



Integrating Seed Quality Traits and Salt Tolerance Indices for Efficient Screening of Salinity-Tolerant Soybean [*Glycine max* (L.) Merrill] Genotypes

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ABSTRACT

Background: Soybean [*Glycine max* (L.) Merrill], popularly known as the “Golden bean,” is a major oilseed crop valued for its high protein and oil content. Soil salinity is a critical abiotic stress that adversely affects germination, growth and productivity in soybean. Identifying salinity-tolerant genotypes remains a key challenge for breeders and seed technologists. Therefore, standardized and reliable screening protocols are essential for the rapid identification of salinity-tolerant genotypes.

Methods: The study was conducted at ICAR-IARI, New Delhi, during the *kharif* season of 2022 and 2023. A total of 156 soybean genotypes were evaluated, including 150 germplasm accessions (42 black-seeded, 105 yellow-seeded and 3 brown-seeded) with one-year-old stored seeds and six freshly harvested yellow-seeded varieties. Two techniques, namely the top-of-paper method and the between-paper method, were assessed. The top-of-paper method, with application of 2.5 ml NaCl solution on alternate days, was selected as a standard method. Salinity stress was imposed using four NaCl concentrations (0, 3, 6 and 9 dS/m) and seed quality traits [germination, seedling length, fresh and dry weight, Seedling Vigour Indices (SVI-I, SVI-II)] were integrated with the Salt Tolerance Index (STI) for genotypic categorisation.

Result: Significant genotypic variation was observed for seed germination and seed quality under salinity stress. Black-seeded accessions and freshly harvested varieties showed higher germination and vigour than yellow-seeded accessions, which were poor storers. STI correlated positively with vigour indices at 6 dS/m in black accessions and released varieties and at 3 dS/m in yellow accessions. Brown-seeded accessions showed inconsistent responses. Based on STI and correlation, 20 genotypes were categorised as highly tolerant, moderately tolerant, or susceptible. Principal Component Analysis (PCA) identified seedling length and dry weight as key contributors to variation, while MGIDI analysis improved the efficiency of multivariate selection, identifying superior genotypes under both control and stress. The integration of physiological parameters with the Salt Tolerance Index (STI) served as an effective screening method for categorising genotypes into tolerant and susceptible groups. These findings highlight the importance of using a reliable screening protocol using STI for successful soybean cultivation under salinity stress conditions.

Key words: Germination, MGIDI, NaCl, PCA, Salinity stress, Salt tolerance index, Soybean.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is a self-pollinated crop that belongs to the family Leguminosae and is believed to have originated in eastern Asia and is native to China. In India, soybean has surpassed other major oilseed crops and has been cultivated for centuries under different names (Dupare *et al.*, 2008). In the 2023-24 crop season, global soybean production reached a record 399 million tons, representing a significant increase of 10.8% compared to the previous year's 370 billion tons, with the US, Brazil, Argentina, China and India being the leading producers of soybean in the world. In India, soybean was cultivated on an area of 123.85 lakh hectares, with a total production of 130.62 lakh tonnes, with Madhya Pradesh, Maharashtra, Rajasthan, Karnataka and Telangana being the top five soybean-producing states in India. Despite technological advancements in agriculture, global food security is increasingly threatened by climate change, which exerts significant pressure on India's limited land and water resources (Godfray *et al.*, 2010). In India, about 147 million hectares of land are affected by soil degradation, of which

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nearly 23 million hectares suffer from salinity, alkalinity, or acidification, posing a major constraint to agricultural sustainability (Bhattacharyya *et al.*, 2015). Around 6.74 million hectares are salt-affected, accounting for about 2.1% of the country's geographical area, including 2.95 million hectares of saline soils and 3.77 million hectares of sodic

soils. It is estimated that by 2050, nearly 50% of arable land will be salt-affected (Kumar and Sharma, 2020). In South Asia, around 52 million hectares of land are affected by salt (Mandal *et al.*, 2018). Osmotic stress caused by salinity disrupts several physiological processes, including nutrient balance, membrane function and the ability to detoxify reactive oxygen species (Essa, 2002).

Soybean is considered moderately salt-sensitive crop. Elevated salt levels exert toxic effect on the embryo, adversely influencing several aspects of plant development. Salinity reduces germination percentage, root length, seedling growth and callus formation (Agnihotri *et al.*, 2006; Munns and Tester, 2008 and Ramana *et al.*, 2012). Screening of salt-tolerant genotypes for enhanced yield is a key objective for plant breeders and agronomists, but it is often costly and time-consuming. However, assessing salt tolerance at the early stages of plant development can significantly save time and reduce costs. This is particularly relevant if the degree of salt tolerance at the early stage of development is correlated with other stages of growth and yield (Hu *et al.*, 2005). The productivity of crops, including soybean, is critically determined by germination and early growth stages. The germination stage is particularly sensitive to salinity and is often used for selecting salinity tolerance in plants (Çirka *et al.*, 2021). Seeds that exhibit rapid and normal germination in saline conditions are expected to be tolerant to salinity (Bybordi and Tabatabaei, 2009). However, limited work has been performed to screen the existing soybean germplasm accessions as well as a few promising varieties for salinity tolerance. Therefore, the present study aimed to decipher the genetic relationship between selection indices such as the salt tolerance index (STI), yield, morpho-physiological traits and seed quality parameters. The outcomes will contribute to improving the efficiency of breeding programmes under saline environments and suggest an effective screening protocol for soybean.

