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NON-PARAMETRIC METHODS FOR ANALYZING STABILITY OF CHICKPEA (*Cicer arietinum* L.) GENOTYPES

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ABSTRACT

A study was conducted to identify chickpea genotypes with stable performance in multi-locations through non-parametric stability parameters. Twenty chickpea genotypes along with three checks were evaluated for pod yield in three locations. Non-parametric stability statistics discriminated the chickpea genotypes based on their pod yield and stability into four sections visually on graphs. The Kang's rank sum (rs) method identified the genotypes ICC-19830, GNG-1958, PBC-1103, IPC-02-248, JG-11, RKG-155, GCP-105 as high yielding and stable. The genotypes GNG-1958 and PBC-1103 being more stable but the genotype PBC-1103 most preferred one with high Fox TOP statistic value. According to Huehn's *S1*, *S2*, *S3* and *S6* statistics, the genotypes PBC-1103 and IPC-02-248 were the highly stable and high yielding genotypes. The genotypes IPC-02-248, PG-06102 and JG-11 were high yielding and stable as they lie in section 1 of the graph. Majority of parameters identified the genotypes IPC-02-248 and RKG-155 as high yielding and stable. Spearman's rank correlation among non-parametric statistics showed high degree of positive association of mean yield with Thennarasu's *N2*, *N3* and *N4* and Ketata's sdy statistics. Genotypes with specific adaptation should be selected based on their ranks in individual environments.

INTRODUCTION

Chickpea is the third largest produced food legume globally after common bean and field pea (Kumar *et al*., 2016) in terms of area under cultivation (14.80 m ha), ranks third in production and is currently cultivated more than 50 countries. India is the largest producer of chickpea, grown in an area of 9.51 million hectares with the annual production of 8.83 million tonnes and with productivity of 929 kg/ ha (Johnson *et al*., 2015). In order to boost the productivity, chickpea genotypes that perform stably across environments need to be identified. Stable yield of a genotype implies that its rank relative to other genotypes remains same in a given environment *i.e.*, maximum stability is displayed with equal ranks in diverse environments. A genotype is considered stable if it has a high mean yield but a low magnitude fluctuation in yielding ability when grown in different environment (Tuba and Dogan, 2006). This concept is known as biological or static concept of stability. But many researches prefer the genotypes that can capitalize on existing favorable conditions of the environment to express their full potential. The high yielding performance of released varieties is one of the most important targets of breeders, which explains why they prefer a dynamic concept of stability (Becker and Leon, 1988).

Genotype × environment interaction (G × E) is a major problem in the comparison of genotype performance across environments (Kang, 1988). The non-parametric stability models, which do not have presumptions like normal distribution, homogeneity of variances and additivity and linearity of genotypic and environmental effects, are not so often used in plant breeding (Huehn and Leon, 1995), although these methods are easy to use and interpret (Huehn, 1990a). The most commonly used models to interpret genotype environment interaction are Additive main effects and multiplicative interaction (AMMI) and genotype main effects and genotype × environment interaction effects (GGE). These two parametric models, although robust may not behave well if the underlying assumptions are violated by factors such as outliers. The non-parametric stability models, which do not have presumptions like normal distribution, homogeneity of variances and additivity and linearity of genotypic and environmental effects, are not so often used in plant breeding (Huehn and Leon, 1995), although these methods are easy to use and interpret (Huehn, 1990a).

Non-parametric methods proposed by Huehn (1979), Nassar and Huehn (1987), Kang (1988), Fox *et al*. (1990) and Thennarasu (1995) are based on ranks of genotypes in each environment and genotypes with similar rankings across environments are classified as stable. Huehn (1979), Nassar and Huehn (1987) proposed four different non-parametric stability measures *S1* (mean of the absolute rank differences of a genotypes over *n* environments),*S2* (the variance among the ranks over the *n* environments) and *S3* (the sum of square deviations in yield units of each classification relative to the mean classification) and S6 (the sum of squares of ranks for each genotype relative to the means of ranks). Each of them can be used as stability parameter. Test of significance based on normal distribution was developed by Nassar and Huehn (1987) and Truberg and Huehn (2000) for S1 and S2. Thennarasu (1995) proposed the following non-parametric statistics as a measure of stability: N1, N2, N3 and N4 which are based on orders or ranks of adjusted mean of the genotypes in each environment (Akcura et al., 2008). The objective of the present investigation is to test the presence of interaction for pod yield in three environments, to determine phenotypic stability of chickpea genotypes and to evaluate the level of association among non-parametric stability parameters.

