Non-parametric Measures of Fruit Yield Stability in Spine gourd [*Momordica dioica* (Roxb.) Ex. Willd.] Genotypes

N.K. Jajoriya* and H.L. Raiger
All India Coordinated Research Network on Potential Crops, ICAR-NBPGR,
Pusa Campus (New Delhi), India.

(Corresponding author: N.K. Jajoriya*)

ABSTRACT: Multi environmental trials (MET) play a pivotal role in selecting the best genotype or agronomic practices to be adopted in future years at different locations by assessing the genotype stability across over environments before its commercial release. Objective of this investigation is to identify spine gourd genotypes that have high fruit yield and stable performance across different environments. The genotypes were developed by the various breeders at different centres of AICRN on Underutilized Crops in India. Ten genotypes were tested at four research centres for three years (2005-2007). Two non-parametric statistical tests of significance for genotype × environment (GE) interaction and 10 non-parametric measures of stability analysis were used to identify high yield stable genotype across the 11 environments. The non-parametric measures for G×E interaction were highly significant (p<0.05), suggesting different response of genotypes to the test location/year. Based on low value of non-parametric measures, RMF-17 was identified most stable genotype by Nassar and Huehn (1987); Thennarasu (1995). These non-parametric measures were observed to be associated with high mean fruit yield. The simple correlation coefficient using Spearman's rank correlation, calculated using the ranks was used to measure the relationship between the stability parameters. To understand the nature of relationships among the non-parametric methods, a hierarchical cluster analysis based on non-weighted values of genotypes was performed. The 8 stability measures fell into four groups. At a global level information on genetic improvement, adaptability and genotype environment interaction of spine gourd is restricted to few publications.

Keywords: Spine gourd [*Momordica dioica* (Roxb.) Ex. Willd.], genotype x environment interaction, non-parametric stability measures, hierarchical cluster analysis.

I. INTRODUCTION

Spine gourd is a perennial dioecious climber of cucurbitaceae family having tuberous roots. The vegetable is ideal for patients of diabetics and gout. The consumption of green fruits and tubers, stimulates the activities of pancreas and controls the level of sugar. Its green fruits contain 12-14% protein. It is also rich in ascorbic acid (275.1 mg/100g) and iodine (0.7 mg/100g). The roots, which often weigh around 500g are astringent and contain an alkaloid. The roots of female plants are larger than those of the male and are preferred for medicinal use. They are applied in bleeding piles and used for bowel affection and urinary complaints. The root is pasted and applied over the body as a sedative in fevers [6]. In India, an adult needs about 280g of vegetable per day for balanced diet, but the national consumption is <175 g, actual production of the vegetable is not even of the required quantity. The vegetable production must be increased by growing spine gourd to meet the demand of vegetables in India. Spine gourd is not cultivated on large scale in India. While diploid is collected from hill forests, tetraploid is cultivated in Assam and Nadia district of West Bengal. Tribal people in several states like Orissa, Chhattisgarh,

Jharkhand, Gujarat cultivate this vegetable in their backyards. The vegetable is very costly and is always in demand. The crop is not grown on large scale because of want of sufficient planting material and lack of appropriate cultivation technique [7]. Development of the genotypes or varieties, which can be adopted on a wide range of diversified environments, it is the ultimate aim of the vegetable breeders in a crop improvement programme. The major goal of plant breeding programme is to increase stability and stabilize crop yield over average of environments. The improved genotypes were evaluated in multilocation environment trials to test their performance across different environments. The genotype × environment interaction is a major problem when comparing the performance of genotypes across the environments [8]. Genotype environment interaction occurs when the performance of cultivar is not consistent from one location/year to another. A significant genotype x environment interaction for a quantitative trait such as a yield can decrease the correlation between phenotypic and genotypic, and reduces the progress in selection [9]. A more stable genotype, as compared to other should give relatively more stable yield across the

environments. The information of the nature of GE interaction can be utilized to reduce breeding and testing costs. The stability measures/indices have allowed breeders to identify widely adapted cultivars from those that are environment specific. This information is required by breeding programme and can help to improve recommendations to breeders [10].