MATERIALS AND METHODS

Seeds of 156 soybean germplasm accessions, comprising one-year stored seeds of 150 accessions (Table 1) and six freshly harvested varieties with yellow seed coat colour, viz. Pusa 9712, Pusa 12, SL 958, PS 1347, PS 24 and SL 1074 were procured from the Division of Genetics, ICAR-IARI, New Delhi and evaluated for salinity studies during the kharif season of two consecutive years, 2022 and 2023. The experiment was conducted in the Division of Seed Science and Technology, ICAR-IARI, New Delhi. The 150 accessions were categorized into three groups based on seed coat colour, viz., yellow-seeded (105 accessions), black-seeded (42 accessions) and brown-seeded (3 accessions). The study was broadly divided into two groups: (i) freshly harvested six released varieties and (ii) one-year stored seeds of 150 accessions. Among the 105 yellow-seeded accessions, only 84 accessions were considered for further evaluation, as the remaining failed to germinate due to reduced viability after storage. The classification by seed

coat colour was done to account for the known variability in seed longevity associated with pigmentation (Kuchlan *et al.*, 2010).

A standardisation experiment was conducted under laboratory conditions on three randomly selected genotypes using two techniques, viz. top-of-paper (petri plate) method and standard germination testing using the between-paper method. Four different NaCl solutions with salinity levels of 0, 3, 6 and 9 dS/m were used for the experiment. For the top-of-paper method, 15 cm diameter sterile petri dishes were lined with Whatman No.1 filter paper and soaked with 10 ml of distilled water (control) and NaCl solutions (3, 6 and 9 dS/m). Each petri dish contained 20 uniform seeds of soybean cultivars, arranged equidistantly in a randomised design with three replications. To standardise the top-of-paper method, three techniques were evaluated for the first time in soybean: (i) alternate day application of 2.5 ml solution, (ii) a single application of 10 ml solution at the beginning and (iii) alternate day application of 10 ml solution until the end of the experiment. For germination testing using between-paper method, the International Seed Testing Association (ISTA) recommended procedure was followed. Germination paper was soaked with distilled water (control) and NaCl solutions (3, 6, 9 dS/m) and seeds were placed between folds of paper before rolling. To assess the response of the soybean cultivars under different NaCl concentrations, seeds were placed on germination paper and the effect of salinity on various seed quality parameters viz. germination percentage, seedling length (cm), seedling fresh weight (mg) and seedling dry weight (mg) was observed on the final day of germination. Seedling vigour indices were calculated as per the standard procedure of Abdul-Baki and Anderson (1973) for all four salinity treatments. Seedling Vigour Index-I (SVI-I) was obtained by multiplying the germination percentage with the mean of total seedling length (cm) and Seedling Vigour Index-II (SVI-II) was determined by multiplying the germination percentage with the mean seedling dry weight (mg). Salt Tolerance Index (STI) was calculated as the ratio of total plant (shoot + root) dry weight obtained from 10 seeds grown under different salt concentrations (3, 6 and 9 dS/m NaCl) to the total plant dry weight obtained under control conditions (0 dS/m NaCl) (Khatun *et al.*, 2013).

RESULTS AND DISCUSSION

Standardization of protocol for salinity stress evaluation

The top-of-paper method was found superior to the between-paper method for evaluating seed quality parameters under varied salinity stress levels (0 to 9 dS/m), due to several advantages, including ease of handling, precise application of salt solutions at required concentrations and practical in execution. Among the different protocols tested for the top-of-paper method, the addition of 2.5ml NaCl solution every alternate day was proved to be most effective, maintaining adequate moisture without water stagnation during germination testing.

Table 1: List of germplasm accessions categorised into varied seed coat colours.

