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Genotype × Environment Interaction for Germination in Spine gourd (*Momordica dioica* Roxb.)

JK TIWARI[1]* AND NK RASTOGI[2]

[1]Section of Genetics and Plant Breeding, RMD Collage of Agriculture and Research Station, IGKV, Ambikapur, C.G.-497001, India

[2]Department of Genetics and Plant Breeding, Collage of Agriculture, IGKV, Raipur, C.G.-492012, India

*tiwarijk5@gmail.com

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ABSTRACT: Seed dormancy is a major limiting factor constraining commercial cultivation of spine gourd and hence the present study was undertaken to identify better environment for the germination. The germination of fifteen spine gourd genotypes, grown at three different locations was analyzed. All the combinations were tested under optimal conditions for germination. We have evaluated the seed germination response to different environment along with different physical factors which influence seed germination and found that the genotype, G10 did perform well under all the three environments which was followed by G2, G4, G8 and G12. Among the environments, E2 was a best suited environment for germination and E1 is at par with E2. Germination deciding factors like soil pH, temperature, N, P, K and organic matter content did affect germination in some extent and these factors can be additive, synergistic or inhibitory, depending on the combination, providing an almost infinite spectrum of dormancy levels. The location caused the greatest variation across all the experiments and the impact was greater for germination than for vitality. The results should encourage breeding for seed vitality and some reconsideration of provenance effects on seed vitality.

Key words: AMMI, G × E interaction, Seed germination, Spine gourd, Stability

Spine gourd (*Momordica dioica* Roxb.) is a highly nutritious vegetable containing high amount of protein as compared to other cucurbitaceous vegetables with a high medicinal value, mainly cultivated for its fruits. It is widely distributed in tropical and sub-tropical parts of India and adapted to a wide range of soil and climatic conditions [1]. Because of its immense medicinal and nutraceutical value, spine gourd is now gaining much importance in *ayurvedic* and allopathic medicine preparations.

Seed dormancy is a major limiting factor constraining commercial cultivation of spine gourd [2]. It is characterized by partial metabolic arrest with its inception and termination under endogenous hormonal control. Low temperature and seed hardiness are also reported to be the reason for slow and poor germination in *M. dioica* [2]. Good quality seed is very essential to get high germination percentage and seedling establishment which are the most critical stages in the life cycle of plants. Knowledge of seed germination with response to environmental factors is required not only to understand and predict the ecological adaptation of the species, but also to formulate effective strategies for restoration [5].

There are limited data available regarding differences in impact of environmental signals on dormancy in spine gourd. Therefore, the present work was undertaken to study the effect on seed germination in response to different environments and to better understand varying environmental adaptation.

Sowing environment is a key factor in breaking seed dormancy and initiation of seed germination. During germination seeds take different senses from the soil *viz*., temperature, pH, nitrate, phosphorus, potash etc. In response to these signals, seeds alter their depth of dormancy and their sensitivity to other signals that inform of the spatial environment. These spatial signals indicate in a more immediate way that conditions are suitable for germination and so trigger the termination of dormancy and therefore improve germination.

The growing conditions may affect the degree of dormancy of cultivated seeds. A survey of the literature shows that some well defined patterns emerge, with certain environmental factors tending to have similar effects over a wide range of species. Lower dormancy (i.e., increased germination) is generally associated with the following environmental conditions during seed development: high temperatures, short days, red light, drought and high nitrogen levels [4].

The development of improved genotypes, which can be adapted to a wide range of environments, is one of the final goals of researchers in plant breeding program. Varietal adaptability to environmental fluctuations is important for the stabilization of crop production over both the regions and years. An information on genotype x environment interaction leads to successful evaluation of stable genotype, which could be used for general cultivation. Yield is a complex quantitative character and is greatly influenced by environmental fluctuations; hence, the selection for superior genotypes based on yield *per se* at a single location in a year may be very effective. The additive main effects and multiplicative interaction (AMMI) and GGE biplot models can be powerful tools for effective analysis and interpretation of multi environment data structure in breeding programs [3, 8].The utilization of both AMMI and GGE biplot methods simultaneously in confirmation of stable genotypes with high yield conducted by many researchers. Therefore, purpose of the present study was to apply GGE biplot and AMMI techniques to study the patterns of GEI in 15 advanced winged bean genotypes; to graphically display means, adaptability and stability, to identify suitable genotypes, to the relationship among the test environments and to compare result of GGE biplot and AMMI models.

