



## Assessing the Effect of Salt Stress on Soybean [*Glycine max* (L.) Merrillis] Genotypes Using AMMI and GGE Biplot Analysis

Vijayata Singh<sup>1</sup>, SK Sanwal<sup>1</sup>, Giriraj Kumawat<sup>2</sup>, M Shiva Kumar<sup>2</sup>, GK Satpute<sup>2</sup>, BS Gill<sup>2</sup>, Sachin Panwar<sup>1</sup>, Jogendra Singh<sup>1\*</sup> and PC Sharma<sup>1\*</sup>

<sup>1</sup>ICAR-Central Soil Salinity Research Institute, Karnal-132001, Haryana, India

<sup>2</sup>ICAR-Indian Institute of Soybean Research, Indore- 452001, Madhya Pradesh, India

\*Corresponding authors Email: jogendra.singh@icar.gov.in, pcsharma.knl@gmail.com

### Abstract

The genotype × environment interaction manipulates the selection criteria in a multipurpose crop like soybean. A total 108 soybean genotypes were evaluated at normal tap water (Control), field sodicity conditions (pH 9.0 and 9.3) and saline water ( $EC_{iw}$  5.0 and 8.0 dS m<sup>-1</sup>) at ICAR-CSSRI, Karnal from 2017-2020. Yield and associated data were analyzed using the AMMI and GGE biplot. The AMMI analysis of variance for seed yield detected significant effects for genotype, environment and genotype × environment interaction. The environment effect was responsible for the greatest part of the variation, followed by genotype and genotype × environment interaction effects. The 'which-won-where' feature of the GGE biplot identified winning genotypes SL-1226 and SL-1258 in the saline (up to  $EC_{iw}$  8 dS m<sup>-1</sup>) and sodic (up to pH 9.3) and SL-1242 in control conditions whereas, PS-1225 across the environment was the most ideal and these genotypes could be used as donor for breeding soybean for salt tolerance. This indicates that characterization of germplasm using GGE and AMMI model is important for determining visual comparisons, adaptability/stability focusing on overall performance to identify superior genotypes.

**Key words:** Soybean, Salt stress, AMMI analysis, GGE biplot, Genotypes

**Abbreviations:** AMMI= additive main effects and multiplicative interaction; GGE= genotype plus genotype by environment interaction; AEC= average environment coordination; IPCA= interaction principal component axis

### Introduction

Globally, about 1 billion ha area is salt-affected. Further, 20% of irrigated land and 2.5% of dryland agriculture is adjudged salt-affected (FAO, 2016). In India, nearly 6.73 million ha area is reported to be affected with salinity and sodicity stresses covering various states of the country (Mandal *et al.*, 2010). Reclamation and preventable measures for transforming salt-affected lands into arable land are very expensive. The development and use of plant species that can tolerate high salt level is cost effective and important for sustainable crop production in such abandoned area. This may be achieved by making use of variations in tolerance both, between and within cultivars. Soybean is one of the globe's most important legume crops classed as an oilseed rather than a pulse by the UN Food and Agriculture Organization. It is valued for its high protein (38–

45%) and oil contents (20%). Approximately 85% of the world's soybean crop is processed into meal and oil, the remainder processed in other ways or eaten whole. United States, Brazil, Argentina, China, India and Paraguay represent more than 87% of the global soybean production (USDA, 2020). Soybean is grown as a *Kharif* crop in India. The top three largest Soybean growing states are Madhya Pradesh, Maharashtra and Rajasthan sharing 45, 40 and 8.2% production, respectively (<http://www.gktoday.in/blog/key-facts-about-soyabean-production-in-india/>). However, there is 1.30 mha salt-affected area lies in these major growing states [Madhya Pradesh (0.14 mha) Maharashtra (0.61 mha) and Rajasthan (0.38 mha)].

Relative to other crops, little is known about intrinsic ability of soybean to tolerate salt stress. High salt significantly hampered its productivity and imposes negative impacts on growth,

nodulation, agronomy traits, seed quality and quantity and thus reduce the yield of soybean (El-Sabagh *et al.*, 2015). However, threshold limit is 5 dS m<sup>-1</sup> (Maas and Hoffman, 1977; Chinusamy *et al.*, 2005). Out of 126 soybean varieties released in India till date, none of the variety was released for salt-affect areas. Hence, there is an opportunity to develop salt tolerant soybean genotype and expanding cultivation in about 1.30 million ha salt-affected area lying in major producing states of the country in additional to 11 million ha currently cultivated.