S.no.	Acc.no	Pedigree	Yellow	S.no.	Acc.no	Pedigree	Yellow	S.no.	Acc.no	Pedigree	Black
1	1	AVRDC-5	Y	56	108	JS-90-29	Y	106	3	BJ-1	BL
2	2	AVRDC-516	Y	57	111	JS-93-29	Y	107	4	BJJF-8	BL
3	10	DS-172	Y	58	112	JS-CSH/91-16	Y	108	5	DS-61	BL
4	11	DS-37-3-8	Y	59	113	JS-94-23	Y	109	8	DS-241	BL
5	12	DS-74-37	Y	60	115	KG-83-1	Y	110	9	DS-371	BL
6	13	DS-76-1-3	Y	61	116	KG-831-A	Y	111	14	DS-76-1-2-2	BL
7	16	DS-9802	Y	62	117	KHSb-3	Y	112	15	DS-76-1-2-3	BL
8	17	DS-76-35-4	Y	63	118	KHSb-6	Y	113	18	DS-76-37-2	BL
9	20	DS-76-1-2-1	Y	64	143	MAUS-311	Y	114	19	DS-76-37-3	BL
10	23	DS-93-108A	Y	65	145	NRC-7	Y	115	28	EC-109514	BL
11	24	DS-91-3	Y	66	147	NRC-21	Y	116	31	EC-113396	BL
12	25	DS-9703	Y	67	152	PK-1169	Y	117	34	EC-114526	BL
13	27	DSb-2-Y	Y	68	153	PK-1180	Y	118	35	EC-252720	BL
14	29	EC-109565	Y	69	154	PK-1197	Y	119	36	EC-75758	BL
15	30	EC-11-2897	Y	70	155	PK-1223	Y	120	42	EC-439599	BL
16	33	EC-113398-8	Y	71	156	PK-1240	Y	121	57	EC-39779	BL
17	37	EC-1137	Y	72	157	EC-389148	Y	122	64	EC-98299	BL
18	38	EC-3891796	Y	73	159	PK-1042	Y	123	67	G-267	BL
19	39	EC-389392	Y	74	160	PK-292	Y	124	69	G-395	BL
20	40	EC-390981	Y	75	162	PK-1225A	Y	125	71	G-2215	BL
21	43	EC-439608	Y	76	163	PK-1241	Y	126	73	G-2265	BL
22	44	EC-439618	Y	77	164	PK-1135	Y	127	74	G-2344	BL
23	45	EC-439619	Y	78	165	Bragg	Y	128	75	G-26-1	BL
24	46	EC-44303	Y	79	169	S-1	Y	129	76	G-2602	BL
25	47	EC-13969	Y	80	171	SL-46	Y	130	79	G-2651	BL
26	48	EC-14427	Y	81	183	PK-1084	Y	131	80	G-2656	BL
27	49	EC-85286	Y	82	662	TS-56	Y	132	81	G-2638	BL
28	51	EC-34-3301 A	Y	83	679	PS-1450	Y	133	82	G-2670	BL
29	52	EC-28635-A	Y	84	680	DS-2614	Y	134	83	G-2685	BL
30	53	EC-1001	Y	85	688	VLS-75	Y	135	84	G-3023	BL
31	54	EC-50082	Y	86	715	VLS-85	Y	136	98	G-390	BL
31	55	EC-14436	Y	87	724	AMS-MB-5-19	Y	137	99	IC-141446	BL
33	56	EC-1023	Y	88	730	DS-27-11	Y	138	114	KALITUR	BL
34	58	EC-30221	Y	89	749	PS-1503	Y	139	119	L-416	BL
35	59	EC-34141	Y	90	750	Himso-1681	Y	140	120	L-440	BL
36	60	EC-39873	Y	91	751	KDS-701	Y	141	121	L-587	BL
37	61	EC-97351	Y	92	793	NRC-92	Y	142	122	L-52	BL
38	62	EC-105790	Y	93	813	DS-3047	Y	143	161	PK-7427B	BL
39	63	EC-18761	Y	94	814	JS-20-79	Y	144	166	DS-76-1-20-1	BL
40	65	EC-80064	Y	95	815	AMCS-1419	Y	145	167	PL50-6A	BL
41	68	IC-144409	Y	96	860	PS-1569	Y	146	168	PL50-79	B
42	70	G-2133	Y	97	877	DS-3104	Y	147	170	PL50-91	BL
43	85	G-2126	Y	98	878	SL-1569	Y				
44	87	G-2130	Y	99	896	DS-3105	Y	S.no	Acc.no	Pedigree	Brown
45	88	G-2132	Y	100	897	SL-1104	Y	148	7	DS-201	BR
46	89	G-2144	Y	101	906	MAUS-711	Y	149	32	EC-113397	BR
47	91	HIMSO-1574	Y	102	907	N50-626	Y	150	72	G-2252	BR
48	93	IC-24069	Y	103	908	AMS-MB5-19	Y				
49	94	IC-81655	Y	104	910	RSC-10-71	Y				
50	95	IC-101449	Y	105	934	SL-1123	Y				
51	101	J-231	Y								
52	103	JS-76-257	Y								
53	104	JS-81-303	Y								
54	105	JS-81-607	Y								
55	106	JS-91-4	Y								

Analysis of variance for seedling quality parameters

The statistical analysis (ANOVA) revealed that treatment, accession and their interaction exerted highly significant effects ($p < 0.001$) on all measured traits, including germination percentage, seedling length, fresh and dry weight (Table 2). These results underscore the strong influence of salt stress on seedling performance. The significant accession $\times$ treatment interaction further indicates that responses to salinity vary widely among accessions, reflecting a complex genetic basis for salt tolerance and emphasizing the role of both genetic background and environmental conditions for stress responses.

