Biplot graphic of non-parametric stability measures was created by JMP Version 13 (JMP 2016). Interpretations about relationships between non-parametric measures were based on Torres-Salinas et al. (2013).

Results

Assessment of genotype reactions by non-parametric stability statistics

Leaf rust severities with means and standard deviations are given in Table 2. Disease reactions of landrace bread wheat pure lines varied between 1 and 95 across seven environments (Table 2). Leaf rust disease reactions of many genotypes varied across environments due to the high variation of disease severities. Genotype 25 were

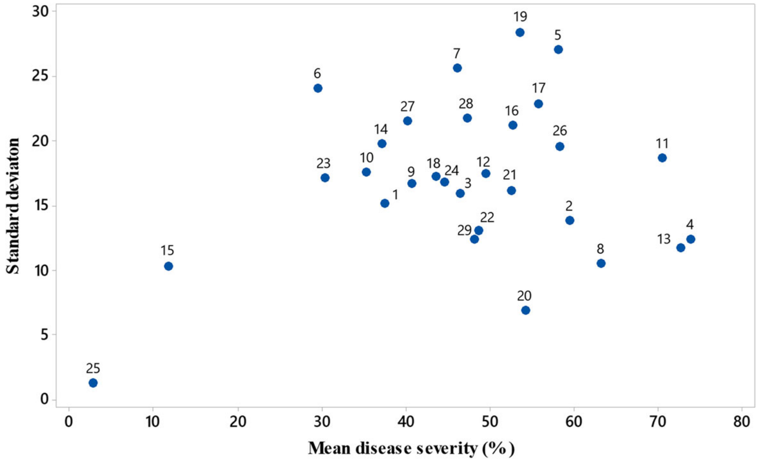


Fig. 1 Scatterplot of mean disease severities of 29 bread wheat pure lines over 7 environments with standard deviation

found resistant to leaf rust in all environments when reaction groups of Genotype 15 were Resistant in E2, E3, E5 and E7 and moderately resistant for E1, E4 and E6 (Table 2). Genotypes 4, 11 and 13 had the highest MDS in our study, thus consistently appeared as being susceptible to leaf rust in all environments. Mean disease severities and standard deviations across environments from Table 2 were also presented as a scatterplot in Fig. 1. Genotypes with the lowest standard deviations tended to have either the lowest (Genotypes 25 and 15) or the highest (Genotypes 8, 13 and 4 with the exception of Genotype 20) mean disease severities when other genotypes with higher standard deviations were located in between these extreme genotypes (Fig. 1).

Non-parametric measures used in this study were mostly calculated on genotype rankings over environments rather than their actual disease reactions. Given that selecting for resistance to the leaf rust requires negative selection, genotypes with lower values were selected on some non-parametric stability statistics such as TOP (Fox et al. 1990), Percentage Availability (PA) (St.Pierre et al. 1967), RS (Kang 1988) and RM (Ketata et al. 1989). According to the results of Fox's stability statistics, the most resistant landrace pure lines were separated from others by having lower disease reactions, thus appearing in the lower third of all genotypes more often. Genotypes 9, 10, 15, 23, 25 and 29 scored highest (100) in LOWER and lowest (0) in both TOP and MIDDLE since they consistently appeared among the least infected genotypes in all environments. PA, R1i, R2i, RM and RSD separated those genotypes further, narrowing the preferable genotypes down to 15 and 25. PA values of 25 and 15 were 0.0, meaning that both of these genotypes never exceeded the mean disease severity in any environment. Genotypes 25 and 15 also had the lowest R1i scores and closest R2i scores to zero of all genotypes indicating that their leaf rust severities had minimal deviation across environments. Moreover, low RM and RSD values of Genotypes 25 and 15 are also an indication of their consistently lower leaf rust severities across environments.

Other commonly used non-parametric stability statistics, NP^{1}_{i}, NP^{2}_{i}, NP^{3}_{i} and NP^{4}_{i} (Thennarasu 1995) are based on adjusted ranks while S_1, S_2, S_3, S_6 (Huehn 1979), NS_1, NS_2 (Sabaghnia 2015) are based on absolute rank differences and variances between genotype rankings across environments. The majority of the stability statistics confirmed that genotypes 15, 22, 23, 25 and 29 had relatively stable disease reactions across environments. Since some of these genotypes were undesirable due to higher disease scores, resistant – stable genotypes needed to be defined. NP^{1}_{i}, NP^{2}_{i}, NP^{3}_{i}, NP^{4}_{i}, S_1, S_2, S_3, S_4, NS_1 and NS_2 failed to recognize the lower disease reactions of Genotypes 15 and 25 (the resistant-stable genotypes) and favored them along with other stable but susceptible genotypes (Tables 2 and 3). Genotype 29 could be selected by Kang's, Thennarasu's, Huehn's and Fox's stability measures with Genotype 20, which also delivered significantly lower results in Huehn's statistics and RS. RS also took high performance into consideration but failed to provide meaningful outcomes.

