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Stability Analysis for Biological Nitrogen Fixation and Seed Yield in Mungbean [*Vigna radiata* (L.) Wilczek] Genotypes

Madhuri Arya[1], S.B. Mishra[1], Kanshouwa Modunshim Maring[2], Ravi Kant[1] 10.18805/LR-5256

ABSTRACT

Background: Mungbean is a versatile legume crop grown in various cropping systems. Despite its adaptability, the yield is hindered by the low yielding capacity of undifferentiated varieties and their limited ability to fix nitrogen. The current study aims to identify stable genotypes through continuous cultivation in eight different environments over two years.

Methods: To assess the stability for BNF and seed yield, twenty-five mungbean genotypes were measured at eight diverse agro-ecological conditions, represented as two different seasons during spring and summer in two consecutive years (2016 and 2017) at two locations - TCA, Dholi, Muzaffarpur and Pusa Farm, RPCAU, Pusa, Samastipur, Bihar. To evaluate the most stable and superior genotypes, three parameters, *viz*., $\bar{x}_i$, b_i and S^2d_i were calculated.

Result: There is a substantial amount of variability across genotypes and environments, as shown by the pooled analysis of variance. The genotype, Pusa 11-31 had shown regression near to unity, comprised with non-significant standard deviation along with higher mean seed yield and biological nitrogen fixation indicates that this is the most stable genotypes for the above traits.

Key words: Environment, Interaction, Mungbean, Stability analysis, Yield.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is a short duration legume crop cultivated in all the three seasons round the year in mono, double and multiple cropping systems. Due to its short lifespan and broad range of adaptation, it is planted as a mixed, inter and relay crop. The cultivation of mungbean is done particularly in regions where dryland farming methods are predominant. However, this crop faces numerous challenges, primarily stemming from its susceptibility to moisture stress due to unpredictable rainfall. These challenges include low intrinsic yield capacity, poor nitrogen-fixing ability from the environment and genetic instability within the crop's narrow genetic base. The phenotypic expression of mungbean genotypes varies significantly across different environments, highlighting the importance of understanding genotype-environment (G × E) interactions and yield stability. To enhance mungbean crop output, it is imperative to develop or identify high-yielding and consistently performing cultivars tailored to different seasons and conditions. Therefore, the current study aims to identify such genotypes through continuous cultivation in eight different environments over two years at two locations. This research seeks to address the challenges faced by mungbean cultivation and ultimately benefit both farmers and the Indian market.

An essential factor for plant growth and development is nitrogen, but its conventional source, inorganic fertilizers, poses environmental risks such as energy depletion and contamination. Globally, symbiotic nitrogen fixation (BNF) by legumes plays a pivotal role in sustainable agriculture, fixing over 70 million metric tonnes of nitrogen annually. Microbial inoculants, including *Rhizobium*, *Azotobacter* and Azuspirillum for nitrogen fixation, *Bacillus* and *Pseudomonas* for phosphorus solubilization and *Pseudomonas* and *Rhizobium* for rhizospheric organism development and disease suppression, have been crucial for enhancing crop yields. Among these, *Rhizobium* stands out as it forms root nodules in most legumes, facilitating atmospheric nitrogen fixation that benefits the plant. Therefore, this study was also conducted to evaluate the nitrogen-fixing capabilities of various mungbean genotypes to identify those with superior performance across different environments.

[1]Department of Genetics and Plant Breeding, Tirhut College of Agriculture, Dholi, Muzaffarpur-843 121, Bihar, India.
[2]Department of Genetics and Plant Breeding, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur-848 125, Bihar, India.