Germination percentage declined progressively with increasing salinity across all genotype groups (Fig 1A-1D). At 0 dS/m (control), black-seeded accessions recorded a mean germination of 75.51%, which declined to 67.81% at 3 dS/m, 52.77% at 6 dS/m and 36.46% at 9 dS/m. In contrast, yellow-seeded accessions recorded markedly lower germination values of 32.32%, 27.11%, 15.06% and 7.23% at 0, 3, 6 and 9 dS/m, respectively confirming that genotypes with yellow seed coat color were poor storers compared to black-seeded genotypes. For brown-seeded accessions, germination percentages were 68.33%, 56.66%, 38.33% and 23.33% at 0, 3, 6 and 9 dS/m, respectively. Similarly, for released varieties, values were 91.66%, 86.66%, 66.66% and 45.00% at corresponding salinity levels. Variation in germination percentage among genotypes may be attributed to differences in genetic makeup, metabolic activity and enzyme regulation (Bojovic *et al.*, 2010; Kondetti *et al.*, 2012 and Kandil *et al.*, 2016).

A similar trend was observed for seedling length, fresh weight and dry weight, all of which decreased linearly with increasing salinity from 0 to 9 dS/m. This reduction may be attributed due to the osmotic stress limiting water uptake, restricted elongation and reduced enzyme activity involved in reserve mobilization from seeds to seedlings (Kandil *et al.*, 2012; Kondetti *et al.*, 2012; Ayed *et al.*, 2014 and Tesfaye *et al.*, 2014). Interestingly, few genotypes recorded highest germination percentage, seedling length and shoot and root dry weights at 3 dS/m, as low concentrations of NaCl may act as a nutrient, improving water uptake, promoting elongation, enhancing ion uptake, or facilitating better reserve mobilization under mild stress (Essa, 2002; Tamura and Chen, 2009 and Gogile *et al.*, 2013). Both Seedling Vigour Index-I (SVI-I) and Seedling Vigour Index-II (SVI-II) followed the same declining trend with increasing salinity across all genotypic groups. The variation in vigour indices among soybean genotypes reflected differential genotypic responses to salinity stress, with the lowest values of SVI-I and SVI-II recorded at the highest salinity level (9 dS/m).

Formulation of screening criteria for selection of tolerant and susceptible genotypes

Earlier studies have reported that seedling dry weight, which is the ultimate result of all physiological and biochemical

Table 2: Analysis of variance for seedling vigour and seed quality parameters.

SV	Df	SL	RL	TSL	SDW	RDW	TSDW	G%	SFW	RFW	TSFW	SR_ratio
Treatment	3	1793.69***	1720.46***	7006.8***	5209.3***	1184.79***	12520***	62005***	605044***	110577***	1232568***	10.7303***
Replication	2	2.68***	0.99***	6.3***	68***	22.52***	183.4***	848***	1079***	502***	3010***	0.0571
Accession	149	50.99***	73.9***	238.1***	178.5***	48.76***	606.4***	6004***	18014***	4885***	40159***	3.1813***
Treatment:Accession	447	5.84***	8.88***	25.7***	17.4***	5.48***	61.8***	234***	2335***	525***	4433***	0.6438***
Residuals	1198	0.11	0.09	0.20	0.70	0.18	1.20	16.00	23.00	9.00	33.00	0.03

[SL: Shoot length (cm), RL: Root length (cm), TSL: Total seedling length (cm), SDW: Shoot dry weight (mg), RDW: Root dry weight (mg), TSDW: Total seedling dry weight (mg), G%: Germination percentage, SFW: Shoot fresh weight (mg), RFW: Root fresh weight (mg), TSFW: Total seedling fresh weight (mg), SR_ratio: Shoot to root ratio].

activities in plant, is markedly reduced under high salt concentrations (Akbarimoghaddam *et al.*, 2011 and Rumena, 2006). Physiological and seed quality parameters, along with their relationship to salt tolerant indices, have been proved as an applicable tool for selecting salt-tolerant genotypes (Kagan *et al.*, 2010; Khatun *et al.*, 2013 and Putri *et al.*, 2022). For black seeded genotypes, the present study revealed a progressive decline in the Salt Tolerance Index (STI) with increasing salinity (0–9 dS/m). At 6 dS/m, the highest STI value was 90.19% in accession no. 80, while the lowest was 36.43% in accession no. 71. At 9 dS/m, accession no. 3 recorded the highest STI (71.69%), whereas accession no. 9 recorded the lowest (23.81%) (Fig 2A). Similarly, for yellow and brown seeded accessions, the STI averaged across all genotypic treatments decreased with an increase in the salinity level from 0 - 9 dS/m (Fig 2B and 2C). Among freshly harvested varieties, the highest STI value at 6 dS/m was 74.55% in variety Pusa 9712, while the lowest was 56.78% in SL 958. At 9 dS/m, Pusa 9712 again recorded the highest value (48.60%), whereas SL 958 had the lowest (28.50%) (Fig 2D).