Results of some non-parametric stability statistics were not useful in terms of detecting susceptibility. Genotypes 4, 13 and 11 had high disease scores with low standard deviations, thus consistently marked as

Table 1 Non-parametric stability statistic symbols, formula and references

Non-parametric Stability Statistic	Definitions and References
$\begin{cases} n_{ij} = 1, & DS_{ij} > \overline{DS}_j \\ n_{ij} = 0 & else \end{cases}$, $PA_i = \frac{\sum_{j=1}^{n} n_{ij}}{n}$ x 100	PA: Percentage Availability, from St.Pierre et al. (1967)
$R1i = DS_{iMax} - DS_{iMin}$ $R2i = DS_{ib} - DS_{il}$ DS_{iMax} = Maximum disease severity of genotype *i* in a series of environments, DS_{iMin} = Minimum disease severity of genotype *i* in a series of environments, DS_{ib} = Disease severity of genotype *i* in the best environment, DS_{il} = Disease severity of genotype *i* in the lowest environment.	R1i and R2i stand for the first and second stability index from Langer et al. (1979).
$RS = R_{\overline{X_i}} + R_{\sigma_i^2}$ $R_{\overline{X_i}}$ =Ranking of genotype *i* in genotype rankings by disease severity. Genotype with the highest value ranked first. $R_{\sigma_i^2}$= Ranking of genotype *i* by Shukla's stability variance (Shukla, 1972). Genotype with the lowest value ranked first. $\sigma_i^2 = \frac{P}{(p-2)(q-1)} \sum_{j=1}^{q} \left(X_{ij} - \overline{X}_i - \overline{X}_j + \overline{X}..\right)^2 \frac{SS(GXE)}{(p-1)(p-2)(q-1)}$	RS: Rank Sum. from Kang (1988). Shukla's stability variance formula was derived from (Lin et al. 1986).
$\begin{cases} t_{ij} = 1, & r_{ij} > {}^{2}/_{3}k \\ t_{ij} = 0, & else \end{cases}$, $TOP = {}^{\sum_{j=1}^{n} t_{ij}}/_{n}\ x\ 100$ $\begin{cases} m_{ij} = 1, & {}^{1}/_{3}k < r_{ij} < {}^{2}/_{3}k \\ m_{ij} = 0, & else \end{cases}$, $MIDDLE = {}^{\sum_{j=1}^{n} m_{ij}}/_{n}\ x\ 100$ $\begin{cases} l_{ij} = 1, & r_{ij} < {}^{1}/_{3}k \\ l_{ij} = 0, & else \end{cases}$, $LOWER = {}^{\sum_{j=1}^{n} l_{ij}}/_{n}\ x\ 100$	The top, middle and lower third of entries (TOP, MIDDLE and LOWER) were proposed by Fox et al. (1990).
$S_1 = 2\sum_{j}^{n-1} \sum_{j'=j+1}^{n} \lvert r_{ij} - r_{ij'} \rvert / [n(n-1)]$	S_1: First Stability statistic. (Huehn 1979)
$S_2 = \sum_{j=1}^{n} \left(r_{ij} - \overline{r}_i\right)^2 / (n-1)$	S_2: Second Stability statistic (Huehn 1979)
$S_3 = \sum_{j=1}^{n} \left(r_{ij} - \overline{r}_i\right)^2 / r_i$	S_3: Third Stability statistic (Huehn 1979)
$S_6 = \sum_{j=1}^{n} \lvert r_{ij} - \overline{r}_i \rvert / \overline{r}_i$	S_6: Sixth Stability statistic (Huehn 1979)
$RM = {}^{r_{ij}}/_{n}$	RM: Rank means (Ketata et al. 1989), formula delivered from Akcura and Kaya (2008)
$RSD = \sqrt{\sum_{i=1}^{j} (r_i - \overline{r})^2 / n-1}$	RSD: Ranks standard deviation (Ketata et al. 1989), formula derived from (Akcura and Kaya 2008)
$NP_i^1 = \sum_{j=1}^{n} \lvert r_{ij}^* - M_{di}^* \rvert / n$	NP^1_i: First Non-Parametric statistic (Thennarasu 1995)
$NP_i^2 = \left[\sum_{j=1}^{n} \lvert r_{ij}^* - M_{di}^* \rvert / M_{di}\right] / n$	NP^2_i: Second Non-Parametric statistic (Thennarasu 1995)

Table 1 (continued)

Non-parametric Stability Statistic	Definitions and References
$NP_i^3 = \sqrt{\left(r_{ij}^* - r_i^*\right)^2 / n} \Big/ \overline{r_i}$	NP^3_i: Third Non-Parametric statistic (Thennarasu 1995)
$NP_i^4 = \frac{2}{n(n-1)} \left[\sum_{j=1}^{n-1} \sum_{[j' = j+1]}^{n} \left\lvert r_{ij}^* - r_{ij'}^* \right\rvert \Big/ \overline{r_i} \right]$	NP^4_i: Fourth Non-Parametric statistic (Thennarasu 1995)
$NS_1 = {}^{(Q_3 - Q_1)} \big/ {}_{M_{di}}$	NS_1:First non parametric stability statistic from Sabaghnia 2015.
$NS_2 = {}^{(D_9 - D_1)} \big/ {}_{M_{di}}$	NS_2:Second non parametric stability statistic from Sabaghnia 2015.