MATERIALS AND METHODS

This experiment included 23 chickpea genotypes including three checks JG-11, JAKI-9218 and KAK-2 selected based on their seed yield per plant during July - October 2014 and October-March 2014-2015 germplasm evaluation. During October-March 2015-2016, all the 23 genotypes were sown in randomized complete block design (RCBD) with two replications at two locations in Karnataka (GKVK-Bangalore and farmer's field at Viduraswatha village, Gauribidanur) and one location in Andhra Pradesh (Regional Agricultural Research Station-Nandyal). Each genotype was sown in four rows of 4 meter length with a spacing of 30 × 10 cm. All the recommenced management practices were followed to raise a healthy crop. Seed yield per plot was recorded in both replications of three environments.

To remove the genotypic effect from phenotypic value, S1 and S2 statistics are two rank stability measures (Huehn, 1990b), the S1 statistic measuring the mean absolute rank difference of a genotype over environments, with S1 = 0 for a genotype with maximum stability, while S2 gives the variance between the ranks over environments, with zero variance being an indication of maximum stability (Akcura et al., 2008). The statistics S3 and S6 are the sum of the absolute deviations and sum of squares of ranks, respectively. Among 23 chickpea genotypes, genotype with highest adjusted yield was assigned a rank 23 and lowest adjusted yield was given a rank of 1. Huehn's statistics based on yield ranks of genotypes in each environment were computed as follows.

$$S1 = 2\sum_{j}^{n-1}\sum_{j=j+1}^{n}\left|r_{ij} - r_{ijr}\right| / [n(n-1)]$$

$$S2 = \sum_{j=1}^{n}(r_{ij} - \bar{r}_i)^2 / \sum_{j=1}^{n}\left|r_{ij} - \bar{r}_i\right|$$

$$S3 = \sum_{j=1}^{n}(r_{ij} - \bar{r}_i)^2 / \bar{r}_i$$

$$S6 = \sum_{j=1}^{n}\left|r_{ij} - \bar{r}_i\right| / \bar{r}_i$$

For a two-way dataset with k genotypes and n environments, it was de-noted the phenotypic value of i^{th} genotype in j^{th} environment as x_{ij}, where, $i = 1, 2, 3,...k$, $j = 1, 2, 3,...n$, r_{ij} as the rank of the i^{th} genotype in the j^{th} environment, and $\bar{r}_{ij}$ as the mean rank of i^{th} geno-type across all environments.

Other two non-parametric stability measures were computed as per Ketata et al., 1989, in the first method, the rank (rm) is plotted against standard deviation of ranks (rsd), whereas in the latter, mean yield is plotted against standard deviation of yield (sdy). Genotypes with minimum rm and rsd are considered as most stable. Kang's (1988) rank-sum is another method of non-parametric stability analysis in which both the genotype mean rank and Shukla's (1972) stability variance are used as selection criteria. This statistics assigned a weight of one to both mean yield and stability and it helps to identify the high yielding stable chickpea genotypes. Based on the corrected ranks of genotypes in each environment, Thennarasu (1995) proposed four non-parametric stability measures, which were also computed in the present experiment. The ranks of genotypes in each location were adjusted as ($x^*_{ij} = x_{ij} - \bar{x}_i$).

$$N1 = \frac{1}{m}\sum_{j=1}^{m}\left|r^*_{ij} - M^*_{ij}\right|$$

$$N2 = \frac{1}{m} / \left(\frac{1}{m}\sum_{j=1}^{m}\left|r^*_{ij} - M^*_{ij}\right| / M_{di}\right)$$

$$N3 = \frac{\sqrt{\sum(r^*_{ij} - \bar{r}_i^*)^2 / m}}{\bar{r}_i} M^*_{di}$$

$$N4 = \frac{2}{m(m-1)}\left(\sum_{j=1}^{m-1}\sum_{j=j+1}^{m}\left|r^*_{ij} - r^*ij\right|\right)$$

In the above formulae, r^*_{ij} is the rank of $X^*ij = Xij - Xi\ r_{i.}^*$ and M_{di}^* are the mean and median ranks for adjusted values, where and are the same parameters computed from the original (unadjusted) data.

Spearman's rank correlation was calculated to statistically compare the stability indices used in this experiment. All non-parametric stability analysis was calculated using RStudio Team (2015).