Corresponding Author: Madhuri Arya, Department of Genetics and Plant Breeding, Tirhut College of Agriculture, Dholi, Muzaffarpur-843 121, Bihar, India. Email: madhuriaryatca@gmail.com

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MATERIALS AND METHODS

The field experiments were conducted by using twenty-five mungbean genotypes (Table 1) at TCA, Dholi and Pusa Farm during spring and summer seasons of 2016 and 2017 (Fig 1). In the year 2016, four experiments were conducted at two different dates of sowing in two locations (16.02.2016 and 27.03.16 at TCA, Dholi and 15.02.2016 and 28.03.2016 at

Pusa Farm). In the year 2017, again same experiments were repeated at two different sowing dates (14.02.2017 and 27.03.2017 at TCA, Dholi and 16.02.17 and 25.03.2017 at Pusa Farm). At each date of sowing, a randomized complete block design with three replications was applied. The experimental units are a four-row plot with a length of 4 meters. The distance between plants and rows were fixed at 10 and 30 cm, respectively. In each growing season and locations, all recommended agronomic practices were followed. Observations were recorded on five randomly selected plants from each plot. Number of nodules was counted before flowering which is peak period of nodulation (Murakami *et al*., 1990). This is the perfect stage for counting nodule number and measuring fresh and dry weight of nodules. Nitrogen activity was measured using an acetylene reduction assay (ARA) in a gas chromatograph at the pod-developing stage to determine N_2-fixation. The seed yield per plant was taken in gram after the crop was harvested at physiological maturity. The data obtained for these characters were subjected to statistical analysis.

Statistical methods/tools

In each different environment mean performance of the characters were estimated. Each location was regarded as a distinct environment. Combined analysis of variance was done to determine genotype and environment interaction from replicated multi-location trials. A trial where the yield of (G) genotypes is calculated in an environment (E) with (R) replicates is mathematically modelled as:

$$Y_{ij} = u + G_i + E_j + GE_{ij} + e_{ij}$$

Where,

u = Overall mean.

G_i, E_j and GE_{ij} = Effect of genotypes, environment and the G × E interaction.

Whereas,

e_{ij} = Average of the random errors associated with the r^{th} plot that receives the i^{th} genotype in the j^{th} environment.

The capacity of a genotype to exhibit minimal interaction with the environment is known as stability. Stability analysis is carried out by utilizing the Eberhart and Russell (1966) method. Everhart and Russell defined stable genotypes as those with a regression coefficient close to unity (b_i = 1), a minimal non-significant departure from regression (S^2d_i = 0) and greater mean performance. The computer software MSTAT-C and Spreadsheet 2007 were used for the analysis of variance and stability respectively.

RESULTS AND DISCUSSION

The mean differences between genotypes and environments were highly significant for all five characters, namely nodule number, seed yield (g), fresh weight (g), dry weight (g) and biological nitrogen fixation, according to a pooled analysis of variance of mungbean genotypes across the eight different environments (Table 2). This shows that there is a significant degree of genetic and environmental variation for these traits. All of the characters' mean sums of squares attributable to G × E interactions were significant, indicating that the presence of interaction between genotypes and environments. As a result, it is required to prescribe certain genotypes for particular place (Sarma *et al*. 1993). This shows that the genotypes are not entirely adapted for all the features to the examined environment. None of the characters' variance attributable to G × E (Linear) components was statistically significant except for the dry weight, indicating that most of the characters of the genotypes responded similarly across environments. For nodule number, fresh weight and biological nitrogen fixation, the magnitude of G × E (linear) was less than the non-linear component (pooled deviation), showing the significance of unpredictable components in influencing the G × E and vice versa for the remaining character. All of the traits had significant variation due to pooled deviation, which indicated large deviations from each trait's linear path response to the environment. These findings corroborated with Worku