"susceptible" across all environments (Table 2, Fig. 1). Since the non-parametric analysis was based on genotype rankings rather than the reactions of groups shown in letters from S (susceptible) to R (resistant) in Table 2, only a few of non-parametric statistics were able to classify them as susceptible-stable (Table 3) even though their susceptibility to leaf rust in all environments was unquestionable. Therefore, the difference between the reaction of groups and non-parametric assessment should be taken into consideration when non-parametric statistics are used to determine susceptibility.

Interpreting the relationships between non-parametric stability statistics

Spearman correlations of non-parametric stability statistics were presented in Table 4. NP^1_i, NP^3_i, R1i, RSD, S_1, S_2, S_3, S_6, NS_1, NS_2 and TOP were significantly correlated with each other. Significant and positive correlations between Huehn's statistics were recorded previously by Sabaghnia et al. (2006), Karimizadeh et al. (2012) and Mohammadi et al. (2007). In some instances, this similarity extended to Thennarasu's statistics (Sabaghnia et al. 2013; Kaya and Turkoz 2016; Sabaghnia et al. 2016). Mean disease severity was positively correlated with PA, NP^3_i, S_6 and TOP when negatively correlated with RM, LOWER and RS, R1i of Langer et al. (1979) and RSD of Ketata et al. (1989) were both aimed at responding differences between maximum and minimum values of a genotype over all environments. Naturally, results obtained from those statistics were found to be aligned (Fig. 2 and Table 4).

A PCA biplot of non-parametric statistics calculated from the output matrix is given in Table 3 is presented in Fig. 2. PC 1 (49.5%) and PC2 (21.5%) were accounted for 71% of total variance. Interpretations can be deducted on the biplot from the distribution of non-parametric statistics with regard to mean disease severity (MDS). Non-parametric statistics included by the same group can be interpreted as being positively related to each other (Zali et al. 2011). It should be noted that NP^1_i, NP^3_i, S_2, S_3, S_6 and NS_2 were excluded from PCA for their unusually high correlations with other variables such as S_1, R1i, MDS and NS_1 (Table 4). In addition, NP^2_i, R2i and MIDDLE were also excluded from the PCA due to their short vector sizes, which were an indication that these parameters didn't contribute significantly to the overall variation (Yan 2014).

Non-parametric measures were concentrated into five groups in PCA Biplot (Fig. 2). First group contained R1i and S_1. Another strong and positive relationship was visible between R1i and RSD. The second group consisted of NS_1, and TOP when MDS, PA and NP_4, were included by the third group. Fourth group (RM, LOWER and MR) were located opposite from these measures with respect to the biplot origin. This is a clear indication that non-parametric statistics of Group III were positively and strongly associated with MDS when statistics included by Group IV demonstrated a negative and strong association. In other words, higher scores for the statistics included by Group IV reflected mean disease severities of wheat genotypes when the lower scores for the Group III statistics is an indication for their leaf rust resistance (Fig. 2). Relationships between non-parametric stability statistics were generally

Table 2 Materials pedigree and leaf rust disease reactions of bread wheat landrace genotypes in Çanakkale, Edirne between 2011 and 2014 and Samsun location in 2011–2012