The genotype environment interaction has been extensively studied by a galaxy of biometricians who have developed several methods to analyse it. The genotype variance [11], interaction sum of squares [12], slope of regression on environmental index [13], the non-parametric measures [4]. However, there are many questions about spine gourd productions, commercialization and genetic improvement in India. Hardly any work has been taken up on genetic improvement of this species so far in India. At a global level information on genetic improvement, adaptability and genotype environment interaction of spine gourd is restricted to few publications. There are two major approaches for studying G×E interaction. The first one is parametric approach which is more common and popular and involves relating observed genotype response, e.g., yield, to the sample of environmental conditions; the second one is the non-parametric approach, which defines environments and phenotypes relatively to biotic and abiotic factors. The parametric stability have good properties under certain statistical assumptions, like normal distribution of error and interaction effect; they may not perform where these assumptions are violated [14]. Non-parametric measures for stability based on ranks provide a viable alternative to existing parametric measures based on absolute data [4]. For many applications, including selection and breeding and testing programme, the rank orders of genotypes are the most essential information.

There is ample justification for the use of non-parametric measures in the assessment of yield stability of crop varieties. The chief motivation for the use of non-parametric measures is that they are useful in a number of problem situations. The non-parametric measures do not require any tacit assumptions about the normality and independence of observations as well as homogeneity of error variances. When sample size is very small non-parametric method is the obvious choice, unless the nature of the population is exactly known. Non-parametric measures are also less sensitive to measurement errors or to outliers than parametric measures. Above all, the use of non-parametric method becomes inevitable when the parametric method fails to provide valid interpretations due to the presence of large nonlinear genotype-environment interactions. For these reasons non-parametric measures are widely employed in the selection of crop varieties especially when the interest lies in genotypes which excel in both yield and stability. However, it is a known fact that the non-parametric methods are less powerful than their parametric counterparts. Nevertheless, an empirical investigation [15] has shown that when the number of genotypes in the trial is fairly large, the power efficiency of the non-parametric measures will be quite close to those of the parametric measures. So in

situations which are commonly encountered, i.e. those involving a good number of genotypes being performance-tested in a set of environments whose number is neither too small nor too large, the risk of selecting inferior genotypes from the use of non-parametric measures is minimal.

Several non-parametric methods have been developed to describe and interpret the responses of a variety to environmental variation [16, 17, 18, 19]. Huehn (1979)[16] proposed four non-parametric measures of phenotypic stability ($S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$) based on the classification of genotypes in each environments, and define stable genotype as those who position in relation to others remained unaltered in the set of environments assessed. Thannarashu (1995) [5] proposed as non-parametric stability measure based on ranks of adjusted means of the genotypes in each environment, and defined stable genotypes as those whose position in relation to the remained unaltered in the set of environments assessed. Truberg and Huehn 2000 [20] reported two approaches for the test of significant genotype × environment interaction; parametric and non-parametric approaches. For data set with more than two environments, the genotype x environment interactions are commonly calculated by analysis of variance (ANOVA). Non-parametric statistical procedures for the test of crossover interactions have been developed in the field of medicine and can be applied to GE interaction. The objectives of this study were: (i) to identify spine gourd genotype that has high fruit yield and stable performance across different locations; (ii) to study the relationship among non-parametric stability measures.

II. MATERIALS AND METHODS

A. Experimental Design and plant materials
Data for this study was obtained from set of spine gourd fruit yield trials conducted for three years (2005-07) at four locations in India. The locations consisted of Bhubaneswar, Faizabad, Ranchi and Ambikapur. For Ambikapur location trial were conducted for only two years (2005-06). The detailed description of these test locations is given in Table 1. In each environment and year (year × location), ten genotypes were tested. These genotypes were developed by various breeders at different research centre of AICRN on Underutilized Crops in India. The names, origin and genotype codes of these genotypes are given in Table 2. At each environment a randomized complete block design was used with three replications. The experimental plot consisted of 4m length. Row to row and plant to plant distances was kept at 200 cm and 200 cm respectively at all environments. Data on fruit yield were taken on plot basis. At harvest fruit yield was determined for each genotype at each test environment.

B. Statistical analysis
Three non-parametric statistical procedures were used to test the significance of genotype environment interaction [1]. The methods [2-3] were used to examine whether any non-cross over interaction was present while the method[1] defined interaction according to the

crossover interaction model. The test statistics for testing GE interaction is approximately χ^2-distribution with (t-1) (s-1) degrees of freedom, where t = number of genotypes, and s = number of environments. These statistical methods have been described in details elsewhere [21-22] and shall not be repeated here.