Hence, based on the significant correlation of STI with several seed quality traits, genotypes were categorized into three groups: highly tolerant, moderately tolerant and highly susceptible. At salinity level of 6 dS/m, seed quality parameters (germination percentage, SVI-I, SVI-II and related traits) showed a positive correlation with STI for both black-seeded accessions and released varieties (Fig 3 and 4), whereas in yellow-seeded accessions, significant correlations were recorded at 3 dS/m (Fig 5).

In contrast, no significant correlations were detected for brown-seeded accessions at any salinity level; therefore, no categorization was made for this group. Further selection of tolerant and susceptible genotypes within the three groups: black-seeded accessions, yellow-seeded accessions and released varieties was carried out based on STI values at their respective salinity levels. In total, 20 genotypes were selected: 7 black-seeded, 7 yellow-seeded and all 6 released varieties. The selection was carried out at 3 dS/m for yellow-seeded accessions and at 6 dS/m for black-seeded accessions and released varieties. The calculated STI values were then arranged in increasing

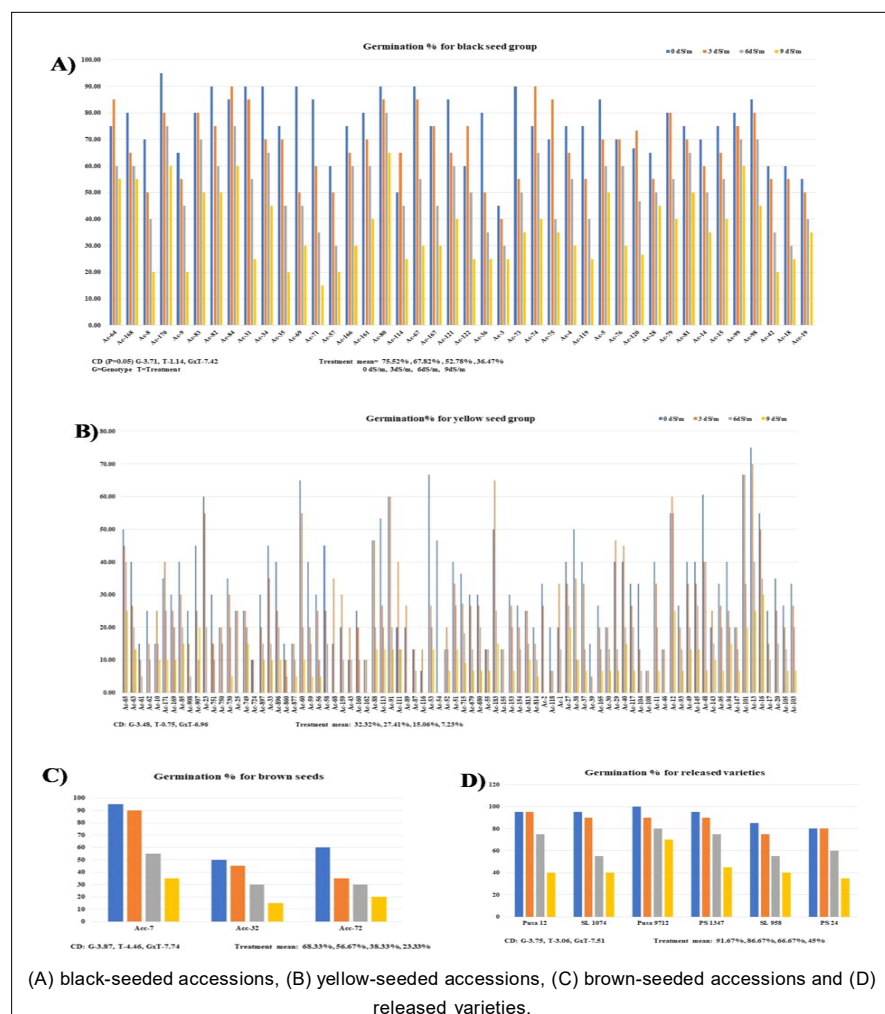


Fig 1: Germination percentage.