	Registration Codes	E1		E2		E3		E4		E5		E6		E7		MDS	STD
		CI	RG	CI	RG	CI	RG	CI	RG	CI	RG	CI	RG	CI	RG		
1	ADIYAMAN TR 49034/2	60.0	S	20.0	MR	21.0	MR	30.0	MS	55.0	S	29.0	MR	47.0	S	37.43	15.16
2	ADIYAMAN TR 50457/6	47.0	S	54.0	S	60.0	S	60.0	S	45.0	S	60.0	S	90.0	S	59.43	13.77
3	ADIYAMAN TR 50476/1	53.0	S	55.0	S	18.0	MR	53.0	S	60.0	S	26.0	MR	60.0	S	46.43	15.83
4	ADIYAMAN TR 50455/1	90.0	S	80.0	S	70.0	S	90.0	S	57.0	S	70.0	S	60.0	S	73.86	12.33
5	ADIYAMAN TR 46810/6	46.0	S	80.0	S	20.0	MR	54.0	S	90.0	S	27.0	MR	90.0	S	58.14	26.98
6	ADIYAMAN TR 49029/3	46.0	S	6.0	R	45.0	S	72.0	S	5.2	R	27.0	MR	5.6	R	29.54	24.02
7	ADIYAMAN TR 50465/6	60.0	S	12.0	MR	12.8	MR	66.0	S	72.0	S	27.0	MR	72.0	S	45.97	25.54
8	ADIYAMAN TR 49034/3	65.0	S	70.0	S	80.0	S	70.0	S	57.0	S	50.0	S	50.0	S	63.14	10.45
9	ADIYAMAN TR 46822/3	54.0	S	20.0	MR	26.0	MR	55.0	S	45.0	S	21.0	MR	63.0	S	40.57	16.60
10	ADIYAMAN TR 50464/5	54.0	S	8.0	R	8.7	R	49.0	S	41.0	S	45.0	S	41.0	S	35.24	17.52
11	ADIYAMAN TR 50465/1	85.0	S	90.0	S	90.0	S	45.0	S	43.0	S	75.0	S	65.0	S	70.43	18.62
12	ADIYAMAN TR 49040/5	53.0	S	70.0	S	10.0	MR	56.0	S	56.0	S	45.0	S	56.0	S	49.43	17.48
13	ADIYAMAN TR 49040/4	60.0	S	80.0	S	70.0	S	70.0	S	58.0	S	95.0	S	75.0	S	72.57	11.64
14	ADIYAMAN TR 49040/6	65.0	S	6.0	R	10.0	MR	45.0	S	46.0	S	41.0	S	46.0	S	37.00	19.70
15	ADIYAMAN TR 50465/4	21.0	MR	2.0	R	3.0	R	22.0	MR	4.0	R	27.0	MR	2.5	R	11.64	10.28
16	ADIYAMAN TR 50476/4	56.0	S	21.0	MR	21.0	MR	55.0	S	74.0	S	67.0	S	74.0	S	52.57	21.17
17	ADIYAMAN TR 49029/5	65.0	S	19.0	MR	24.0	MR	80.0	S	57.0	S	67.0	S	78.0	S	55.71	22.85
18	ADIYAMAN TR 49029/6	53.0	S	18.0	MR	19.0	MR	60.0	S	45.0	S	65.0	S	45.0	S	43.57	17.24
19	ADIYAMAN TR 46822/5	46.0	S	17.0	MR	18.0	MR	45.0	S	90.0	S	69.0	S	90.0	S	53.57	28.35
20	ADIYAMAN TR 49029/1	54.0	S	64.0	S	44.0	S	57.0	S	55.0	S	45.0	S	60.0	S	54.14	6.83
21	ADIYAMAN TR 50476/5	56.0	S	20.0	MR	43.0	S	60.0	S	72.0	S	49.0	S	67.0	S	52.43	16.11
22	K.MARAŞ M-396/6	45.0	S	54.0	S	20.0	MR	60.0	S	58.0	S	45.0	S	58.0	S	48.57	12.98
23	K.MARAŞ M-397/6	10.0	MR	11.0	MR	11.0	MR	47.0	S	46.0	S	41.0	S	46.0	S	30.29	17.09
24	K.MARAŞ TR 32009/1	60.0	S	19.0	MR	43.0	S	60.0	S	55.0	S	20.0	MR	55.0	S	44.57	16.71
25	K.MARAŞ M-397/4	4.5	R	2.8	R	1.9	R	1.0	R	4.2	R	3.0	R	1.9	R	2.76	1.18
26	K.MARAŞ M-388/4	56.0	S	72.0	S	90.0	S	56.0	S	57.0	S	20.0	MR	57.0	S	58.29	19.54
27	K.MARAŞ M-398/3	19.0	MR	17.0	MR	20.0	MR	79.4	S	45.0	S	56.0	S	45.0	S	40.20	21.47
28	K.MARAŞ M-394/6	81.5	S	60.0	S	15.0	MR	45.0	S	55.0	S	19.0	MR	55.0	S	47.21	21.71
29	K.MARAŞ M-391/6	55.0	S	55.0	S	20.0	MR	55.0	S	55.0	S	42.0	S	55.0	S	48.14	12.33