In crop trials, genotype-by-environment (GE) interaction is often analyzed by the additive main effects and multiplicative interaction (AMMI) model [9]. The AMMI model combines the analysis of variance (ANOVA) with additive parameters and the principal component analysis (PCA) with multiplicative parameters in a single analysis. The AMMI biplot graphic display simultaneously both main and interaction effects for genotypes and environments, and enables a single analysis of the genotype by environment interaction. The AMMI is, therefore, also known as interaction PCA [7]. The aim of this work was to assess genotype by environment interaction for germination in spine gourd lines.

MATERIALS AND METHODS

Plant Materials

Fifteen improved spine gourd lines developed for cultivation in sub tropical ecosystem were used in this study. All the genotypes are from Chhattisgarh State, India and the seeds were obtained from the All India

Table 1. Details of the three environments used in the present study

Environments		Soil type	Soil characteristics						Annual average rainfall (mm)	Annual average temp. (°C)		Global position		
			pH	EC (dS/m)	OC (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)		Min.	Max.	Latitude	Longitude	Altitude (m)
Germination under normal field conditions Ambikapur 2018	E1	Sandy loam	5.6	0.10	16.3	150.52	10.57	250.43	1216.30	17.60	30.00	23°09′ N	83°08′ E	623
Germination under agroforestry conditions Ambikapur 2018	E2	Sandy loam	5.8	0.11	17.5	125.44	7.79	269.92	1216.30	18.00	30.40	23°09′ N	83°08′ E	623
Germination under greenhouse conditions Ambikapur 2018	E3	Compost	6.5	0.7	25	120.34	6.87	135.34	Controlled	20.00	30.00	23°09′ N	83°08′ E	632

Coordinated Research Network on Potential Crops, Ambikapur, Chhattisgarh, India.

Experimental location and design

Germination of 15 spine gourd lines were tested under three environments viz., normal field conditions (NFC), agro-forestry conditions (AFC) and green house conditions (GHC). NFC and AFC both the location have distinct soil and climatic characteristics along with a control condition. The experiments in all the locations were done in a single season.

About 200 gram seeds of each line was taken in the month of October, 2017 and sun tried for 15 days and then kept at 4°C for 6 months. Seeds were surface-sterilized in a 0.125% sodium hypochlorite solution for 5 min and washed three times with distilled water and then soaked in 100 ppm Gibrellic acid (GA_3) solution for 12 hours.

Seeds were sown in a temperature- controlled greenhouse (30/20 °C). The greenhouse was vented and heated to control temperature. Compost, sand and vermiculate in the ratio of 4:2:2 was mixed to provide base for germination test. In Each trial there were three replicates of 100 seeds for each line. A seed was considered to have germination when the radical had emerged above the soil surface. Germination percentage and soil test of each trailwas recorded.

Data analysis

Data analysis was carried out using the statistical programme PB Tools, version 1.4. 2014 (bbi.irri.org) and R (R core Team, 2013). Germination percentage data was angular transformed before analysis. The AMMI model (Gauch, 1988) was used in analyzing the GEI (Genotype Environment Interactions) for seed germination. The AMMI model is a combination of analysis of variance (ANOVA) and Principal component analysis (PCA). ANOVA used to analyze the main effects while PCA decomposes the interaction into PCA axes. Biplots was used to visualize the AMMI results. Genotypes group performance plot was constructed by plotting mean germination percentage for genotype groups against environment groups based on the untransformed germination percentage.

RESULTS

Combined analysis

The joint analysis of variance showed significant differences ($p < 0.01$) for environments (E), genotypes (G) and the G×E interaction. A significant effect of the

Table 2. Mean yield, AMMI and biplot scores of the test genotypes and environments

Code	Genotypes and Environments	Mean germination percentage over 3 environments	AMMI Score		
			IPCA I	IPCA II	IPCA III
G1	AJSG-2	51.16	-0.817	-0.773	9.96
G2	RMD 15-3	63.39	0.985	0.648	-3.29
G3	RMDSG-3	48.72	0.2666	1.031	-3.56
G4	RMD 15-4	44.27	2.435	0.693	1.32
G5	AMBIKA 13-5	51.67	1.085	-2.294	1.02
G6	Ambika 13-6	39.17	0.764	-0.675	5.32
G7	ASG 18-4	35.11	-0.094	-0.473	-5.36
G8	Chattisgarh Kankoda-2	50.27	-0.196	-1.084	1.34
G9	ASG 18-2	26.94	-0.131	2.350	3.70
G10	Selection-2	40.06	0.308	0.128	2.62
G11	RMD 15-1	38.50	0.084	1.832	-2.45
G12	Selection-4	41.00	0.503	0.062	-1.03
G13	ASG 18-3	27.33	-3.036	0.338	-3.90
G14	Selection-3	32.61	0.335	-1.351	-9.18
G15	ASG 18-1	44.00	-2.491	-0.431	2.54
E1	Germination under natural field conditionsAmbikapur 2018	34.93#	4.043	-0.675	4.14
E2	Germination under agro forestry conditionsAmbikapur 2018	55.23#	-1.375	3.502	4.14
E3	Germination under greenhouse conditionsAmbikapur 2018	36.48#	-2.668	-2.827	4.14
	Mean				

Mean germination of all the test genotypes in a given environment.

