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Nonparametric approaches for the study of $G \times E$ interaction of pearl millet genotypes

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Abstract

Analysis of multi-environment trials (METs) of crops for cultivar evaluation and recommendation is an important issue in plant breeding research. Evaluating both stability of performance and high yield is essential in MET analyses. The promising pearl millets genotypes were grown in different agroecological regions of Gujarat state to study their adaptability to varying climatic and soil conditions. Yield data of 23 pearl millets genotypes grown at Amreli, Anand, Dhari and Talaja locations during Kharif 2018 were collected. The result of the combined or pooled ANOVA revealed that genotype, environment and genotype-environment interaction were highly significant. Significant genotypic variance indicated genetic diversity among genotypes yield. Further, the results of non-parametric stability analysis indicated that the genotype G23 had the lowest value of $S_i^{(1)}$ and had higher grain yield as compared to overall mean grain yield thus genotype G23 were found to be more stable over location while G21 has been found to be highly unstable genotype. According to $S_i^{(6)}$, G10 was found to be the most stable genotype.

Keywords: Pearl millet, non-parametric methods, Genotype \times Environment Interaction, Multi environment trials

1. Introduction

Pearl millet is gaining importance as a climate-resilient and health-promoting nutritious crop. Pearl millet, commonly known as bulrush millet (*Pennisetum glaucum* (L.) R. Br.), also classified as *P. typhoides*, *P. americanum*, or *P. spicatum*, is a cultivated, small-grain, tropical cereal grass. Vernacular names include: “Bajra” (India), “gero” (Nigeria, Hausa language), “hegni” (Niger, Djerma language), “sanyo” (Mali), “dukhon” (Sudan, Arabic), and “mahangu” (Namibia) that belongs to the family of Gramineae. This grain is considered to be the poor man’s staple nourishment and is suitable to cultivate in drylands. It is the most widely grown drought-tolerant warm-season coarse grain cereal grown on 26 million ha in some of the harshest semi-arid tropical environments of South Asia and sub-Saharan Africa. It is also consumed as feed and fodder for livestock. It is the sixth most important cereal crop in the world next to maize, rice, wheat, barley and sorghum. India stands at the first position in the world for pearl millet market share. The multi environmental trial is one of the most important steps towards the crop improvement programme which shows the performance of the genotype at the multi-environment (multi-location, multi-year or both). The MET data show the performance of genotype in a different environment. A specified difference in the environment may produce a differential effect on phenotypes. This interplay of genetic and non-genetic effects causing differential relative performances of genotypes in different environments is called genotypes \times environment interaction (GEI).

A single pearl millet cultivar can't be expected to perform properly underneath all the environmental conditions, and a cultivar planted outdoors in its adaptation region would go through yield discount due to significant genotype \times environment interactions. The selection of millet variety with its production environment is often challenged by the occurrence of significant GEI in the variety development process. Despite millet's drought tolerance, it is largely affected by GEI, making it difficult and expensive to select and recommend new millet varieties for different environments.

Multi-environmental trials (MET), generally, have significant main effects and significant multiplicative genotype \times environment interaction effect. AMMI (additive main effects and multiplicative interaction) offers a more appropriate statistical analysis to deal with such situations, compared to traditional methods like ANOVA, PCA and linear regression. This study, selects genotypes for both high yield and stability in MET using the AMMI model (Gauch, 2006) [6].

According to Huehn (1996), there are two basic techniques for researching $G \times E$ interaction and determining genotype adaptability. The first and most popular method is parametric, which is based on distributional assumptions regarding genotypic, environmental, and $G \times E$ effects. The nonparametric or analytical clustering technique, which ties environments and phenotypes to biotic and abiotic environmental elements without making explicit modelling assumptions, is the second major approach.