RESULTS AND DISCUSSION

Genotype interaction with environmental factors is an important consideration for plant breeding. The effects that genotypes and environments have on G × E interactions are statistically non-additive, indicating that differences in yields depends mainly on the environment (Yue et al., 1997). The ANOVA for genotypes, locations and their interactions showed significance indicating that genotypes differ significantly for

Table 1: Mean yields of 23 chickpea genotypes and estimates of non-parametric statistics

Sl. No.	Genotypes	Mean seed yield per plot (g)	rs	*S1*	*S2*	*S3*	*S6*	sdy	rm	rsd	*N1*	*N2*	*N3*	*N4*
1	ICCV-07305	1070.45	28	2	25.33	1.6	1.6	492.48	22.33	0.94	3.33	0.14	0.18	0.09
2	ICCV-10110	1540.92	40	6.67	100.33	11.57	1.91	1084.62	16.33	5.44	6.67	0.37	0.5	0.41
3	ICC-19830	1975.67	17	2	105.33	5.64	1	679.9	9.33	5.25	6.67	0.56	0.9	0.21
4	ICC-19336	1166.67	24	4	76.33	3.5	1.5	366.17	20	2.16	5.67	0.27	0.36	0.2
5	RVSSG-10	1647.37	22	7	117	19.93	2.34	556.52	14.33	8.01	7	0.35	0.62	0.49
6	KBG-36	1749.83	31	5.33	64.33	8	1.33	947.52	12	5.66	5.33	0.67	0.55	0.44
7	GNG-1958	2289.62	14	2.33	64.33	0.9	0.3	703.16	4	2.45	5.33	1.33	1.64	0.58
8	PBC-1103	2227.65	17	2	105.33	0.67	0.29	834.08	3	2.16	6.67	3.33	2.79	0.67
9	DCP-92-3	1704	22	0.01	3	1	0.42	583.4	11.33	2.05	1	0.09	0.12	0.01
10	KAK-2	1514.33	31	1	9	6.09	1.45	693.28	16.67	3.86	2	0.13	0.15	0.06
11	BG-2094	1588.5	20	4.67	49	3.94	0.9	480.7	13.67	3.68	4.67	0.33	0.42	0.34
12	GNG-1969	1723.67	15	1	93	6.62	1.08	434.13	11	5.35	6	0.5	0.72	0.09
13	JG-62	1670.33	35	4.33	79	7.75	1.5	962.23	16	4.55	5.67	0.33	0.45	0.27
14	IPC-02-248	1884.83	23	1	16.33	0.93	0.4	749.26	9	2.16	2.67	0.27	0.37	0.11
15	L-550	1414.54	26	1.33	16	1.68	0.84	497.16	17.67	1.89	2.67	0.14	0.18	0.08
16	JG-11	2046.01	19	2.33	31	0.1	0.1	861.57	4	0.82	3.67	0.92	1.14	0.58
17	GNG-1499	1205.77	22	6.33	114.33	3.5	1.5	263.97	20	2.16	6.33	0.33	0.44	0.32
18	JAKI-9218	1962.93	30	5.67	116.33	4.2	0.82	1137.45	7.67	4.78	6.67	0.67	1.15	0.74
19	RKG-155	2034.5	14	5.33	80.33	3.64	0.68	597.11	7.33	4.5	5.33	0.76	1	0.73
20	PG-06102	1922.5	27	3.33	52	0.81	0.34	920.59	8.33	2.05	4.67	0.58	0.71	0.4
21	BG-212	1562.83	25	1.33	7	0.5	0.36	566.52	14.67	1.25	1.67	0.11	0.15	0.09
22	Phule G 0215-2	1883.5	27	0.67	76	4.68	0.91	881.55	9.33	4.78	5.33	0.76	0.76	0.07
23	GCP-105	2014	23	5.67	96.33	6.13	1	897.15	8	5.72	5.67	1.13	1	0.71