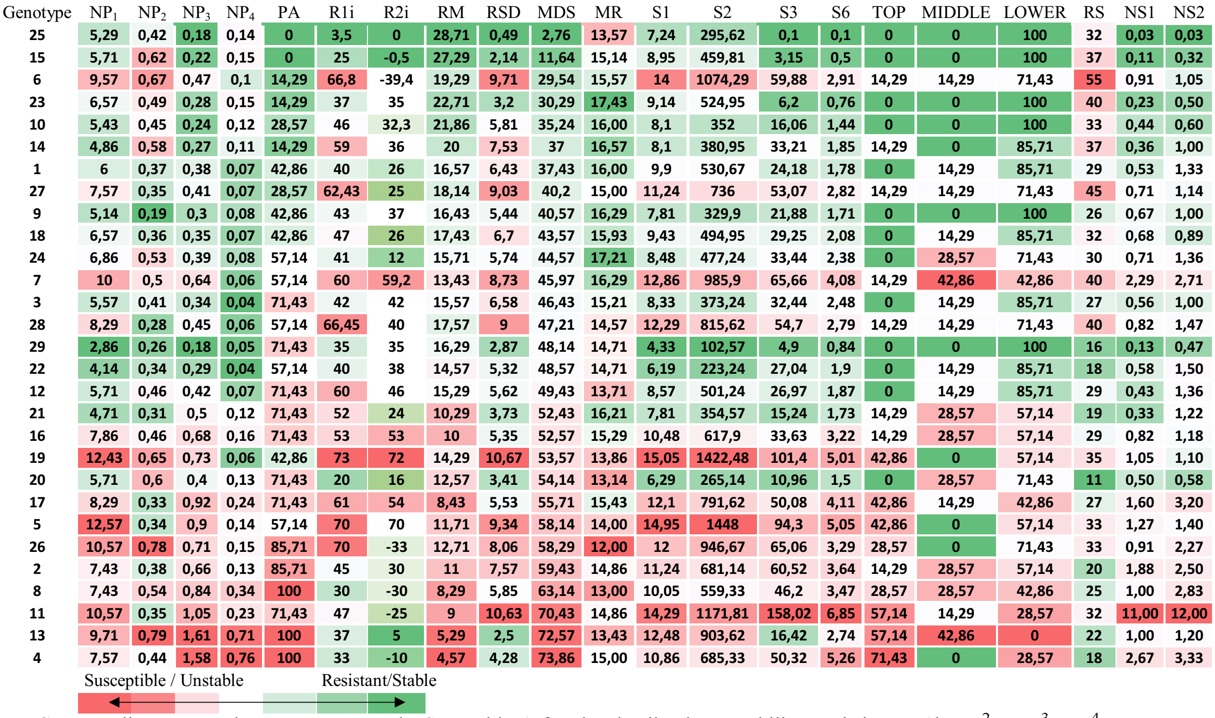
E1 Çanakkale 2011–2012; *E2* Edirne 2011–2012; *E3* Samsun 2011–2012; *E4* Çanakkale 2012–2013; *E5* Edirne 2012–2013; *E6* Çanakkale 2013–2014; *E7* Edirne 2013–2014; *CI* Coefficients of Infection; *RG* Reaction of Group; *MDS* Mean disease severity across seven environments; *STD* Standard deviation of leaf rust reactions across environments

in agreement with the earlier studies (Duarte and Zimmermann 1995; Scapim et al. 2000).

Discussion

There are many statistical approaches available to evaluate multi-environmental data. While research about non-parametric assessments of varying grain yields of wheat genotypes across environments are fairly common, few reports were published about disease reactions (Sabaghnia 2016). In this study, leaf rust reactions of 29 bread wheat pure lines over 7 environments were evaluated by some commonly referenced non-parametric statistics with the aim of selecting resistant and stable genotypes. According to the results of non-parametric stability statistics, leaf rust reactions of genotypes 15, 20, 25 and 29 were found to be stable across all

Table 3 Heatmap of non-parametric stability statistics of 29 pure bread wheat landrace lines