The nonparametric procedures have the following advantages over the parametric stability methods: they reduce the bias caused by outliers, no assumptions are needed about the distribution of the observed values, they are easy to use and interpret, and additions or deletions of one or few genotypes do not cause much variation of results. With this motivation and looking towards the advantages of non-parametric methods, non-parametric methods were used to determine the stability of rice genotypes in the present study.

Huehn (1979) [8] and Nassar and Huehn (1987) [12] focused on four nonparametric measures of phenotypic stability $S_i^{(1)}$ is the mean of the absolute rank differences of a genotype over the n environments, $S_i^{(2)}$ is the variance among the ranks over the environment, $S_i^{(3)}$ and $S_i^{(6)}$ are the sum of the absolute deviations and sum of squares of rank for each genotype relative to the mean of ranks, respectively.

This work aimed to discover pearl millet genotypes with high mean yields and consistent yield performance across a range of environments, to investigate crossover and noncrossover interaction in METs, and to find the relationships between nonparametric stability statistics.

2. Materials and Methods

2.1 Study area

Multi-environment experiments have been conducted at different locations of Gujarat viz., Amreli (E_1), Anand (E_2), Dhari (E_3) and Talaja (E_4). For the study of stability analysis, twenty-three different genotypes of pearl millet were selected from four different environments (Table 1). The experiment was carried out under a large-scale variety trial (LSVT) of pearl millet in the year 2018 in the Kharif season. The experiment was laid out in randomized complete block design (RCBD) with three replications for all the locations and standard agronomic practices were followed.

2.2 Statistical Analysis

Data on grain yield (kg plot^{-1}) of pearl millet genotypes grown at different test locations were collected and subjected to stability analysis by four non-parametric methods which were proposed by Huehn (1979) [8], Nassar and Huehn (1987) [12]. They were based on ranks of genotypes within the environment. Genotypes with similar ranking across environments are classified as the most stable genotype. Huehn (1979) [8] and Nassar and Huehn (1987) [12] proposed following four non-parametric measures of phenotypic stability.

2.2.1 Mean of the absolute rank differences $\{S_i^{(1)}\}$ of a genotype

For a two-way data with k genotypes and n environments, the

statistics based on yield ranks of genotypes in each environment are given below:

$$S_i^{(1)} = \frac{2 \sum_{j=1}^{n-1} \sum_{j'=j+1}^n |r_{ij} - r_{ij'}|}{n(n-1)} \quad (i)$$

Where,

r_{ij} = the rank of the i^{th} genotype in the j^{th} environment based on $(Y_{ij} - \bar{Y}_i + \bar{Y}_.)$ Ranks are assigned from lowest to highest

\bar{r}_i = mean rank across environments for the i^{th} genotype

n = no. of environments

2.2.2 Variance among the ranks over the n environments $\{S_i^{(2)}\}$ (2)

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

2.2.3 Testing of Significance

The statistical properties of $S_i^{(1)}$ and $S_i^{(2)}$ have been investigated by Nassar and Huehn (1987) [12]. Approximate tests of significance based on the normal distribution are developed for these two nonparametric measures. One can compute the following statistic

$$S^{(m)} = \sum_{i=1}^K Z_i^{(m)} = \sum_{i=1}^K \frac{[S_i^{(m)} - E(S_i^{(m)})]^2}{\text{Var}(S_i^{(m)})} \quad (iii)$$

Where,

$m=1, 2$

$$E(S_i^{(1)}) = (k^2 - 1)/3k$$

$$E(S_i^{(2)}) = (k^2 - 1)/12$$

$$\text{Var}(S_i^{(1)}) = (k^2 - 1)[(k^2 - 4)(n + 3) + 30]/45k^2n(n - 1)$$

$$\text{Var}(S_i^{(2)}) = (k^2 - 1)[2(k^2 - 4)(n - 3) + 5(k^2 - 1)]/360n(n - 1)$$

The statistic may be approximated by a chi-square distribution with k degree of freedom with $E(S_i^{(m)})$ expected mean and variance $\text{Var}(S_i^{(m)})$. Under the null hypothesis that all genotypes are equally stable. The mean $E(S_i^{(m)})$ and variances $\text{Var}(S_i^{(m)})$ may be computed from the discrete uniform distribution $(1, 2, \dots, k)$.