The statistical procedures adopted for the stability analysis of the genotype were those proposed [4, 5, 16]. Nassar and Huehn 1987 [4] proposed four non-parametric stability measures ($S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$). $S_i^{(1)}$ statistic measures the mean absolute ranks difference of genotype over environments. $S_i^{(2)}$ gives the variance among the ranks over environments while $S_i^{(3)}$ is the sum of square deviation in yield rank of each classification relatively to the mean classification and $S_i^{(6)}$ is sum of absolute deviation in yield ranks of each classification relatively to mean classification. The stability measures based on yield ranks of genotypes in each environment are expressed as following formulae:

$$S_i^{(1)} = 2\sum_{j}^{s-1} \frac{\sum_{j'=j+1}^{s}\left|r_{ij} - r_{ij'}\right|}{[s(s-1)]}$$

$$S_i^{(2)} = \frac{\sum_{j=1}^{s}\left(r_{ij} - \bar{r}_{i.}\right)^2}{(s-1)}$$

$$S_i^{(3)} = \frac{\sum_{j=1}^{s}\left(r_{ij} - \bar{r}_{i.}\right)^2}{\bar{r}_{i.}}$$

$$S_i^{(6)} = \frac{\sum_{j=1}^{s}\left|r_{ij} - \bar{r}_{i.}\right|}{\bar{r}_{i.}}$$

For a two-way data with t genotypes and s environments, r_{ij} is the rank of the ith genotype in the jth environment, and $\bar{r}_{i.}$ is the mean rank across all environments for the ith genotype.

The rank of genotype in a particular environment can not be based psurely on the phenotypic values, because the stability has to be measured independently genotypic effect. Thus, the rank of the ith genotype in jth environment is determined on the basis of the corrected phenotypic value namely, $\left(Y_{ij}^* = Y_{ij} - \bar{Y}_{i.}\right)$, where $\bar{Y}_{i.}$ is the mean performance of the ith genotype. For ranking purpose, the smallest value of Y_{ij}^* in a particular environment is given rank 1, the next higher value rank 2, and so on. The using the rank values and means, Thennarasu[5] proposed the following stability measures:

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

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

$$NP_i(3) = \frac{\sqrt{\sum\left(r_{ij}^* - \bar{r}_{i.}^*\right)/s}}{\bar{r}_{i.}^*}$$

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

In the formulae, r_{ij}^* is rank of the ith genotype in jth environment based on $\left(Y_{ij}^* = Y_{ij} - \bar{Y}_{i.}\right)$. M_{di}^* and $\bar{r}_{i.}^*$ are the median and mean ranks respectively of the ith genotype in jth environment from the corrected Y_{ij}.

The stability parameters were compared using Spearman's rank correlation. Spearman's rank correlation, calculating the ranks measure the relationship between the parameters. To better understand the relationship between the non-parameters, the hierarchical cluster analysis with an incremental sum of square grouping strategy known as Ward's method [23]. The based on non-weighted values of 10 spine gourds genotypes was perform. The squared Euclidean distance was use as dissimilarity measure required in Ward's method.

III. RESULT

A. The test for significance of genotype x environment interaction

The result of the test for significance of GE interaction for different non-parametric statistical procedures are presented in Table 3. For genotype x environment interaction effect, Cochran, Bredenkamp and Kroon/Van der Laan methods revealed the same level of significance (P<0.01). Cochran and Bredenkamp method depend on concept of non-cross over interaction and Kroon/Van der Laan method depends on cross over interaction concept.

B. Stability analysis

The result of stability analysis for 8 non-parametric statistical parameters are presented in Table 4. Accordingly, the $S_i^{(1)}$ and $S_i^{(2)}$ of the tested genotypes revealed that genotype RMF 37, SKNK 501, RMF 07P1, RMF 17 and Phule MD 051 had the lowest value. The genotype stability evaluated by $S_i^{(1)}$ the values conceded with the classification of the genotype stability given by $S_i^{(2)}$. The unstable genotype based on the statistics $S_i^{(1)}$ and $S_i^{(2)}$ were RMF 27, NDM-1, Phule MD 052, because these genotypes had highest values of $S_i^{(1)}$ and $S_i^{(2)}$. Based on $S_i^{(3)}$ genotype SKNK 501, RMF 07P1 were stable but had the lowest fruit yield while genotype RMF-37 had highest fruit yield but third rank in stability, whereas the genotype NDM-1, RMF 27, Phule MD 051 were the least stable ones. Using the parameter $S_i^{(6)}$ RMF 07P1, SKNK 501, RMF 17 were identified as most stable genotypes, other genotypes such as a NDM-1, RMF 05P4 and Phule MD 051 had relatively high values of $S_i^{(6)}$, indicating lower stability. $S_i^{(2)}$ had positive relationship with fruit yield, the results showed that using $S_i^{(3)}$ and $S_i^{(6)}$, it is possible to select stable genotype- low values of the statistics. However, such stable genotypes selected on the basis of those stability parameter turn out to have low fruit yield. This