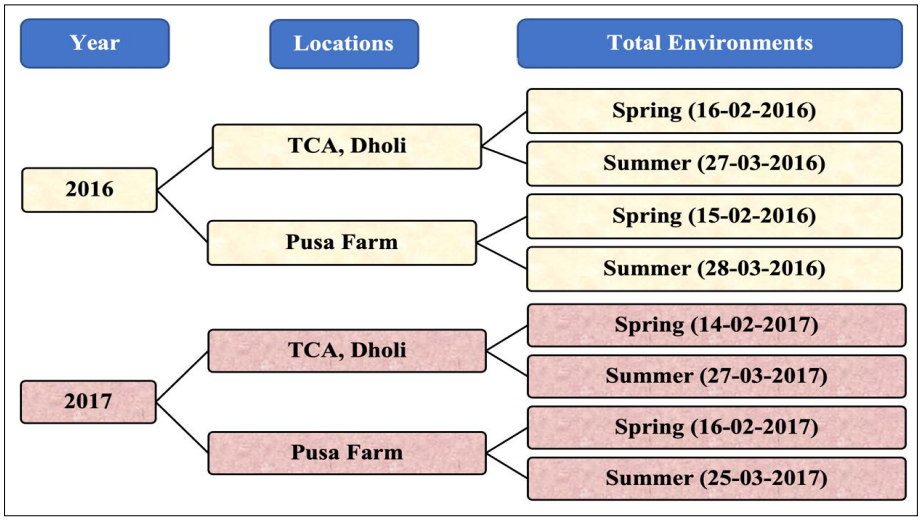


Fig 1: Eight environments across two locations and two years.

Table 1: Mean performance and stability parameters for Nodule Number (NN), seed yield per plant (g), fresh weight (mg), dry weight (mg) and biological nitrogen fixation (BNF) grown in 8 different environments.

Genotypes	NN			Yield (g/pt)			FW (mg)			DW (mg)			BNF		
	$\bar{x}_i$	b_i	S^2d_i	$\bar{x}_i$	b_i	S^2d_i	$\bar{x}_i$	b_i	S^2d_i	$\bar{x}_i$	b_i	S^2d_i	$\bar{x}_i$	b_i	S^2d_i
PDM 178	3.61	0.98	0.07**	6.53	0.93	0.09*	29.81	1.06	0.48**	7.50	0.63	0.42**	3.61	1.00	0.05*
PDM 54	3.84	0.99	-0.01	7.01	1.06	0.07*	27.84	0.90	0.03	6.46	1.34	0.06**	3.84	1.00	-0.01
Pusa 90-72	4.42	1.24	0.42**	6.42	1.01	0.03	31.29	0.91	0.10**	7.77	1.40	0.13**	4.42	1.26	0.41**
PDM 262	3.69	0.94	0.01**	8.23	0.90	0.09*	32.44	0.99	0.75**	8.05	0.70	0.10**	3.69	0.95	0.01
ML 12-57	3.90	0.96	0.00	11.10	0.96	0.06*	35.88	1.14	0.30**	8.74	0.72	0.07**	3.90	0.97	-0.01
IPM 2-3	3.47	0.73	0.47**	10.06	1.08	0.01	32.97	0.94	0.08*	8.12	0.74	0.05**	3.47	0.75	0.45**
HUM 12	4.14	1.04	0.01*	11.99	1.38	0.42**	40.18	0.80	0.04	9.46	1.01	0.01	3.59	0.91	0.12**
DMS 02-11-4	4.36	1.16	0.20**	10.72	0.86	0.13**	37.44	1.05	0.01	9.37	1.25	0.03**	4.36	1.18	0.19**
IPM 99-1-6	4.34	1.18	0.24**	10.75	0.91	0.00	38.15	1.12	0.54**	9.40	1.23	0.04**	4.34	1.20	0.23**
DMS 03-17-2	4.75	1.13	0.12**	12.46	0.92	0.04	43.72	1.08	0.06*	10.36	1.23	0.04**	4.75	1.15	0.11**
IPM 2K-15-4	4.02	1.01	-0.01	11.48	1.02	0.08*	41.14	0.99	0.06*	9.37	0.83	0.05**	4.02	1.03	-0.01
DMS 01-34-2	4.10	0.84	0.15**	13.97	1.02	0.02	47.15	0.99	-0.01	10.42	0.84	0.03**	4.10	0.86	0.14**
IPM 02-17	4.01	1.03	0.01	11.07	0.94	0.01	36.52	1.06	0.01	9.15	1.21	0.03**	4.01	1.05	-0.00
Pusa 871	4.33	1.16	0.20**	11.66	0.94	0.07*	35.82	1.11	0.16**	9.12	1.17	0.03**	4.33	1.18	0.19**
IPM 99-01-10	4.21	1.02	-0.01	12.38	1.11	0.02	40.81	1.12	0.04	9.99	1.11	0.02*	4.21	1.03	-0.01
IPM 03-01	4.46	1.24	0.43**	8.20	0.96	-0.00	31.91	0.85	0.01	8.71	1.16	0.05**	4.46	1.26	0.42**
Pusa 11-31	4.52	0.93	0.01**	16.24	0.97	0.01	48.74	1.12	0.11**	10.85	1.03	0.02**	4.52	0.95	0.01
PM 2	4.54	0.98	-0.01	13.47	1.19	0.99**	46.90	0.97	0.07*	10.50	0.94	0.01	3.75	0.94	0.16**
Pusa 98-71	3.86	0.94	0.01**	11.24	0.91	0.05*	40.70	0.88	0.18**	9.71	0.87	0.05**	3.86	0.95	0.01
Pusa 12-31	4.25	1.00	-0.01	12.02	1.07	0.04	45.21	1.03	0.06*	10.14	1.00	0.01*	4.25	1.02	-0.01
DMS 06-17-2	4.07	0.81	0.23**	12.23	1.37	0.68**	42.06	0.78	0.49**	10.07	0.86	0.01	3.73	0.90	0.04*
SML. 11-51	3.70	0.83	0.18**	10.89	0.74	0.37**	44.00	0.80	0.30**	10.12	0.98	0.01	3.58	0.82	0.08**
DM 05-74-11	3.95	1.04	0.01*	12.20	1.03	0.50**	38.72	1.11	0.05*	9.21	1.02	0.01	3.58	0.84	0.04*
IPM 2K-14-9	3.43	0.81	0.22**	11.04	0.98	0.02	36.85	0.98	0.19**	9.02	0.73	0.05**	3.43	0.83	0.20**
Pusa 171	3.80	0.87	0.09**	11.05	0.61	0.27**	40.47	1.08	0.18**	9.86	0.87	0.00	3.74	0.86	0.07**
Mean	4.071			10.98			38.67			9.259			3.982		