Genotype	NP_1	NP_2	NP_3	NP_4	PA	R1i	R2i	RM	RSD	MDS	MR	S1	S2	S3	S6	TOP	MIDDLE	LOWER	RS	NS1	NS2
25	5,29	0,42	0,18	0,14	0	3,5	0	28,71	0,49	2,76	13,57	7,24	295,62	0,1	0,1	0	0	100	32	0,03	0,03
15	5,71	0,62	0,22	0,15	0	25	-0,5	27,29	2,14	11,64	15,14	8,95	459,81	3,15	0,5	0	0	100	37	0,11	0,32
6	9,57	0,67	0,47	0,1	14,29	66,8	-39,4	19,29	9,71	29,54	15,57	14	1074,29	59,88	2,91	14,29	14,29	71,43	55	0,91	1,05
23	6,57	0,49	0,28	0,15	14,29	37	35	22,71	3,2	30,29	17,43	9,14	524,95	6,2	0,76	0	0	100	40	0,23	0,50
10	5,43	0,45	0,24	0,12	28,57	46	32,3	21,86	5,81	35,24	16,00	8,1	352	16,06	1,44	0	0	100	33	0,44	0,60
14	4,86	0,58	0,27	0,11	14,29	59	36	20	7,53	37	16,57	8,1	380,95	33,21	1,85	14,29	0	85,71	37	0,36	1,00
1	6	0,37	0,38	0,07	42,86	40	26	16,57	6,43	37,43	16,00	9,9	530,67	24,18	1,78	0	14,29	85,71	29	0,53	1,33
27	7,57	0,35	0,41	0,07	28,57	62,43	25	18,14	9,03	40,2	15,00	11,24	736	53,07	2,82	14,29	14,29	71,43	45	0,71	1,14
9	5,14	0,19	0,3	0,08	42,86	43	37	16,43	5,44	40,57	16,29	7,81	329,9	21,88	1,71	0	0	100	26	0,67	1,00
18	6,57	0,36	0,35	0,07	42,86	47	26	17,43	6,7	43,57	15,93	9,43	494,95	29,25	2,08	0	14,29	85,71	32	0,68	0,89
24	6,86	0,53	0,39	0,08	57,14	41	12	15,71	5,74	44,57	17,21	8,48	477,24	33,44	2,38	0	28,57	71,43	30	0,71	1,36
7	10	0,5	0,64	0,06	57,14	60	59,2	13,43	8,73	45,97	16,29	12,86	985,9	65,66	4,08	14,29	42,86	42,86	40	2,29	2,71
3	5,57	0,41	0,34	0,04	71,43	42	42	15,57	6,58	46,43	15,21	8,33	373,24	32,44	2,48	0	14,29	85,71	27	0,56	1,00
28	8,29	0,28	0,45	0,06	57,14	66,45	40	17,57	9	47,21	14,57	12,29	815,62	54,7	2,79	14,29	14,29	71,43	40	0,82	1,47
29	2,86	0,26	0,18	0,05	71,43	35	35	16,29	2,87	48,14	14,71	4,33	102,57	4,9	0,84	0	0	100	16	0,13	0,47
22	4,14	0,34	0,29	0,04	57,14	40	38	14,57	5,32	48,57	14,71	6,19	223,24	27,04	1,9	0	14,29	85,71	18	0,58	1,50
12	5,71	0,46	0,42	0,07	71,43	60	46	15,29	5,62	49,43	13,71	8,57	501,24	26,97	1,87	0	14,29	85,71	29	0,43	1,36
21	4,71	0,31	0,5	0,12	71,43	52	24	10,29	3,73	52,43	16,21	7,81	354,57	15,24	1,73	14,29	28,57	57,14	19	0,33	1,22
16	7,86	0,46	0,68	0,16	71,43	53	53	10	5,35	52,57	15,29	10,48	617,9	33,63	3,22	14,29	28,57	57,14	29	0,82	1,18
19	12,43	0,65	0,73	0,06	42,86	73	72	14,29	10,67	53,57	13,86	15,05	1422,48	101,4	5,01	42,86	0	57,14	35	1,05	1,10
20	5,71	0,6	0,4	0,13	71,43	20	16	12,57	3,41	54,14	13,14	6,29	265,14	10,96	1,5	0	28,57	71,43	11	0,50	0,58
17	8,29	0,33	0,92	0,24	71,43	61	54	8,43	5,53	55,71	15,43	12,1	791,62	50,08	4,11	42,86	14,29	42,86	27	1,60	3,20
5	12,57	0,34	0,9	0,14	57,14	70	70	11,71	9,34	58,14	14,00	14,95	1448	94,3	5,05	42,86	0	57,14	33	1,27	1,40
26	10,57	0,78	0,71	0,15	85,71	70	-33	12,71	8,06	58,29	12,00	12	946,67	65,06	3,29	28,57	0	71,43	33	0,91	2,27
2	7,43	0,38	0,66	0,13	85,71	45	30	11	7,57	59,43	14,86	11,24	681,14	60,52	3,64	14,29	28,57	57,14	20	1,88	2,50
8	7,43	0,54	0,84	0,34	100	30	-30	8,29	5,85	63,14	13,00	10,05	559,33	46,2	3,47	28,57	28,57	42,86	25	1,00	2,83
11	10,57	0,35	1,05	0,23	71,43	47	-25	9	10,63	70,43	14,86	14,29	1171,81	158,02	6,85	57,14	14,29	28,57	32	11,00	12,00
13	9,71	0,79	1,61	0,71	100	37	5	5,29	2,5	72,57	13,43	12,48	903,62	16,42	2,74	57,14	42,86	0	22	1,00	1,20
4	7,57	0,44	1,58	0,76	100	33	-10	4,57	4,28	73,86	15,00	10,86	685,33	50,32	5,26	71,43	0	28,57	18	2,67	3,33

Susceptible / Unstable 🟥 ← → 🟩 Resistant/Stable

MDS Mean disease severity; *MR* Mean rank. See Table 1 for the details about stability statistics NP1i, $NP^2{}_i$, $NP^3{}_i$,$NP^4{}_i$ Percentage Availability (PA), R1i, R2i, Rank Means (RM), Ranks Standard Deviation (RSD), S1, S2, S3, S6, TOP, MIDDLE, LOWER, Ranksum (RS), NS_1 and NS_2. Susceptible/Unstable genotypes are marked with the darker shades of red, indicating that those genotypes poorly comply with the criteria of any given statistic. Genotypes marked with red can be considered as unstable or both unstable and susceptible depending on any given statistic. Similarly, preferable genotypes with high stability and for some cases high leaf rust resistances are marked with shades of green

environments. However, different disease reactions of these four genotypes needed to be taken into consideration. Genotypes 25 and 15 were located apart from other genotypes in Fig. 1 due to their consistent and low leaf rust scores across all environments (Table 2). In addition to being stable and resistant, genotypes 15 and 25 did not contain extreme differences between their minimum and maximum values in comparison to others; which can be seen in their relatively low results of R1i and R2i and lower RSD values. Therefore, Genotype 25 and 15 were chosen as candidates for being the most leaf rust-resistant landrace derived pure lines with high stability.