The success of any breeding programme depends upon the extent of genetic diversity present in germplasm. Therefore, salt tolerance of diverse soybean genotypes must be evaluated. Screening of genotypes for salinity tolerance is necessary to understand the mechanisms of salt tolerance in diverse genotypes (Munns *et al.*, 2006). Various statistical procedures are available to analyze and determine the results of multi-location trials and genotype-environment interaction data. However, two multivariate analysis such as AMMI and GGE bi-plot analysis has been performed in this study. The Additive main effect and multiplicative interaction (AMMI) model (Gauch, 1988) proved to be a powerful tool in diagnosing genotype × environment interaction patterns (Crossa, 1990). AMMI analysis can also be used to determine stability of the genotypes across locations using the PCA scores and AMMI stability value. Moreover, the GGE analysis is an effective method which is based on PCA to fully explore multi-environment trials. GGE analysis partitions G + GE into principal components through singular value decomposition of environmentally centered yield data (Yan, 2001). This study aimed to provide the base material for development of salt tolerant varieties of soybean that will be helpful for farmers for upliftment of their socio-economic aspects and add widely acceptable AMMI and GGE biplot selection approach, which earlier considered only biomass production and ion accumulation.

## Material and Methods

### Plant material and experimental setup

Initial Material for this project comprised of following 108 soybean genotypes (germplasm,

released varieties, wild accession and breeding lines) evaluated from 2017 to 2020 under saline and sodic conditions.

### Field experiment

Soybean accessions were evaluated under the field sodicity conditions (pH 9.0 and 9.3) on completely randomized block design (CRBD) with two replications.

### Pot experiment

Initially, five seeds of each of accessions were sown at depth of 1 cm in 20 kg capacity ceramic pots filled with sand inside the net house facility. The bottom of each pot was delved for drainage of extra water. The pots were irrigated by normal tap water (control), saline water (EC<sub>iw</sub> 5.0 and 8.0 dS m<sup>-1</sup>) and maintained at full strength field capacity. Here, we selected above salinity levels because EC<sub>iw</sub> 5 dS m<sup>-1</sup> is the threshold limit for soybean crop (Chinusamy *et al.*, 2005). The saline water for irrigation was prepared in Hoagland nutrient solution by adding NaCl, Na<sub>2</sub>SO<sub>4</sub> and CaCl<sub>2</sub>, keeping Na:Ca and Cl:SO<sub>4</sub> ratios of 4:1 which reflect the major ion compositions of naturally occurring saline waters/soils. The pots were arranged in a factorial experiment based on completely randomized block design (CRBD) with two replications. The pots were irrigated daily so as to maintain the respective salinity level in the root zone throughout the life cycle of the crop. Saline irrigation was continued until the harvest of the crop for recording yield. Plant sampling for ionic study was done at the harvesting stage. At maturity, three plants per pot were harvested and air dried prior to recording their grain yield. Seed yield of all the genotypes under different salinity regimes was also recorded.

### Measurement of ion concentration

The ion concentrations in shoot, and roots were estimated using di-acid method (Piper, 1942) containing HNO<sub>3</sub> and HClO<sub>4</sub> acid (9:4) for complete understanding of the pattern of ion partitioning under imposed salt stress. The concentrations of Na<sup>+</sup> and K<sup>+</sup> in the samples and standards were estimated using ion specific filters in a flame photometer (Model: 128, Systronics India Ltd., India). The standard curves of Na<sup>+</sup>

**Table 1.** Pooled analysis of variance for yield and associated traits across the salt stress environments

Source of variation	D.F.	Mean squares							
		Number of primary branches	Days to 50% flowering	Number of pods per plant	Seed yield (kg ha <sup>-1</sup> )	100-seed weight (g)	Number of seed per pod	Shoot Na <sup>+</sup> /K <sup>+</sup>	Root Na <sup>+</sup> /K <sup>+</sup>
Replication	1	78.82	1297.66	520.92	104.91	0.28	0.15	0.05	3.38
Genotypes	2	14.45**	2914.03**	9763.55**	568.62**	0.47**	3.33**	0.36**	131.42**
Environments	107	1129.04**	60640.88**	325602.85**	15749.55**	34.87**	119.72**	6.06**	871.98**
Interaction (genotype × environment)	214	5.17**	1309.07**	1762.80**	145.32**	0.27**	1.12**	0.25**	126.09**
Error	323	1.45	12.01	39.58	4.28	0.008	0.07	0.001	0.18

\*p≤0.5, \*\*p≤0.01 level of significance, D.F.=degrees of freedom

and K<sup>+</sup> were plotted and estimated the ion concentrations as mg g<sup>-1</sup> dry weight in the samples and calculated Na<sup>+</sup>/K<sup>+</sup> ratio.