$\bar{x}_i$ = Mean Value; b_i = Regression coefficient; S^2d_i = Deviation from regression; *Significance at 5% and **Significance at 1% level.

et al. (2001) in the maize, Arshad *et al*. (2003) and Patel *et al*. (2009) in the greengram. Both b_i and S^2d_i components of the G × E interaction should be taken into account when assessing the phenotypic stability of a specific genotype according to (Eberhart and Russell, 1966).

Nodule number (NN)

Among the twenty-five genotypes, DMS 03-17-2 (4.75) has the highest mean nodule number (Table 2), whereas the genotypes, IPM 2K-14-9 (3.43) gave the least mean nodule number and is considered as the poorest performing genotype across the different environments. Based on the mean nodule number ranking three genotypes *viz*; DMS 03-17-2 (4.75), PM 2 (4.54) and Pusa 11-51 (4.52), were the most superior genotypes having highest number of nodules across eight different environments. A total of 12 genotypes have nodule number greater than the grand mean value indicating its superior performance over the rest of the genotypes.

The estimates of b_i for nodule number per plant ranged from 0.73 (IPM 2-3) to 1.24 (Pusa 90-72). These results revealed that most genotypes had average response to environments. The genotype, Pusa 12-31 (b_i = 1) is the most stable genotype followed by IPM 2K-15-4 (1.01) and PDM 54 (0.99).

Out of twenty-five genotypes, eighteen genotypes showed a significant deviation from mean square from linear regression indicating that these genotypes were unstable and unpredictable across all the eight different environments. The genotype, ML 12-57 (S^2d_i = 0) is non-significant and has the value equals to zero. Hence, ML 12-57 is the most stable genotypes followed by IPM 02-17 (0.01).