Selecting promising genotypes for future breeding programs requires the assessment of stability and lower disease reactions simultaneously. Non-parametric stability statistics were originally created to evaluate grain yields of genotypes in multi-environmental datasets where higher performances are more favorable. Since our aim was to select genotypes with the least leaf rust disease severities as an implication of higher leaf rust resistances, we reversed the selection criteria of some statistics such as PA, RM and RS to suit our needs. Most of the stability statistics provided information that relatable to stability and/or leaf rust severity of genotypes except for RS. Langer's R1i and Huehn's S_1 (Group I in Fig. 2) were found to account for genotype stabilities across environments, which could be extended to Langer's and Huehn's other statistics such as R2i, S_2, S_3, S_6 due to being highly correlated to R1i and S_1 (Table 4). R2i, for example, is calculated after the definition of two environments: the environment with the highest average of genotype performances is considered as "the best environment" when the opposite is "the lowest environment". Then, R2i of any genotype is calculated by the difference of their disease reactions in these two environments. Absolute stability according to R2i is 0, which means that the performance of a given genotype did not change between the best and the lowest environments where the highest difference is expected.

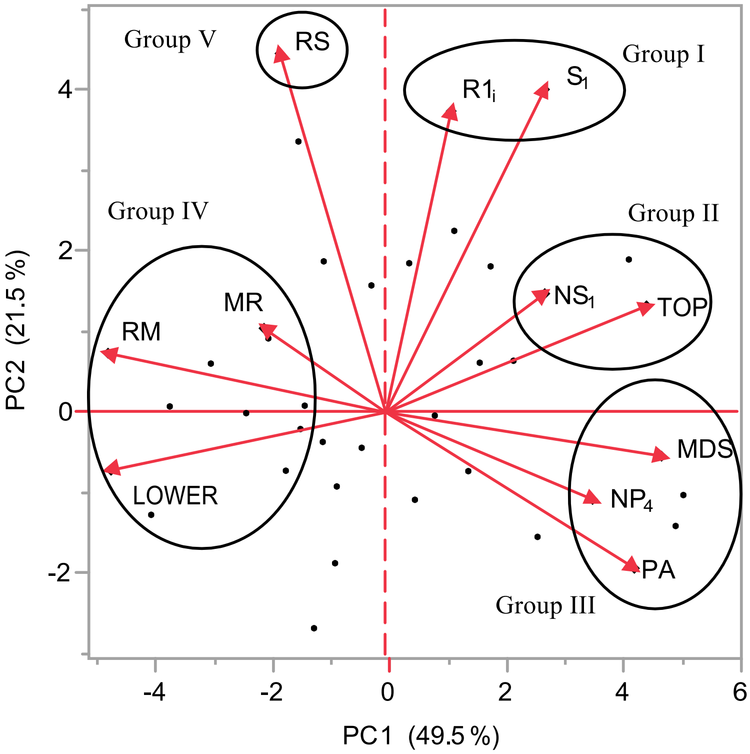
Table 4 Spearman correlations of non-parametric stability statistics with mean disease severity and rank means