### Statistical analysis

Statistical analyses and analysis of variance (ANOVA) was done using the SAS 9.3 software (SAS Institute Inc., Cary, USA). The AMMI and GGE biplot analysis were done using PBTools (v 1.4. 2014, Biometrics and Breeding Informatics, PBGB Division, International Rice Research Institute, Los Baños, Laguna).

## Results and Discussion

### Variable response of morpho-physiological traits to salinity

Analysis of variance exhibited the significant mean square for the studied traits which depicted significant genetic variation for traits days to 50% flowering, number of primary branches, number of pods per plant, number of seed per pod, 100-seed weight (g), seed yield (kg ha<sup>-1</sup>), and Na<sup>+</sup>/K<sup>+</sup> in root and shoot at maturity stages. Mean squares of the salt stress (environment) were significant for all the traits under study indicated significant differences of these traits for control and salt stress. The significant interaction effect of environments (control, salinity and sodicity) × genotypes revealed the variable response of genotypes by expression of traits over the salt stress (Table 1).

Variance analysis of AMMI model for seed yield (kg ha<sup>-1</sup>) detected significant effects for genotype, environment and genotype ×

environment interaction (Table 2). The presence of the genotype × environment interaction was indicated by changes in relative rankings of genotypes over control, saline and sodic conditions. The salt stress effect was responsible for the greatest part of the variation, followed by genotype and genotype × environment interaction effects. Our results are with corroborative of Tarakanovas and Ruzgas (2006).

### Mean performance of genotypes and environments

Results from the present AMMI analysis of variance of the 108 genotypes also revealed that only mean square of the IPCA1 was found to be highly significant (p≤0.01). But, the IPCA2 captured in significant (p≤0.05) portion of the variability. AMMI with two, three or four IPCA axes is the best predictive model (Crossa *et al.*,

**Table 2.** AMMI analysis of variance for seed yield for 108 genotypes and three environments

Source of variation	D.F.	S.S.	M.S.
Replication	1	104.91	104.91
Genotype	107	60842.77	568.62**
Environments	2	31499.10	15749.55**
Genotype × Environment	214	31099.42	145.32**
IPCA 1	108	19843.37	183.73**
IPCA 2	104	3481.20	33.47*
Residuals	2	613.78	306.89
Error	323	1383.29	4.28
Total	647	124929.4853	

\*p≤0.5, \*\*p≤0.01 level of significance, D.F.=degrees of freedom, SS=sum of square, MS=mean square

1990). In the present study, the AMMI analysis further revealed that the first two IPCAs accounted for a total of 100% of the interaction sum of square, with 99.06% of the corresponding degrees of freedom (Table 2). The IPCA1 of the interaction captured 85.10% followed the IPCA2 explained 14.90% of the interaction sum of square and altogether explained 100 of the variability in seed yield of the 108 genotypes tested in control, salinity ( $EC_{iw}$  5 and 8  $dS\ m^{-1}$ ) and sodicity (pH 9.0 and 9.3).

Furthermore, the IPCA scores of genotypes in the AMMI analysis indicated stability of the genotypes across salt stress. All the genotypes respond differentially for different traits at stress level (salinity as well as in sodicity). Hence, reaching on a consensus to identify an ideal accession under stress AMMI and GGE biplot analysis was performed. It indicates multiplicative portion of genotype  $\times$  environment into specific pattern of response of genotypes and the environments.

### Salinity condition

The IPCA1 and IPCA2 explained the 100% (90.1+9.9%) of interaction effects (Fig. 1). The accession G24 (PS-1225), G44 (JS 2029) and G49 (AGS-7513) were considered as stable being falls on base line. G80 (SL-1243) and G83 (SL-1254)

are specifically adapted for salinity stress that occur close to particular environments (E2 and E3;  $EC_{iw}$  5 and 8  $dS\ m^{-1}$ , respectively) on the IPCA2 vs. IPCA1 biplot. Because, IPCAs cover more than 70% variation in salt stress, hence it needed to analyze GGE biplot. Firstly, environment main effects are removed; secondly, genotype and genotype by environment interaction are retained and combined. GGE biplot analysis showed that G24 (PS-1225) is the most ideal genotype as it was located almost on the AEC abscissa and had a near zero projection onto the AEC ordinate. This indicates that its rank was highly consistent across saline environments (Dehghani *et al.*, 2006; Tesfaye *et al.*, 2008; Fetien and Bjornstad, 2009).