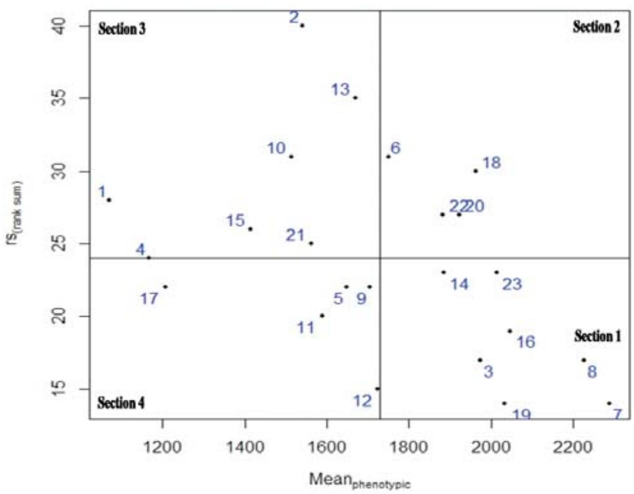

Figure 1 : Plot of Kang's rank sum (rs) *vs*. mean yield for 23 chickpea genotypes over three locations

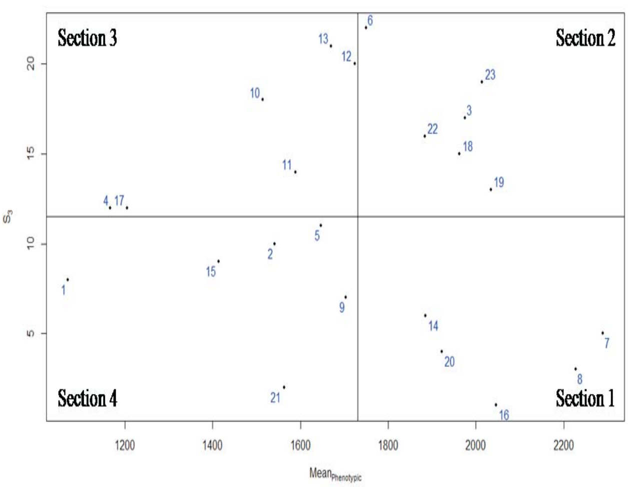

Figure 2 : Plot of Huehn's *S3* statistics *vs*. mean yield of 23 chickpea genotypes over three locations

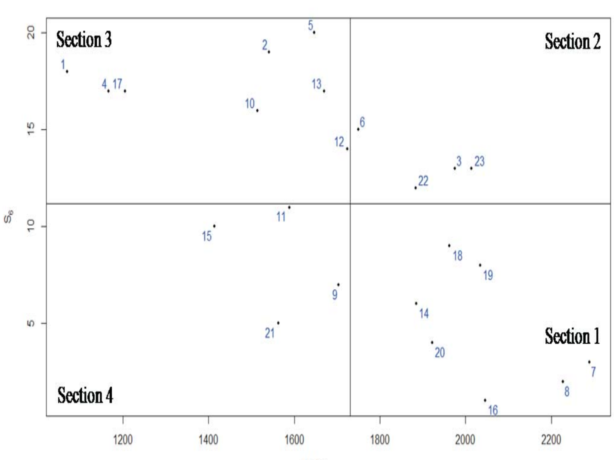

Figure 3: Plot of Huehn's *S6* statistics vs. mean yield of 23 chickpea genotypes over three locations

their stability, locations differ thereby discriminating genotype performance and differential response of genotypes to different environments (data not shown). This provided preliminary evidence for estimating different non-parametric stability parameters and identifying genotypes that are stable. Various non-parametric measures were estimated and presented in the Table 1.

The Kang's rank sum (rs) method that is based on both mean yield and Shukla's stability variance, groups the genotypes into four sections pictorially on a graph. The genotypes that were positioned in section 1 are considered as high yielding and stable. Accordingly the genotypes 3 (ICC-19830), 7 (GNG-1958), 8 (PBC-1103), 14 (IPC-02-248), 16 (JG-11), 19 (RKG-155), 23 (GCP-105) were identified as high yielding and stable (Fig. 1). These are recommended for general adaptation. Among these genotypes, genotypes 19 (RKG-155) and 7 (GNG-1958) had same rank sums but 7 (GNG-1958) has higher mean yield than 19 (RKG-155). Genotypes 6 (KBG-36), 18 (JAKI-9218), 20 (PG-06102), 22 (Phule G 0215-2) also have high mean yields but they are unstable and hence can be specific adapters to high yielding environments. This procedure was also employed for screening stability criteria and quantitative indicators for drought tolerance in wheat (Mohammadi *et al*., 2007 and Farshadfar *et al*., 2012) and in chickpeas (Zali *et al*., 2011 and Mahtabi *et al*., 2013). Because of integrating yield and stability, rs is probably one of the more important criteria for selecting varieties, as compared with other methods (Sabaghnia *et al*., 2014).