correlation coefficients / p values

	NP1	NP2	NP3	NP4	PA	R1i	R2i	RM	RSD	MDS	MR	S1	S2	S3	S6	TOP	MIDDLE	LOWER	RS	NS1	NS2
NP1		*0.11*	*0.00*	*0.08*	*0.21*	*0.00*	*0.97*	*0.02*	*0.00*	*0.01*	*0.17*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.37*	*0.00*	*0.03*	*0.00*	*0.00*
NP2	0.31		*0.45*	*0.11*	*0.93*	*0.79*	*0.07*	*0.97*	*0.98*	*0.98*	*0.35*	*0.24*	*0.20*	*0.79*	*0.82*	*0.46*	*0.59*	*0.45*	*0.17*	*0.79*	*0.51*
NP3	0.79	0.15		*0.01*	*0.00*	*0.04*	*0.80*	*0.00*	*0.05*	*0.00*	*0.07*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.03*	*0.00*	*0.56*	*0.00*	*0.00*
NP4	0.33	0.30	0.49		*0.09*	*0.21*	*0.01*	*0.02*	*0.14*	*0.03*	*0.35*	*0.18*	*0.15*	*0.96*	*0.24*	*0.00*	*0.93*	*0.02*	*0.51*	*0.15*	*0.23*
PA	0.24	-0.02	0.66	0.32		*0.71*	*0.62*	*0.00*	*0.75*	*0.00*	*0.01*	*0.42*	*0.35*	*0.18*	*0.01*	*0.01*	*0.01*	*0.00*	*0.00*	*0.01*	*0.00*
R1i	0.55	-0.05	0.38	-0.24	-0.07		*0.03*	*0.79*	*0.00*	*0.65*	*0.76*	*0.00*	*0.00*	*0.00*	*0.00*	*0.02*	*0.84*	*0.15*	*0.00*	*0.03*	*0.09*
R2i	0.01	-0.35	-0.05	-0.47	-0.10	0.41		*0.99*	*0.38*	*0.92*	*0.34*	*0.77*	*0.85*	*0.45*	*0.59*	*0.65*	*0.80*	*0.73*	*0.73*	*0.87*	*0.97*
RM	-0.42	0.01	-0.84	-0.44	-0.89	-0.05	0.00		*0.84*	*0.00*	*0.06*	*0.06*	*0.05*	*0.03*	*0.00*	*0.00*	*0.01*	*0.00*	*0.00*	*0.00*	*0.00*
RSD	0.60	0.01	0.37	-0.28	-0.06	0.77	0.17	-0.04		*0.55*	*0.97*	*0.00*	*0.00*	*0.00*	*0.00*	*0.03*	*0.88*	*0.08*	*0.00*	*0.00*	*0.03*
MDS	0.46	-0.01	0.82	0.41	0.90	0.09	-0.02	-0.94	0.12		*0.00*	*0.04*	*0.03*	*0.01*	*0.00*	*0.00*	*0.08*	*0.00*	*0.00*	*0.00*	*0.00*
MR	-0.26	-0.18	-0.34	-0.18	-0.46	0.06	0.18	0.36	0.01	-0.54		*0.40*	*0.37*	*0.50*	*0.25*	*0.11*	*0.92*	*0.18*	*0.16*	*0.34*	*0.38*
S1	0.96	0.23	0.76	0.26	0.16	0.61	0.06	-0.35	0.67	0.38	-0.16		*0.00*	*0.00*	*0.00*	*0.00*	*0.47*	*0.00*	*0.01*	*0.00*	*0.00*
S2	0.96	0.24	0.78	0.27	0.18	0.64	0.04	-0.37	0.67	0.40	-0.17	0.99		*0.00*	*0.00*	*0.00*	*0.52*	*0.00*	*0.01*	*0.00*	*0.00*
S3	0.79	0.05	0.69	0.01	0.26	0.71	0.15	-0.41	0.86	0.46	-0.13	0.81	0.82		*0.00*	*0.00*	*0.47*	*0.00*	*0.08*	*0.00*	*0.00*
S6	0.81	0.04	0.87	0.23	0.49	0.53	0.11	-0.68	0.66	0.68	-0.22	0.81	0.82	0.92		*0.00*	*0.20*	*0.00*	*0.70*	*0.00*	*0.00*
TOP	0.74	0.14	0.89	0.52	0.48	0.42	-0.09	-0.68	0.41	0.70	-0.31	0.76	0.78	0.70	0.83		*0.54*	*0.00*	*0.76*	*0.00*	*0.00*
MIDDLE	0.17	0.11	0.40	0.02	0.47	-0.04	-0.05	-0.49	0.03	0.34	-0.02	0.14	0.13	0.14	0.24	0.12		*0.00*	*0.15*	*0.10*	*0.04*
LOWER	-0.70	-0.15	-0.94	-0.44	-0.65	-0.28	0.07	0.85	-0.33	-0.79	0.25	-0.68	-0.69	-0.66	-0.84	-0.87	-0.55		*0.42*	*0.00*	*0.00*
RS	0.41	0.27	-0.11	-0.13	-0.67	0.54	0.07	0.58	0.53	-0.54	0.27	0.47	0.47	0.33	0.08	0.06	-0.27	0.16		*0.90*	*0.43*
NS1	*0.81*	0.05	0.86	0.28	0.50	0.41	0.03	-0.68	0.58	0.67	-0.18	0.80	0.79	0.86	0.95	0.79	0.32	-0.84	0.02		*0.00*
NS2	*0.54*	-0.13	0.77	0.23	0.62	0.33	0.01	-0.72	0.42	0.70	-0.17	0.54	0.57	0.71	0.80	0.66	0.38	-0.76	-0.15	*0.80*	

* p<0.01 p<0.05 positive relationship; p<0.05 p<0.01 negative relationship

Fig. 2 PCA biplot of non-parametric stability statistics



These group of statistics was only partially useful for the genotype selection because another method is needed to complement their failure to assess disease reactions. Some stability statistics such as Fox's parameters were also consistent with MDS but provided imprecise results (favoring many genotypes at once) when others such as NP_3 failed to separate resistant genotypes from susceptible ones. Comparing the results of non-parametric statistics (Table 3) with leaf rust disease reactions (Table 2) revealed that PA and RM were different than other statistics in terms of detecting significantly lower disease severities of Genotypes 15 and 25 and distinguishing them from other genotypes, especially from susceptible and stable Genotypes 20 and 29 (see also Fig. 1). Duarte and Zimmermann (1995) pointed out a similar difference between non-parametric statistics by stating that PA and R2i were uncorrelated and probably investigated different aspects of stability (see Becker and Leon 1988 and Lin et al. 1986 for a more detailed discussion of the different aspects of stability). RM is calculated as the mean of ranks of any genotype across environments and PA is the percentage of how many times any given genotype exceeds the environment average in a multi environmental data set. Hence, the calculations of PA and RM provided insights about both stability and severity. These two methods successfully expressed genotype performances over a series of environments because it was as easy to detect the lowest-performing genotypes as it was for the highest ones, hence these statistics were more useful for negative selection. In addition, PA and RM can easily be adapted to frequently used spreadsheet programs such as MS Excel due to their simpler formulas.

In conclusion, Genotypes 15 and 25 were selected as the most resistant-stable genotypes and PA and RM are recommended as useful selection tools to select for leaf rust resistance in a multi-environmental data set. Using non-parametric stability statistics provided valuable information about G × E interactions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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