Therefore, based on all the parameters *viz.*, $\bar{x}_i$, b_i, S^2d_i, the genotype showing the most stable performance for the trait, nodule number across the 8 different environments is PM 2 ($\bar{x}_i$ = 4.54, b_i = 0.98, S^2d_i=- 0.01), followed by Pusa 12-31 ($\bar{x}_i$ = 4.25, b_i = 1, S^2d_i = - 0.01) and ML 12-57 ($\bar{x}_i$ = 3.90, b_i = 0.96, S^2d_i = 0).

Seed yield per plant (g)

The genotype, Pusa 11-31 (16.24) has the highest seed yield than other remaining genotypes (Table 2), followed by MMS-01-34-2 (13.97) and PM 2 (13.47). A total of 16 genotypes showed its mean seed yield value greater than the grand mean seed yield value indicating its superiority over the rest of the genotypes.

The estimates of b_i for seed yield ranged from 0.61 (Pusa 171) to 1.38 (Hum 12). These results indicated that majority of the genotypes had average response to environments. The genotype, Pusa 90-72 (b_i = 1.01) is the most stable genotype, followed by DMS 01-34-2 (1.02), IPM 2K-15-4 (1.02) and IPM-2K-14-9 (0.98). The extreme variation in responsiveness may help in enhancing higher grain production in specific environment.

Among 25 genotypes, a total of 14 genotypes has significant deviation from the regression line indicating that these genotype performances for seed yield were very unstable and unpredictable across different environments. Two genotypes, IPM 99-1-6 (0) and IPM 03-01 (0) were found to be most stable genotypes followed by Pusa 11-31 (0.01) and IPM 02-17 (0.01) for seed yield.

Based on the above three parameters, the genotype, Pusa 11-31 ($\bar{x}_i$ = 16.24, b_i = 1.01, S^2d_i = 0.01), is found to be the overall best performing genotype for seed yield per plant across different environments.

Fresh weight (g)

The genotype with the highest fresh weight is Pusa11-31 (48.74), followed by DMS 01-34-2 (47.15), (Table 2), indicating that these two genotypes are the best performing genotypes among others. A total of thirteen genotypes exhibited mean fresh weight greater than the grand mean fresh weight value indicating that these genotypes were having superior performance across the environments.

The value of regression coefficient for fresh weight ranges from DMS 06-17-2 (0.78) to ML 12-57 (1.14). This finding indicates the genotypes having average response to different environments. Three genotypes, PDM 262, IPM 2K-15-4 and DMS 01-34-2 each having regression coefficient value of 0.99 were found to be the most stable genotypes for the trait, fresh weight across the test environments. The greater variation in responsiveness is the indicative of higher fresh weight may be achieved.

Table 2: Pooled ANOVA of stability as per Eberhart and Russell (1966) for characters under studied.

Source of variation	d.f.	MSS				
		NN	Yield (g/pt.)	FW (mg)	DW (mg)	BNF
Genotype	24	0.9765**	41.8534**	254.1211**	8.8385**	1.1079**
Environment	7	11.7095**	35.5485**	36.8557**	14.8272**	11.2561**
G × E	168	0.1255**	0.2168**	0.2045**	0.0850**	0.1322**
Evt. + (G × E)	175	1.1948**	1.6301*	1.6705**	0.6746**	0.5772*
Evt. (Linear)	1	186.3925**	248.8392**	257.9900**	103.7903**	78.7928**
G × E (Linear)	24	0.1276	0.2596	0.1290	0.1937**	0.0641
Pooled deviation	150	0.1308**	0.2013**	0.2086**	0.0642**	0.1378**
Pooled error	384	0.0591	0.1033	0.0964	0.0220	0.0664

NN = Nodule number; Yield = Seed yield per plant; FW = Fresh weight of nodule; DW = Dry weight of nodule; BNF = Biological nitrogen fixation; MSS = Mean sum of square; *Significance at 5% and **Significance at 1% level.