G×E interaction demonstrates the differential performance of genotypes in different environments. Therefore, the change in the average germination of spine gourd varieties as a consequence of the environment justifies the need for a more refined analysis to increase selection efficiency and varietal recommendations. The combined analysis indicated that the genotype and GEI accounted for 40.41% and 10.69% of the total variance, respectively (Table 3). The relatively low magnitude of the GEI variance to the genotypic component was unexpected. All the three environments and different component of soil viz., pH, temperature, N,P, K, EC, and Organic content are found to be the most important factors influencing germination.

AMMI

A wide range of variation was observed for seed germination among the genotypes and across the environments. The range among environment for average germination was 34.93 to 55.23 (Table 2). AMMI analysis showed that the first principle component axis accounted for 60% of the total variation and the second accounted for 40%. The first two PCA collectively accounted for 100% of GEI. Based on results from many other studies [3,10,11] in different crops AMMI I and the AMMI 2 model were used to explain the present data. The AMMI I bioplot (Figure 1) indicated that most of the test genotype tend to have IPCA 1 scores of nearly zero, and their mean germination percentage were close to around 0.57.

The position and perpendicular projection of genotype points relative to environment vectors could be used to determine whether a cultivar was specifically adapted to given environment. Genotypes that were positioned further along the positive direction of a vector tend to show higher germination, reflecting better adaptation to that environment. The genotypes G10, G 12, G2, G4, G3, G11 and G9 were positioned along the positive direction of E2, suggesting that they specifically adapted to E2. This result was consistence with the result of AMMI 1 biplot.

Response plot

Genotype performance across the three environment groups plot was presented in Figure 1. It could be seen that G10 followed by G2, G4, G8 and G12 perform well under all the three environment. In contrast, G13, G14, G5 and G6 had the lowest average germination across the environments.

Discussion

Seed germination, in a strict sense, is completed by radical emergence, which is determined by the balance of the growth potential of the embryo and the mechanical resistance of the covering tissues, such as the endosperm

Table 3. Means squares of individual analysis of variance by environment and Combined analysis of variance for 15 genotypes in 3 environments

Source of variation	DF	Means squares of individual analysis of variance by environment			Combined analysis of variance			
		E1	E2	E3	Source of variation	DF	SS	SS (%)
Block	2	317.26	376.71	108.88	Genotypes	14	12169.59**	40.41
Genotype	14	355.50**	430.00**	313.65**	Environments	2	11341.49*	37.66
Error	28	8.35	42.54	12.46	Replications in environment	6	1605.74	
					G X E	28	3218.61**	10.69
					Error	84	1773.92	5.89
					Total	134	30109.37	

**, * and ns significant at the 0.01 and 0.05 probability level, respectively and non-significant.

Table 4. Analysis of variance for AMMI and GGE bioplot model in Germination of Spine gourd genotypes

Source	DF	AMMI			
		Sum of squares	Mean square	F	SS(%)
IPCA1	15	1930.529	128.70196**	6.09	58.98
IPCA2	13	1288.089	99.08378*	4.69	
IPCA3	11	0.00	0.00	0.00	