### Sodicity condition

The IPCA1 and IPCA2 explained the 100% (85.10+14.9%) of interaction effects (Fig. 2). The accession G57 (SL-1205) and G75 (SL-1234) were considered as stable being falls on base line. G51 (SL-1113), G60 (SL-1210), G68 (SL-1226) and G81 (SL-1258) are specifically adapted for sodicity stress that occur close to particular environments (E2 and E3; pH 9 and 9.3, respectively) on the IPCA2 vs. IPCA1 biplot. GGE biplot analysis showed that no genotype was ideal as none of them was located on the AEC abscissa and had

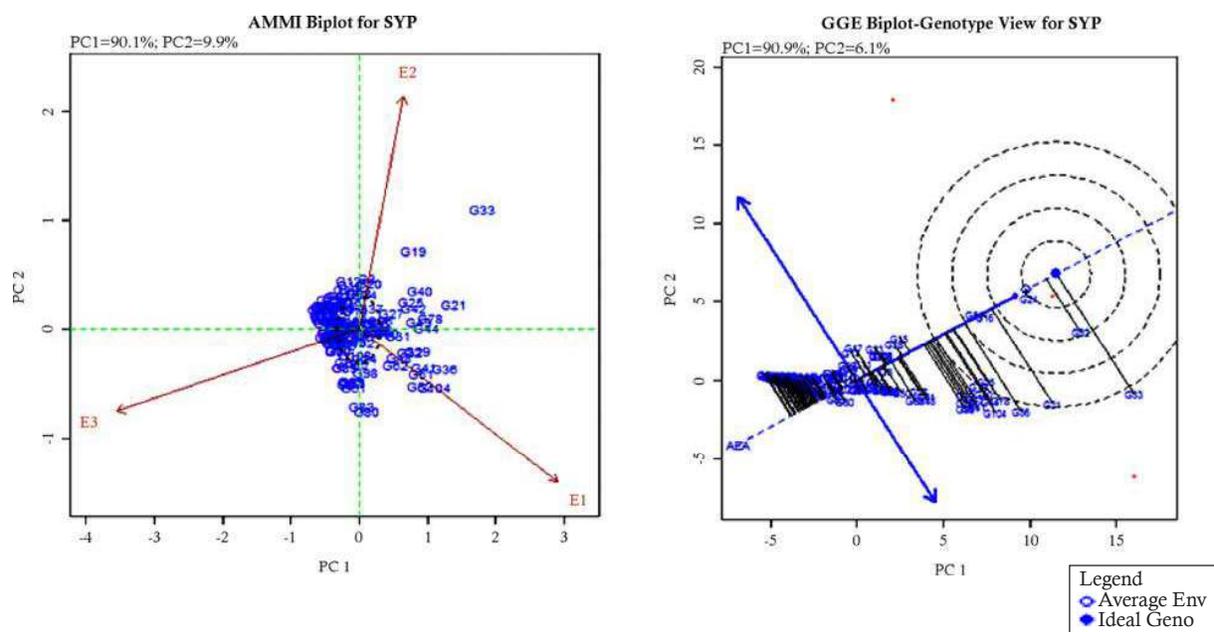


Fig. 1 AMMI and GGE biplot analysis for identification of ideal accession for salinity condition



vertex genotypes G68 (SL-1226) and G81 (SL-1258) were the most responsive genotypes, as they have the longest distance from the origin in their direction (Yan and Tinker, 2006). On the other hand, the result also showed some genotypes which fall in sectors where there was no environment at all; these genotypes are poorly adapted to salt stress.

## Conclusions

From this study it can be concluded that the significant genotype  $\times$  environment interaction in seed yield among the soybean genotypes revealed differential response of the genotypes across the testing environments which are exposed to variations in climate and edaphic factors. It is therefore, difficult to identify one superior genotype for saline and sodic environment. This indicates that particular genotypes tended to rank differently in seed yields at different salt stress due to the presence of either genetic diversity or variation in salt stresses. Thus, testing genotypes under various salt stresses and analysis using GGE and AMMI is important for evaluating adaptability/stability of performance and range of adaptation. Therefore, based on study, the genotypes SL-1226, SL-1258 in the saline (up to  $EC_{iw}$  8 dS m<sup>-1</sup>) and sodic (up to pH 9.3) condition while SL-1242 in normal environment and PS-1225 across the environment are the most ideal and could be used as donor for breeding soybean for salt tolerance.

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