2.2.3 Mean of the absolute rank differences $\{S_i^{(3)}\}$ of a genotype

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

2.2.4 Variance among the ranks over the n environments $\{S_i^{(6)}\}$ (6)

$$S_i^{(6)} = \frac{\sum_{j=1}^n |r_{ij} - \bar{r}_i|}{\bar{r}_i} \quad (v)$$

Kang's (1988) rank-sum is another nonparametric stability procedure where both yield and Shukla's (1972) stability variance are used as selection criteria. This index assigns a weight of one to both yield and stability statistics to identify high-yielding and stable genotypes. The genotype with the highest yield is given a rank of 1 and a genotype with the lowest stability variance is assigned a rank of 1. All genotypes are ranked in this manner, and the ranks by yield and by

stability variance are added for each genotype. The genotype with the lowest rank-sum is the most desirable one. Thennarasu (1995) proposed four non-parametric stability parameters based on the ranks of adjusted means of the genotypes in each environment. Low values of these statistics reflect high stability. The adjusted rank, r_{ij}^* is determined based on the adjusted phenotypic values ($x_{ij}^* = x_{ij} - \bar{x}_i$), where \bar{x}_i is the mean performance of the i^{th} genotype. The ranks, obtained from these adjusted values (x_{ij}^*), depend only on $G \times E$ interaction and error effects.

$$NP_i^{(1)} = \frac{1}{n} \sum_{j=1}^n |r_{ij}^* - M_{di}^*| \tag{vi}$$

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

$$NP_i^{(3)} = \frac{\sqrt{\sum (r_{ij}^* - \bar{r}_i)^2 / n}}{\bar{r}_i} \tag{viii}$$

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

Where,

r_{ij}^* = rank of x_{ij}^* ,

\bar{r}_i^* = mean ranks for the adjusted value

M_{di}^* = median ranks for the adjusted values

\bar{r}_i = mean ranks for the unadjusted values

M_{di} = median ranks for the unadjusted values

Table 1: The names of 23 pearl millet genotypes tested in four different environments.

Code	Genotypes
G1	GHB 1129
G2	GHB 1203
G3	GHB 1214
G4	GHB 1225
G5	GHB 1231
G6	GHB 1232
G7	GHB 1234
G8	GHB 1237
G9	GHB 1239
G10	GHB 1240
G11	GHB 1241
G12	GHB 1242
G13	GHB 1245
G14	GHB 1247
G15	GDBH 1
G16	GHB 538 (C)
G17	GHB 558 (C)
G18	GHB 732 (C)
G19	GHB 744 (C)
G20	GHB 905 (C)
G21	86M11 (C)
G22	9444 (C)
G23	Dhanshakti (C)

3. Results and Discussion

Yield data of 23 pearl millets genotypes grown at Amreli (E₁), Anand (E₂), Dhari (E₃) and Talaja (E₄) locations during kharif-2018 were collected. These data were subjected to analysis of variance for individual location as well as pooled over locations.

3.1 Analysis of Genotype × Environment Interaction

The Analysis of Variance (ANOVA) for individual environment indicated that the variance for genotypes was found significant in all the environments. This suggests the

presence of genetic variability among the genotypes under study at all the environments. Similar findings were reported by Anandan *et al.* (2009) ^[1] in rice, Ozberk, (2005) ^[13] in wheat, Sharma *et al.* (1998) ^[15] in Pearl millet and Shinde *et al.* (2002) ^[16] in pearl millet crop. The environment E₂ was high yielding environment for the genotypes under study, whereas the environment E₁, E₃ and E₄ were found to below yielding environment as indicated by the environmental index. Bartlett's Chi-square test (38.615, p-value-0.000) for testing of homogeneity of variances suggests data is heterogeneous at 5% level of Significance. In this condition the data need to be transform before performing pooled analysis. The transformation involves dividing observations of each environment/year by the square root of MSE of that environment/year which makes the error variances homogeneous and hence usual pooled analysis is performed on transformed Data.