makes the parameters were not so useful for identification of high yielding stable genotype.

According to stability parameters[5] [$NP_i(1)$, $NP_i(2)$, $NP_i(3)$, $NP_i(4)$], genotypes with minimum low values are considered more stable. According the first measure $NP_i(1)$, the genotype RMF 01, RMF 17 and Phule MD 052 having relatively lower values of the parameters, were considered stable in comparison to other genotypes. However, the genotype RMF 37, NDM-1 and SKNK 501 having highest value of the $NP_i(1)$. Based on values of $NP_i(2)$ RMF 17 had the lowest value and was therefore considered high stable followed by RMF 01 and Phule MD 052 in that order. The stability of RMF 37 and NDM-1 due to high value of $NP_i(2)$ were low. According to $NP_i(3)$ value, genotypes RMF 17 and RMF 01 were considered high stable because of low values of $NP_i(3)$. The genotypes RMF 37, Phule MD 051 and NDM-1 with maximum values were unstable. According to $NP_i(4)$ statistics Phule MD 052 had the minimum value and therefore was the most stable genotype, followed by RMF 17 and RMF 07P1. According to this parameter, the genotype RMF 37 and NDM-1 had the highest value of $NP_i(4)$ and therefore were the unstable genotypes.

The result showed that $S_i^{(2)}$, and $NP_i(1)$ were similar because all had positive relationship with fruit yield.

C. *Relationship between mean fruit yield and stability measures*

The result of rank correlation between mean fruit yield and 8 non-parametric stability measures are presented in Table 5. The correlation between mean fruit yield and all non-parametric measures were negatively correlated except $S_i^{(2)}$ and $NP_i(1)$. The high correlation between mean fruit yield and stability measures expected as the values of these measures were higher for high fruit yielding genotypes. The non-significant correlation and negative significant correlation between yield and stability measures suggest that the stability parameters provide information that can not be gleaned from average yield [24]. The stability parameters $S_i^{(1)}$, $S_i^{(2)}$ and $S_i^{(3)}$ were positively significant correlated with each other (p<0.01) and $NP_i(1)$, $NP_i(2)$, $NP_i(3)$ and $NP_i(4)$ were positively and highly significant correlated with each other. Scapim *et al.* (2000) and Segherloo [25,26] also reported significantly positive correlation between $S_i^{(1)}$ and $S_i^{(2)}$. $S_i^{(1)}$ and $S_i^{(2)}$ were uncorrelated with Thennarasu's stability parameters [$NP_i(1)$, $NP_i(2)$, $NP_i(3)$ and $NP_i(4)$].

IV. DISCUSSION

In our study, eight non-parametric stability measures were employed for classification into four groups (Fig. 1). We found those two non-parametric stability measures[16] cluster together, indicating that the two measures were similar in their power of classifying genotypes according to stability under different environmental conditions. It is possible to use only one of them to select stable genotypes in breeding programmes. In group-II include $S_i^{(3)}$ and $S_i^{(6)}$ for stability measures. The mean was ungrouped from these parameters. In group-III consist of $NP_i(2)$, $NP_i(3)$ and $NP_i(4)$ and mean and $NP_i(1)$ were clustered in group-IV.