**, * and ns significant at the 0.01 and 0.05 probability level, respectively and non-significant.

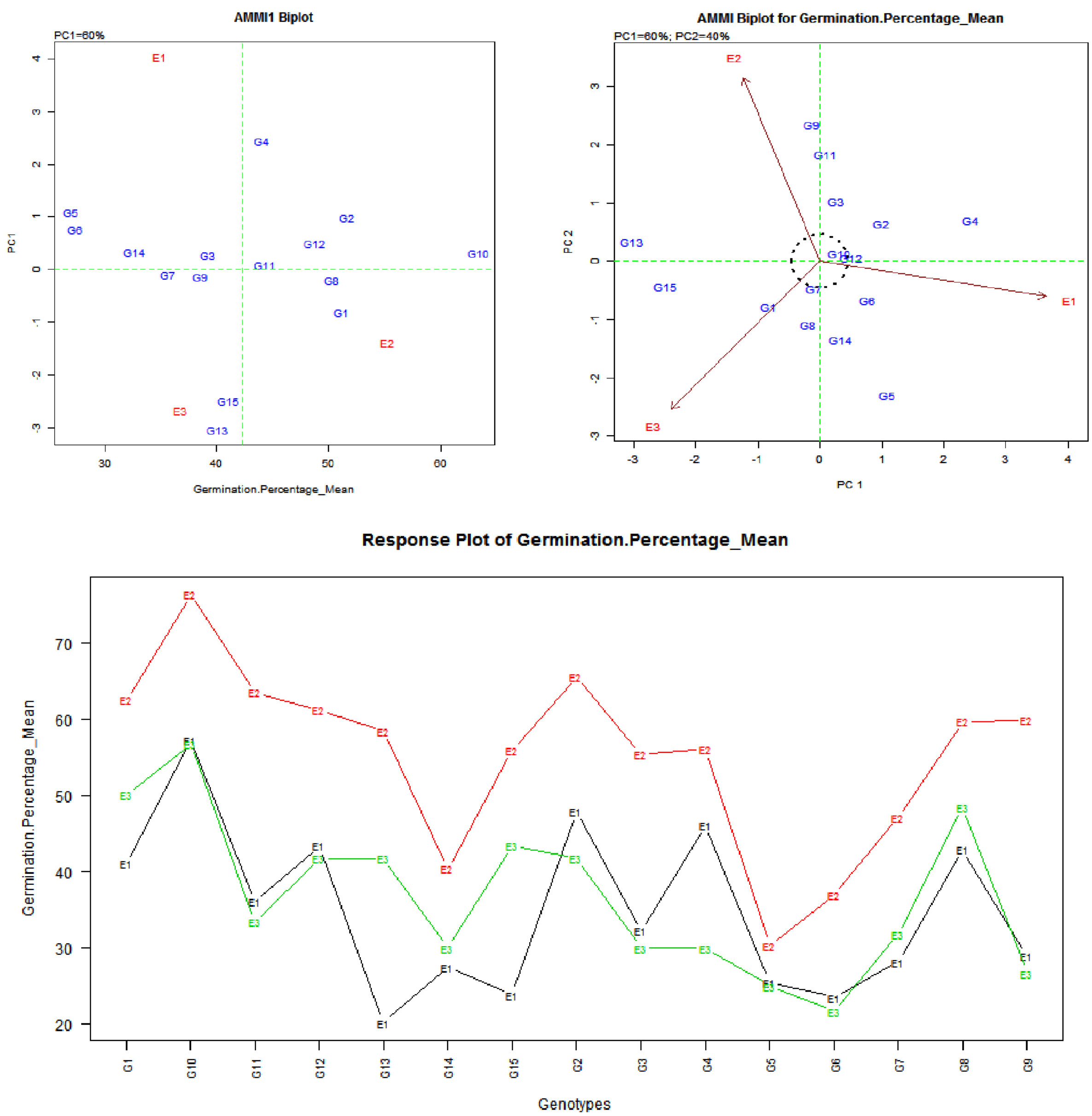

Figure 1. AMMI biplot and response plot of germination in spine gourd

and the testa [13]. An active embryo still can not emerge from a seed due to the firmness of the covering tissue, which is referred to as coat-imposed dormancy [12].

Seeds have evolved to be highly efficient environmental sensors that respond not only to their prevailing environment, but also their environmental history, to regulate dormancy and the initiation of germination. In the present work we investigate the combined impact of a number of environmental signals (temperature, nitrate, light) during seed development on the mother plant, during post-shedding imbibitions and during prolonged post-shedding exposure in both dry and imbibed seed states.

We have evaluated the seed germination response to different environment along with different physical factors which influence seed germination and found that G10 followed by G2, G4, G8 and G12 perform well under all the three environment. E2 was a best suited environment for germination and E1 is at par with E2. Factors like soil pH, Temperature, N, P, K and organic matter content affect germination in some extent and these factors can be additive, synergistic or inhibitory, depending on the combination, providing an almost infinite spectrum of dormancy levels. Seed germination and dormancy research has brought historical discoveries in plant and molecular biology in the past and hormonal regulation of gene expression [13]. Though, this study provides opportunity to further study of effect of different environmental singles in context to seed germination and construct a frame work for our understanding to linked genes and their function at advance level of research i.e. transcriptome analysis and comparative profiling of expressed genes during germination.

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