The impact of environmental conditions, genotypes and their interactions on grain production of pearl millet genotypes were investigated using analysis of variance, presented in Table 2. The combined ANOVA revealed that genotype, environment and genotype-environment interaction were highly significant and contributed 13.46, 45.00 and 22.73 per cent of trial or total variation. Gauch and Zobel (1997) ^[7] reported that in normal multi-environment yield trials (MEYTs), GE accounted about 10% of the total variation. Present findings are in agreement with these reports. Significant genotypic variance indicated genetic diversity among genotypes.

Table 2: ANOVA table for pearl millet grain yield in 4 environments

Source of Variation	DF	Sum of Squares	SS (%)	Mean Squares
Environments	3	505.298	45.00	168.433**
Rep/ environment	8	35.057	-	4.382
Genotypes	22	151.206	13.46	6.873**
G × E	66	255.292	22.73	3.868**
Pooled Error	176	176.002	-	1.000
Total	275	-	-	-

**significant at 1% level of significance

3.2 Stability Analysis

The results of various nonparametric stability measures and genotypes mean yield are summarized in Table 4. The significance tests for $S_i^{(1)}$ and $S_i^{(2)}$ were developed by Nassar and Huehn (1987) ^[12] and were highly significant ($p < 0.01$) for all genotypes (data not shown). The $S_i^{(1)}$ and $S_i^{(2)}$ statistics are based on ranks of the genotypes across environments and they give equal weight to each environment. Genotypes that have the least values are considered as most stable genotypes. The $S_i^{(1)}$ estimates are based on all possible pair-wise rank differences across environments for each genotype, whereas $S_i^{(2)}$ is based on variances of ranks for each genotype across environments (Nassar and Huehn, 1987) ^[12]. Nevertheless, these two statistics ranked genotypes similarly for stability. According to $S_i^{(1)}$ and $S_i^{(2)}$, G23 had the least value and is the most stable genotype of all genotypes whereas G21 had the highest value and is indicated as the most unstable genotype. The next most stable genotype was G10, followed by G17 (Table 3).

Two other nonparametric statistics of Huehn (1979) ^[8], $S_i^{(3)}$ and $S_i^{(6)}$ combine yield and stability based on yield ranks of genotypes in each environment. These parameters measure

stability in units of the mean rank of each genotype. The maximum stability was found in genotypes that have the least value. The genotype G23 was most stable according to $S_i^{(3)}$, but according to $S_i^{(6)}$, G10 was the most stable genotype. The

highest and lowest mean yield was founded on genotypes G5 and G23. The genotype G21 was the most unstable genotype across all genotypes (Table 4).

Table 3: Mean value (Y) and nonparametric stability parameters (NP) for grain yield of pearl millet genotypes evaluated in 4 environments.