The non-parametric approaches use in our study did not however seem to provide an overall picture of the individual genotype responses to environments. Some of genotypes exhibited stability using some parameters and instability for others [26]. In our study for example, the genotype RMF-37 was assessed as a stable using $S_i^{(1)}$ and $S_i^{(2)}$ but unstable with non-parametric stability measures $NP_i(1)$, $NP_i(2)$, $NP_i(3)$ and $NP_i(4)$. Thus, making it difficult to reconcile these assessment into a unified conclusion. This is a problem that has been identify in genotype environment interaction study (Lin *et al.*, 1986) [27]. This problem is brought about by the use of parametric approaches for the analysis, which transform a genotype response to environments from its multivariate state to univariate one. One method of getting over this problem is to assign genotypes into qualitative, homogenous stability subset through cluster analysis [27, 28]. This emphasis in this method is to identify and group genotypes, which shows similar pattern of response across the environments. In our study the hierarchy cluster analysis[23] based on non-weighted values of 10 non-parametric stability measures and mean fruit yield, was used to classification the genotypes into four major groups (Fig. 2). Using the squared eucliden as a dissimilarity measures.

The group-I include the very high yielding genotype RMF-37, this genotype was identify as a stable by $S_i^{(1)}$ and $S_i^{(2)}$ parameters. The group-II most of the genotypes which had high yield clustered in group-II included genotypes Phule MD-051, RMF-27, RMF-05 P4.

Table 1: Agro-climatic characteristics of testing environments.

Environments		Mean fruit yield (kg ha^{-1})	Latitude longitude	Altitude (m)	Soil condition (Texture)
Location	Year				
Bhubaneswar	2005	337.70	21°15' N 85°15' E	230 m	Clay
	2006	837.52			
	2007	1025.03			
Faizabad	2005	1890.40	26°47' N 82°12' E	113 m	Clay
	2006	1081.77			
	2007	751.60			
Ranchi	2005	1510.55	23°17' N 85°19' E	625 m	Sandy loam
	2006	617.93			
	2007	563.40			
Ambikapur	2005	2055.30	23°07' N 83°12' E	603 m	Sandy loam
	2006	1827.83			

Table 2: Genotype code, name and origin of 10 spine gourd genotypes.

Genotype code	Name	Origin	Mean (kg ha^{-1})
GK1	NDM-1	NDUAT, Faizabad, U.P.	1008.26
GK2	Phule MD 051	MPKV, Rahuri, Maharashtra	1204.14
GK3	Phule MD 052	MPKV, Rahuri, Maharashtra	1033.35
GK4	RMF 01	IGKV, Ambikapur, Chhatisgarh	1037.19
GK5	RMF 17	IGKV, Ambikapur, Chhatisgarh	1197.71
GK6	RMF 27	IGKV, Ambikapur, Chhatisgarh	1324.92
GK7	RMF 05P4	IGKV, Ambikapur, Chhatisgarh	1289.97
GK8	RMF 07P1	IGKV, Ambikapur, Chhatisgarh	982.51
GK9	SKNK 501	SDAU, S.K. Nagar, Gujarat	843.31
GK10	RMF 37 (Indira Kankoda)	IGKV, Ambikapur, Chhattisgarh	1441.41

Table 3: The test of significant genotype x environment interaction for fruit yields of 10 genotype tested in 11 environments.

Statistics	df	χ^2
Cochran	51	693.76*
Bredenkamp	90	1026.50*
Kroon/Van der Laan	90	1164.03*

* significant at the 0.01 probability level

Table 4: Mean fruit yields and stability parameter for 10 genotypes of spine gourd tested in 11 environments.

Genotype name	Mean (kg ha^{-1})	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i(1)$	$NP_i(2)$	$NP_i(3)$	$NP_i(4)$
NDM-1	1008.26	3.53	8.87	19.92	6.16	3.00	0.71	0.77	0.81
Phule MD 051	1204.14	2.91	6.09	15.58	5.44	2.55	0.64	0.79	0.61
Phule MD 052	1033.35	3.20	7.29	11.97	4.09	1.73	0.26	0.38	0.31
RMF 01	1037.19	3.02	6.69	13.63	4.74	1.36	0.25	0.37	0.37
RMF 17	1197.71	2.76	6.07	9.41	3.35	1.55	0.22	0.28	0.33
RMF 27	1324.92	3.93	11.60	19.33	5.33	2.27	0.41	0.51	0.58
RMF 05P4	1289.97	3.02	6.67	14.98	5.51	1.73	0.37	0.47	0.46
RMF 07P1	982.51	2.73	6.56	8.40	2.67	2.36	0.28	0.37	0.36
SKNK 501	843.31	2.84	5.87	7.88	3.02	2.91	0.38	0.43	0.50
RMF 37 (Indira Kankoda)	1441.41	1.96	3.07	8.89	4.16	3.09	1.03	0.99	1.15

Table 5: Spearman's coefficient of rank correlation for the mean fruit yield and 8 non-parametric stability measures of 10 spine gourd genotype tested in 11 environments of India.