According to Fox (1990) TOP statistic, the genotype that ranks among top three in highest proportion of environments is taken as most stable one. So, the genotype having high mean yield and high TOP value is more stable. The genotypes 7 (GNG-1958) and 8 (PBC-1103) are having high mean yield and high TOP value, with genotype 8 (PBC-1103) being the most preferred one with high TOP value. The genotype 8 (PBC-1103) had relatively high mean value and it was among top three in two locations. Other genotypes 7 (GNG-1958), 23 (GCP-105), 16 (JG-11), 19 (RKG-155) and 18 (JAKI-9218) are also high yielding but they were among TOP three in only one location. As genotypes were evaluated in only three locations, other genotypes were on par in terms of TOP value and hence, lay horizontally on the graph. Evaluating in more environments would have better discriminated these genotypes.

The *S1* and *S2* (Nassar and Huehn, 1987) statistics are two rank stability measures, the *S1* statistic measuring the mean absolute rank difference of a genotype over environments, with *S1* = 0 for a genotype with maximum stability, while *S2* gives the variance between the ranks over environments,with zero variance being an indication of maximum stability (Akcura *et al*., 2008). The exact variance and expectation of *S1* and *S2* were given by Huehn (1990a). According to Huehn's *S1* statistic, the genotype 22 (Phule G 0215-2) and 14 (IPC-02-248) were the highly stable and high yielding genotypes as they fall in section 1 of the graph. The genotypes 8 (PBC-1103) and 7 (GNG-1958) were high yielding but they were not as stable as the above as the value of *S1* statistic is higher. The genotypes 18 (JAKI-9218), 23 (GCP-105) and 19 (RKG-155) that fell in section 2 also have high means and can be

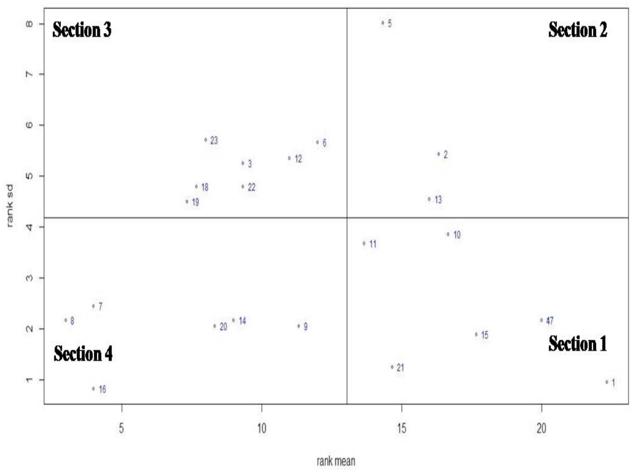

Figure 4: Plot of rank mean *vs.* rank SD for 23 chickpea genotypes over three locations

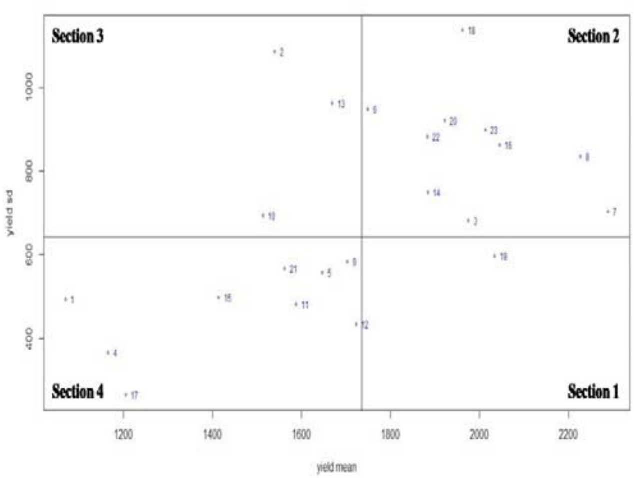

Figure 5: Plot of mean yield *vs.* yield SD for 23 chickpea genotypes over three locations