Genotype	Y	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$	KR
G1	2.88	8.33	43.00	8.90	1.38	5.50	0.48	0.48	0.57	20
G2	2.71	9.33	56.33	11.66	1.66	6.50	0.38	0.51	0.64	23
G3	2.56	9.00	52.33	14.95	2.29	7.50	0.63	0.75	0.86	34
G4	2.88	3.67	8.67	1.44	0.44	2.25	0.43	0.17	0.20	4
G5	3.03	3.83	10.25	1.56	0.56	7.00	0.42	0.36	0.19	11
G6	2.82	7.00	33.67	6.52	1.29	5.75	0.35	0.41	0.45	15
G7	2.66	4.00	11.00	2.64	0.80	3.25	0.19	0.27	0.32	14
G8	2.56	8.00	38.67	9.67	1.67	6.75	0.42	0.58	0.67	29
G9	2.72	10.33	68.67	14.71	1.57	5.25	0.38	0.51	0.74	27
G10	2.63	2.00	3.00	0.78	0.43	1.50	0.10	0.16	0.17	14
G11	2.56	7.17	32.25	9.92	1.95	6.25	0.50	0.69	0.74	9
G12	2.75	5.67	21.67	4.48	1.10	3.25	0.28	0.26	0.39	10
G13	2.41	8.83	49.58	17.00	2.17	8.00	0.78	0.98	1.01	42
G14	2.57	10.50	67.58	17.26	2.30	8.00	0.59	0.71	0.89	31
G15	2.63	6.50	26.25	7.00	1.51	6.00	0.39	0.56	0.58	7
G16	2.50	9.50	66.92	19.59	2.29	6.75	0.63	0.76	0.93	39
G17	2.22	2.33	3.33	2.00	1.20	4.00	1.30	0.86	0.47	27
G18	2.77	9.50	64.25	14.55	1.74	6.00	0.36	0.55	0.72	21
G19	2.65	8.50	50.25	13.40	1.73	5.00	0.53	0.57	0.76	24
G20	2.65	6.50	25.58	6.53	1.28	4.75	0.33	0.49	0.55	18
G21	2.46	11.00	102.00	38.25	3.75	6.50	1.93	1.03	1.38	43
G22	2.80	8.33	43.00	8.32	1.29	4.50	0.35	0.32	0.54	13
G23	1.64	0.50	0.25	0.60	1.20	5.25	17.75	6.38	0.40	45

KR = The rank sum of Kang

Table 4: Ranks of 23 pearl millet genotypes after yield data from 4 environments were analyzed for G×E interaction and stability using different nonparametric methods.

Genotype	Y	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$	KR
G1	3	13	13	12	11	11	14	8	11	11
G2	9	18	18	15	14	16	8	10	13	13
G3	16	17	17	19	20	21	19	18	19	19
G4	2	4	4	3	2	2	13	2	3	1
G5	1	5	5	4	3	20	12	6	2	5
G6	4	10	11	8	9	12	6	7	7	9
G7	10	6	6	6	4	3	2	4	4	7
G8	17	12	12	13	15	18	11	15	14	17
G9	8	21	22	18	13	9	9	11	17	15
G10	13	2	2	2	1	1	1	1	1	7
G11	17	11	10	14	18	15	15	16	16	3
G12	7	7	7	7	5	3	3	3	5	4
G13	21	16	15	20	19	22	20	21	22	21
G14	15	22	21	21	22	22	17	17	20	18
G15	13	8	9	10	12	13	10	13	12	2
G16	19	19	20	22	21	18	18	19	21	20
G17	22	3	3	5	6	5	21	20	8	15
G18	6	19	19	17	17	13	7	12	15	12
G19	11	15	16	16	16	8	16	14	18	14
G20	12	8	8	9	8	7	4	9	10	10
G21	20	23	23	23	23	16	22	22	23	22
G22	5	13	13	11	9	6	5	5	9	6
G23	23	1	1	1	6	9	23	23	6	23

The nonparametric stability measure (rank-sum) developed by Kang (1988) incorporates both yield and stability variance. The genotype with the lowest rank-sum is the most favorable one. The genotype G4 had a minimum value for rank-sum and therefore was the most stable genotype, followed by genotypes G15 and G11. The genotype G23 was the most

undesirable genotype according to Kang's rank-sum statistics (Table 3).

Table 3 shows the results of Thenarasu's nonparametric stability statistics, which are obtained using rankings of adjusted yield means and Table 4 shows the rankings of genotypes based on these characteristics. The most stable genotype was G10, which was followed by genotypes G4 and

G7, however the most unstable genotypes were G13 and G14 according to $NP_i^{(1)}$.