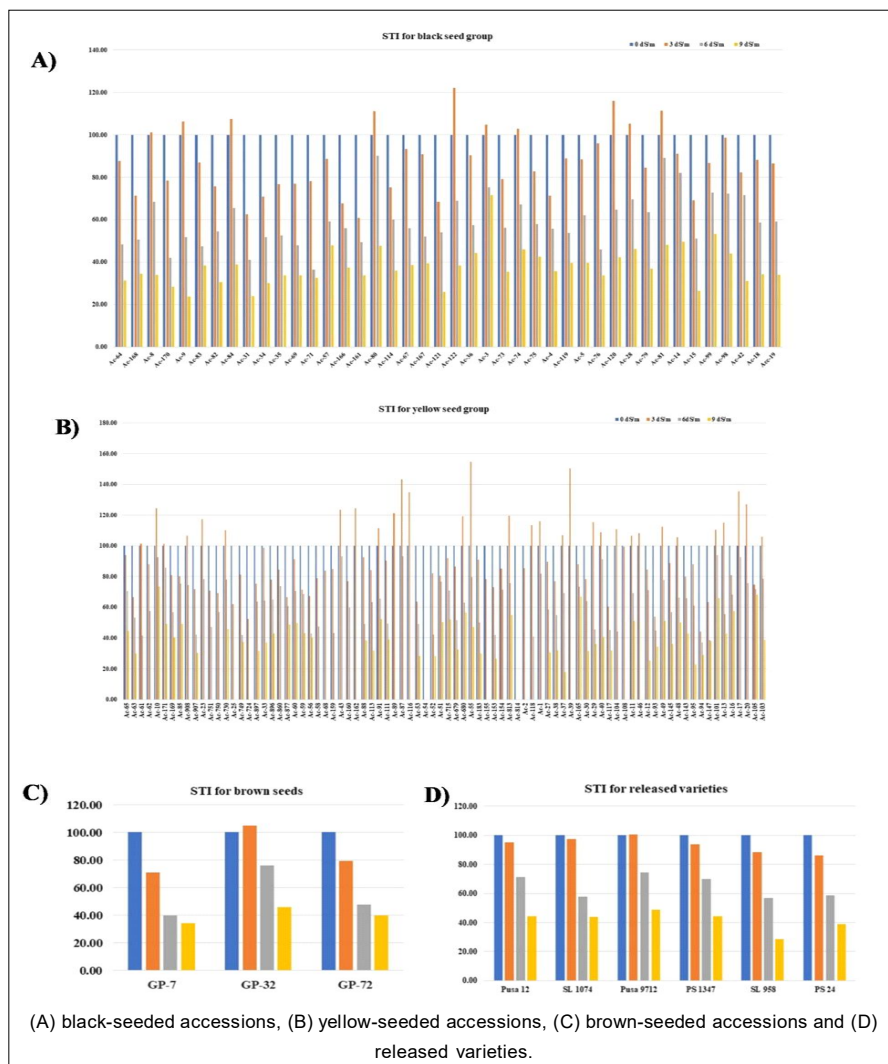


Fig 2: Salt tolerance index (STI).

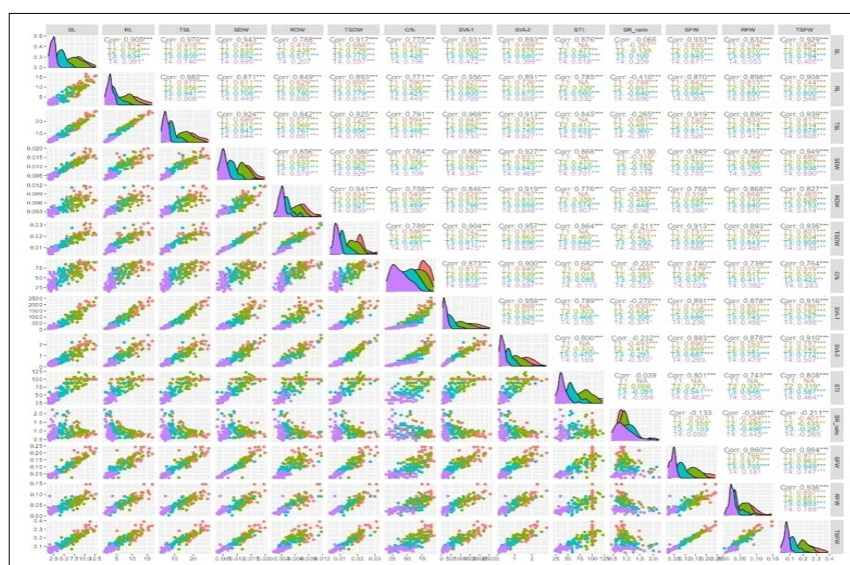


Fig 3: Correlation values of black genotypes at 6 dS/m salinity level.

order and the genotypes were classified into three categories based on their ability to withstand salinity stress: highly tolerant, moderately tolerant and highly susceptible (Table 3 and Fig 6-8).

Correlation analysis of seed quality traits and salt tolerance index

Principal component analysis (PCA)

PCA (Fig 9) revealed that the first principal component explained 79% of the variance, largely driven by shoot

length, root length, total seedling length and seedling dry weight. The second component explained an additional 8.8%. The clustering of these traits indicates their interrelated roles in salinity tolerance. Control treatments clustered separately from salinity treatments, reflecting baseline physiological responses. Similar clustering of stress-related traits has been reported in drought and salinity studies (Lee *et al.*, 2017; Gupta *et al.*, 2020). These results suggest that PCA is effective in identifying key traits contributing to variation among genotypes under salinity