Parameters	Mean	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i(1)$	$NP_i(2)$	$NP_i(3)$	$NP_i(4)$
Mean	1.00								
$S_i^{(1)}$	-0.04	1.00							
$S_i^{(2)}$	0.03	0.90**	1.00						
$S_i^{(3)}$	-0.36	0.79**	0.73**	1.00					
$S_i^{(6)}$	-0.44	0.64**	0.49	0.90**	1.00				
$NP_i(1)$	0.01	-0.22	-0.32	-0.05	0.13	1.00			
$NP_i(2)$	-0.31	0.05	-0.08	0.30	0.47	0.90**	1.00		
$NP_i(3)$	-0.47	0.12	-0.05	0.38	0.56	0.79**	0.96**	1.00	
$NP_i(4)$	-0.38	0.02	-0.13	0.35	0.54	0.81**	0.94**	0.89**	1.00

* significant at the 0.01 probability level; ** significant at the 0.05 probability level
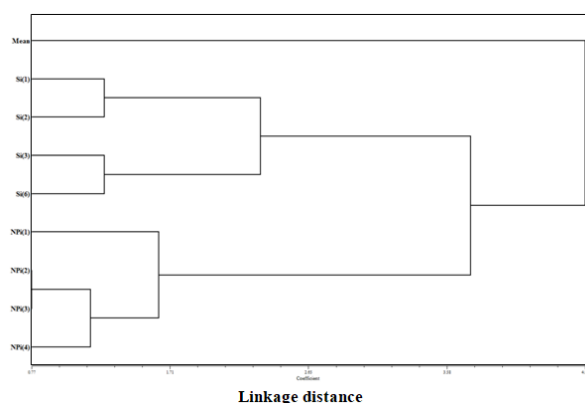

Fig. 1. Dendogram showing hierarchical classification of eight non-parametric stability measures and mean fruit yield on non-weighted values of 10 genotypes.
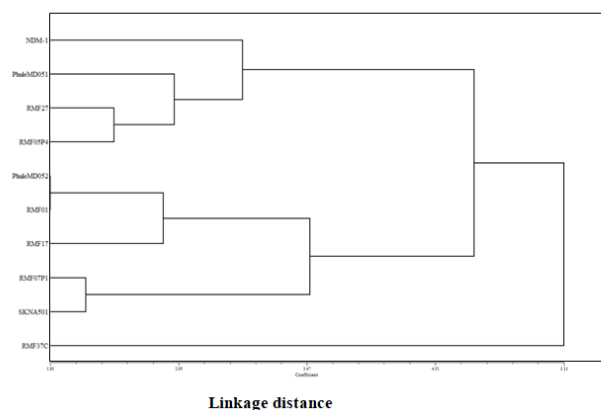
Linkage distance

Fig. 2. Dendogram showing hierarchical classification of ten spine-gourd genotypes based on non-weighted values of eight non-parametric stability measures and mean fruit yield.

These genotypes were identified as a moderate stable genotypes [5, 16] stability parameters. The moderate yielding genotypes Phule MD-052, RMF-01, RMF-17 were clustered in group-III. These genotypes were identify stable genotypes [5] stability parameters. The low yielding genotypes RMF-07 P1 and SKNK-501 were clustered in the group-IV which had high stability.

CONCLUSION

RMF-17 was identified most stable genotype. These non-parametric measures were observed to be associated with high mean fruit yield. The simple correlation coefficient using Spearman's rank correlation, calculated using the ranks was used to measure the relationship between the stability parameters.

FUTURE SCOPE

This method of analysis therefore proved useful for the identification of the high yielding genotypes for breeding programmes as well as for commercial exploitation in conclusion, the several of stability statistics that have been employed in this study quantified stability of varieties with respect to yield, stability and both of them. Both fruit yield and stability of performance should be considered simultaneously to exploit the useful effect of genotype environmental interaction and to make a selection of the genotypes more precise and refined manure. The non-parametric measures for GxE interaction were highly significant (p<0.05), suggesting different response of genotypes to the test location/year. Based on low value of non-parametric measures, RMF-17 was identified most stable genotype.

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