According to $NP_i^{(2)}$, The genotype G10 had the lowest value and was the most stable. The genotypes G21 and G23 had the highest value and were the most unfavorable genotypes. $NP_i^{(3)}$, like $NP_i^{(2)}$, found G10 to be the most stable genotype followed by G4, G12 and G7. The genotype G21

was the most unstable genotype with the lowest mean yield. The genotype G10 was found most stable genotype with the lowest value and G21 was identified as the most unstable genotype highest value according to $NP_i^{(4)}$. Three NPs ($NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$) were highly comparable to each other, indicating that G10 was the most stable.

Table 5: Spearman’s rank correlation coefficients between the different nonparametric stability parameters for grain yield of 23 pearl millet genotypes.

Methods	Y	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$
$S_i^{(1)}$	-0.07								
$S_i^{(2)}$	-0.05	0.99**							
$S_i^{(3)}$	-0.28	0.96**	0.95**						
$S_i^{(6)}$	-0.44*	0.87**	0.85**	0.95**					
$NP_i^{(1)}$	-0.30	0.61**	0.58**	0.68**	0.75**				
$NP_i^{(2)}$	-0.63**	0.24	0.22	0.37	0.52**	0.51*			
$NP_i^{(3)}$	-0.82**	0.39	0.37	0.54**	0.71**	0.60**	0.86**		
$NP_i^{(4)}$	-0.48*	0.86**	0.85**	0.96**	0.97**	0.68**	0.55**	0.72**	
KR	-0.66**	0.48*	0.48*	0.54**	0.60**	0.50*	0.66**	0.79**	0.63**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

3.3 Relationship among Different Stability Statistics

Each pair of nonparametric stability parameters had their Spearman's rank correlations computed (Table 5) and demonstrate a highly significant ($p < 0.01$) rank correlation between

$S_i^{(1)}$ and $S_i^{(2)}$, $S_i^{(1)}$ and $S_i^{(3)}$, $S_i^{(3)}$ and $S_i^{(6)}$. The mean yield was negatively significant correlated with $NP_i^{(4)}$ while it was negatively highly significantly correlated with

$NP_i^{(2)}$ and Kang rank-sum. The correlation also highly significant between $S_i^{(6)}$ and

$NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$. Two parameters $NP_i^{(1)}$ and $NP_i^{(2)}$ were positively correlated with each other.

A PC analysis based on the rank correlation matrix was used to better understand the relationship among the nonparametric approaches (Table 6). According to PC analysis, the first two PCs explained 88.36% of the variation in the original variables (PC1 and PC2 explained 54.72 per cent and 33.64 per cent, respectively).

Table 6: First two principal components loadings of ranks obtained from 9 nonparametric methods used to analyze G × E interaction of lentil genotype yields.

Stability methods	Principal component	
	PC1	PC2
Y	-0.110	-0.938
$S^{(1)}$	0.868	-0.403
$S^{(2)}$	0.942	-0.209
$S^{(3)}$	0.957	-0.068
$S^{(6)}$	0.963	0.111
$NP^{(1)}$	0.745	0.100
$NP^{(2)}$	-0.125	0.952
$NP^{(3)}$	-0.033	0.971
$NP^{(4)}$	0.979	0.059
KR	0.668	0.631

4. Conclusions

Interactions between genotype and environment are key sources of variation in any crop and the word "stability" is

commonly used to describe a genotype that has a generally consistent yield regardless of changing environmental conditions. The genotype which has a minimum variance for yield was more stable. The results of non-parametric stability analysis indicated that the genotype G23 had the lowest value of $S_i^{(1)}$ and had higher grain yield as compared to overall mean grain yield thus genotype G23 were found to be more stable over location while G21 has been found to be highly unstable genotype. According to $S_i^{(6)}$, G10 was found to be the most stable genotype.

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