Out of total 25 genotypes, 4 genotypes, namely, DMS 02-11-4 (0.01), IPM 02-17 (0.01), IPM 03-01 (0.01) and DMS 01-34-2 (-0.01), were found non-significant with least deviation from the regression line for the trait, fresh weight and considered as the most stable genotypes. A total of eighteen genotypes showed significant mean square deviation from the linear regression indicating that these genotype performances for seed yield were very unstable and unpredictable across different environments.

The genotype DMS 01-34-2 ($\bar{x}_i$ = 47.15, b_i = 0.99, S^2d_i = 0.01), turned out to be the most suitable genotype for the trait, fresh weight across the eight different environments.

Dry weight (g)

The genotype, Pusa 11-31 (10.85) exhibits the highest mean dry weight (Table 2), followed by the genotypes, PM 2 (10.50), DMS 01-34-2 (10.42), DMS 03-17-2 (10.36). A total of fourteen genotypes showed its mean dry weight value greater than the grand mean value which indicates that these genotypes exhibited superior performance over the rest of the genotypes.

The regression coefficient for the trait dry weight ranges from 0.63 (PDM 178) to 1.40 (Pusa 90-72). These results imply that most genotypes showed average response to different environments. The genotype, Pusa 12-31 (1), followed by HUM 12 (1.01) were found to be the most stable genotypes across the environments.

The genotype, Pusa 171 was found to be the most stable genotype for the trait, dry weight across the environment. Nineteen genotypes exhibited significant mean square deviation from the regression line indicating that performance of these genotypes unstable and unpredictable across the different environments.

The genotype, SML 11-51 ($\bar{x}_i$ = 10.12, b_i = 0.98, S^2d_i = 0.01), gave the overall best performance for the trait, dry weight with respect to the above three parameters in all the eight different tested environments.

Biological nitrogen fixation (BNF)

The highest BNF is observed in the genotype DMS 03-17-2 (4.75), followed by Pusa 11-31 and IPM 03-01 (Table 2). About twelve genotypes had nitrogen fixing capacity greater than the average nitrogen fixing capabilities of twenty-five genotypes indicating the superior performance of these genotypes across different environments.

The regression coefficient for biological nitrogen fixing ranges from 0.75 (IPM 2-3) to 1.26 (Pusa 90-72) and PDM 178 (1) and PDM 54 (1) were two most suitable genotypes due to the regression co-efficient value equal to unity. The genotypes with extreme values and showing greater deviation from the unity imply that these genotypes are unstable and sensitive to different environments.

The genotype IPM 02-17 has the mean square deviation from the linear regression equals to zero and is also non-significant. Hence, IPM 02-17 is the most suitable genotype for biological nitrogen fixation across the test environments. Other genotypes showing non-significant and least mean square deviation from regression line were PDM 262 (0.01), Pusa 11-31 (0.01), Pusa 98-71 (0.01), PDM 54 (- 0.01), ML 12-57 (- 0.01), IPM 2K-15-4 (- 0.01), IPM 99-01-10 (- 0.01) and Pusa 12-31 (- 0.01). Among twenty-five genotypes, a total of sixteen genotypes had significant deviation from regression line indicating that these genotypes were very unstable and unpredictable for the trait.

Based on the above three parameters, Pusa 11-31 ($\bar{x}_i$ = 4.52, b_i = 0.95, S^2d_i = 0.01), was found to be the genotype showing most stable biological nitrogen fixation ability across different environments.

CONCLUSION

The experiment showed the presence of G × E interaction among mungbean genotypes. Based on the three stability parameters, the genotype, Pusa 11-31 gave the overall most stable performance for seed yield per plant and other components traits. Hence, Pusa 11-31 can be recommended for cultivation in diverse range of different environments.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this paper. The authors have no personal, professional, or financial relationships that could influence the design, conduct, or reporting of the research. The authors have adhered to the ethical standards and principles of scientific integrity in conducting and presenting their work.

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