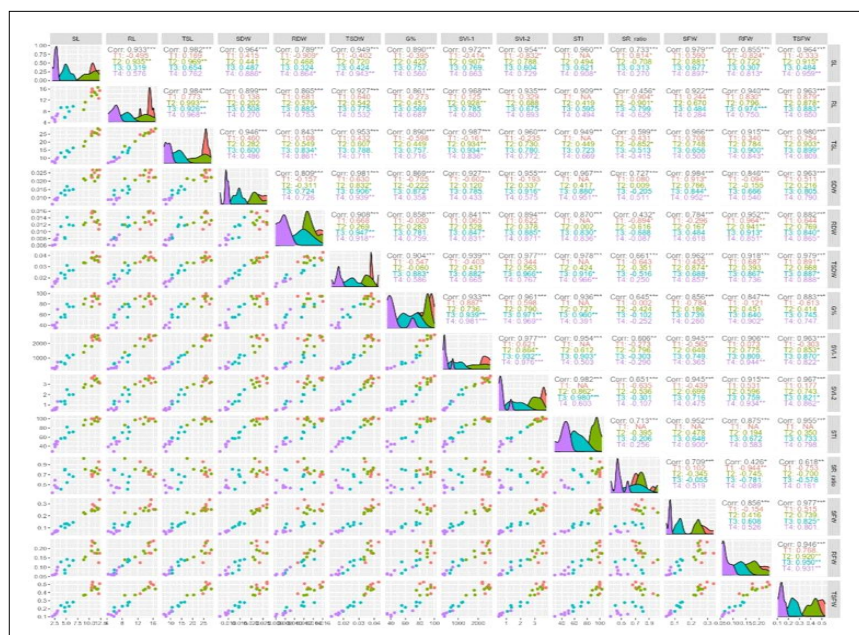


Fig 4: Correlation values of released varieties at 6 dS/m salinity level.

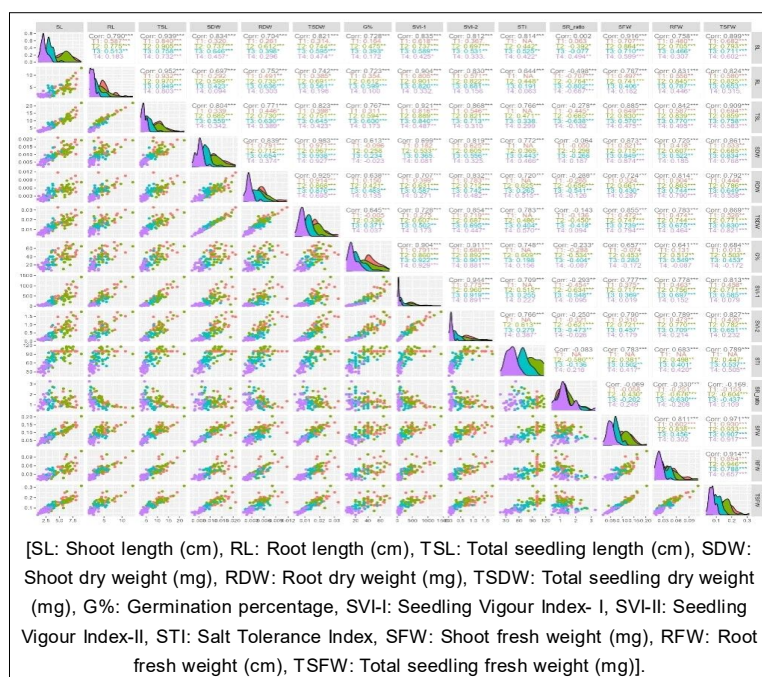


Fig 5: Correlation values of yellow genotypes at 3 dS/m salinity level.

stress. Traits that maintain their position closer to the control across increasing salinity levels might be more tolerant and hence valuable for breeding programs.

Multivariate selection using MGIDI

The Multi-Trait Genotype-Ideotype Distance Index (MGIDI) was employed to rank genotypes under control and salinity

stress. By integrating multiple seedling traits, MGIDI enhanced selection efficiency and identified genotypes resilient under both optimal and stress conditions. A total of 156 genotypes were assessed for seed quality parameters under both normal and salt stress conditions for selecting the genotypes with high vigour. This approach identified eight superior soybean genotypes in each condition,

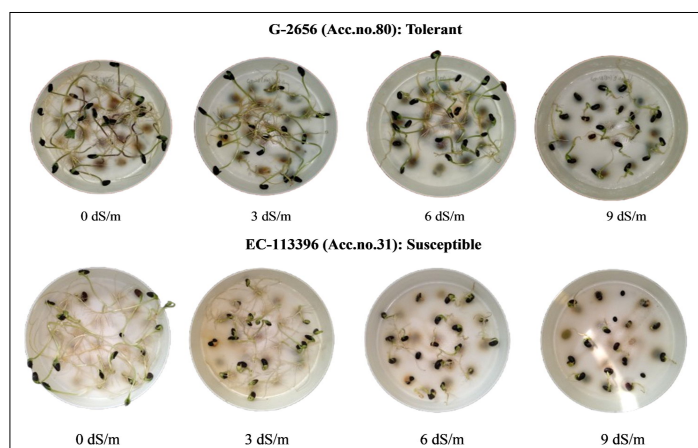


Fig 6: Effect of salinity stress (0-9 dS/m) on seed germination of black-seeded genotypes.