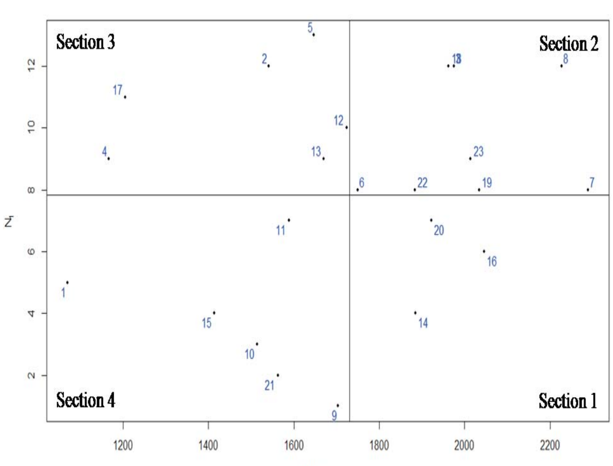

Figure 6: Plot of Thennarasu's *N1* statistics vs. mean yield of 23 chickpea genotypes over three locations

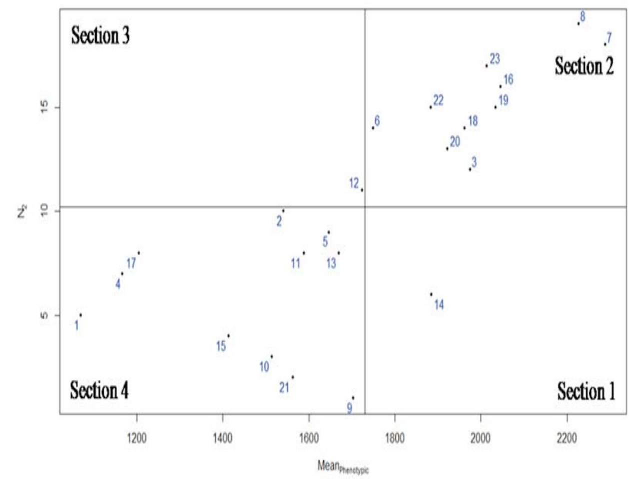

Figure 7: Plot of Thennarasu's *N2* statistics vs. mean yield of 23 chickpea genotypes over three locations

specifically recommended to high yielding environments. The *S2* statistic also yielded similar results but projected 8 (PBC-1103) and 3 (ICC-19830) as high yielding and stable genotypes on the graph. The *S1* statistic is preferred for practical applications because it is very easy to calculate and allows a clear and objective interpretation. It represents the mean absolute rank difference between the environments. Furthermore, an efficient test of significance is available for this statistic (Farshadfar *et al.*, 2012).

Two other Huehn's statistics, *S3* and *S6* combine yield and stability based on the yield ranks of genotypes in each environment. These statistics measures the stability in units of the mean rank of each genotype, with the lowest value for each of these statistics indicating maximum stability for a certain genotype (Rea et *al.*, 2015). Both these statistics indicated genotypes 14 (IPC-02-248), 20 (PG-06102), 16 (JG-11), 8(PBC-1103) and 7 (GNG-1958) as high yielding and stable with 16 (JG-11) as best recommended for general adaptation (Fig. 2 & Fig. 2 3).

When rank mean plotted against rank standard deviation (rsd), the genotypes that fell in section 4 of the graph are considered high yielding and stable, while those in section 1 are low yielders. Thus genotypes 8 (PBC-1103), 7 (GNG-1958), 16 (JG-11), 20 (PG-06102), 14 (IPC-02-248) are high yielding and stable (Fig. 4). These results were consistent with Huehn's *S3* and *S6* stability measures. The results of mean yield and yield standard deviation (sdy) are presented in Figure 5, where the genotype 19 (RKG-155) being in section 1 was high yielding and stable. However, the mean yield of genotypes 14 (IPC-02-248) and 7 (GNG-1958) were greater than grand mean of all genotypes, and their sdy values are close to mean, indicating that these genotypes can also be considered stable. Both these measures projected genotypes 2 (ICCV-10110) and 13 (JG-62) as low yielding and unstable, and was relatively better adapted to poor environments and insensitive to environmental changes.

The ranks of adjusted yield means were used to calculate Thennarasu's non-parametric stability statistics. The genotypes 14 (IPC-02-248), 20 (PG-06102) and 16 (JG-11) were high

Table 2: Spearman's correlation among yield mean and other non-parametric parameters

	Mean	rs	*S1*	*S2*	*S3*	*S6*	sdy	rm	rsd	*N1*	*N2*	*N3*
rs	-0.50*	1										
S1	0	0.11	1									
S2	0.24	-0.16	0.66**	1								
S3	-0.22	0.34	0.41*	0.49*	1							
S6	-0.68**	0.44*	0.39	0.35	0.78**	1						
sdy	0.51*	0.46*	0.19	0.15	0.17	-0.2	1					
rm	-0.98**	0.51*	0.04	-0.19	0.30*	0.75**	-0.47**	1				
rsd	0.18	0.09	0.44*	0.65**	0.89**	0.49*	0.32*	-0.11	1			
N1	0.2	-0.12	0.62**	0.98**	0.52*	0.39	0.17	-0.14	0.66**	1		
N2	0.81**	-0.35	0.33	0.55**	0.03	-0.33	0.48*	-0.77**	0.37	0.52*	1	
N3	0.83**	-0.42*	0.32	0.63**	0.01	-0.36	0.45*	-0.81**	0.36	0.60**	0.96**	1
N4	0.62**	-0.26	0.74**	0.61**	0.05	-0.21	0.42*	-0.60**	0.33	0.56**	0.76**	0.78**

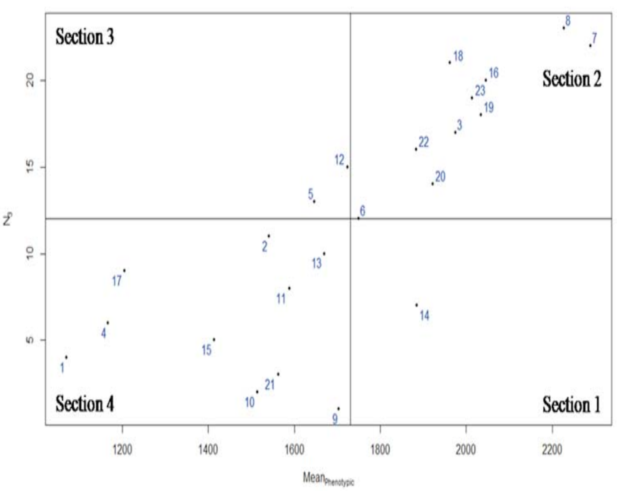

Figure 8. Plot of Thennarasu's *N3* statistics vs. mean yield of 23 chickpea genotypes over three locations

yielding and stable as they lie in section 1 of the graph (Fig. 6) plotted between mean yield and *N1* statistic, with 14 (IPC-02-248) regarded as the best. Section 2 consists of genotypes that are high yielding but are unstable. These genotypes could be recommended in specific type of environments based on their relative ranks in corresponding locations. Thennarus's *N2* and *N3* statistics provided identical results indicating the genotype 14 (IPC-02-248) as the best (Fig. 7 & 8), whereas according to *N4* statistics, genotypes 4 (ICC-19336), 22 (Phule G 0215-2) and 3 (ICC-19830) are considered stable and high yielders. Although genotypes 14 (IPC-02-248) and 22 (Phule G 0215-2) displayed same mean yield value but Phule G 0215-2 had less *N4* statistic value stating it more stable. As a whole, Thennarasu's NPI statistics revealed IPC-02-248 as cultivar that has general genotypic adaptation.

Spearman's rank correlation coefficient was also calculated between mean yield and various stability parameters (Table 2). These coefficients were used to statistically compare stability indices within themselves and also with respect to mean yield. Mean yield was significantly positively correlated with Thennarasu's statistics *N2, N3* and *N4* but had significant negative correlation with Kang's rank sum (rs), *S6* and rm statistics. These results were contradictory to those reported by Akcura (2008) and Kang and Pham (1991) where significant positive correlation was found between mean yield and *S6* in wheat genotypes. Selection for increased pod yield in chickpea would, therefore, be expected to change pod yield stability by increasing the stability statistics *N2, N3* and *N4*. This would lead to the development of genotypes specifically adapted to environments with optimal growing conditions (Akcura, 2008). These genotypes may not perform well under below average or poor environments. On the flip side, significant negative correlation for rs and *S6* with mean yield suggests that selection for higher yield will change stability by decreasing the values of *rs* and *S6*. The non-parametric statistics *S1, S2* and *S3* were significantly correlated among themselves, which was earlier reported by Scapim *et al.* (2000). All the Thennarasu's non-parametric stability statistics have significant and positive correlation among themselves. It suggested that these estimates were similar in classifying the genotypes as per their stability across locations. Further it can also be said that one of these measures would be sufficient to discriminate among the genotypes.

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