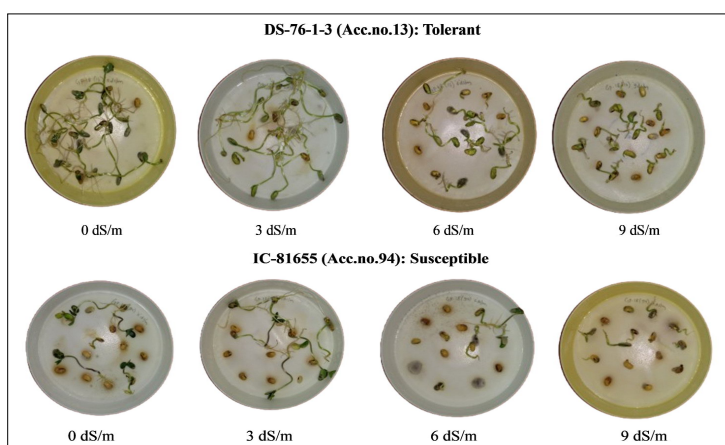


Fig 7: Effect of salinity stress (0-9 dS/m) on seed germination of yellow-seeded genotypes.

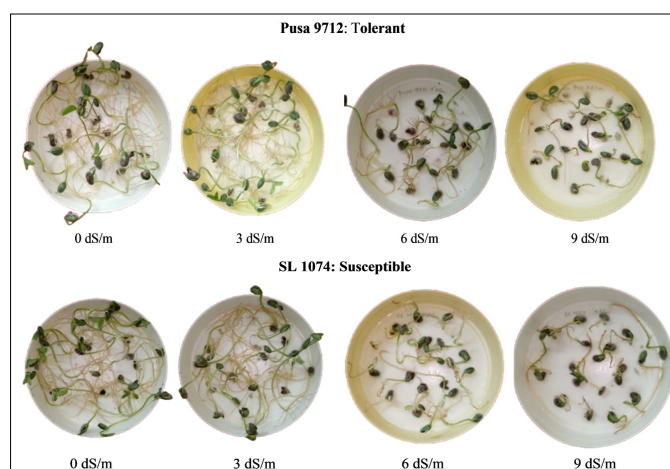


Fig 8: Effect of salinity stress (0-9 dS/m) on seed germination of released varieties.

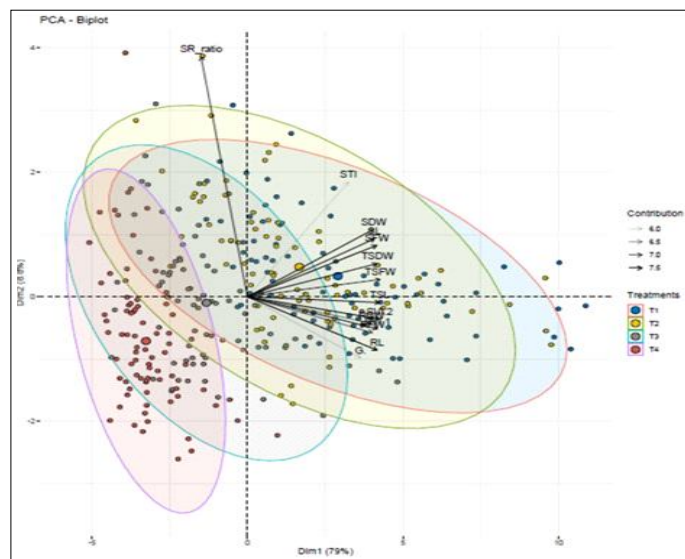


Fig 9: Principal component analysis identifying crucial traits affecting genetic diversity in response to salinity stress.

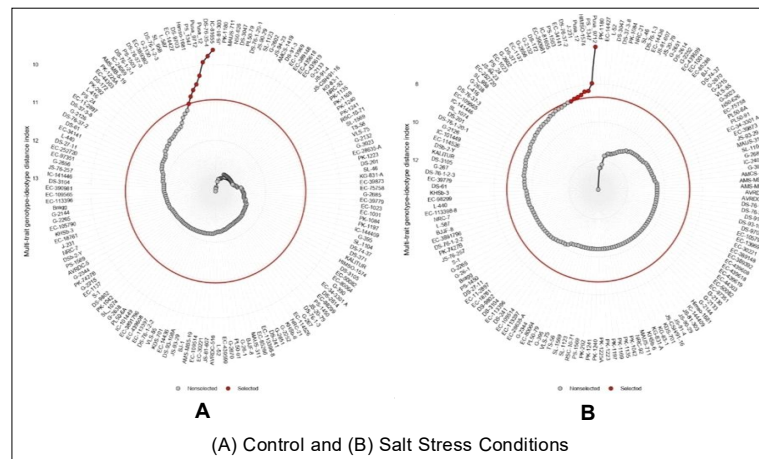


Fig 10: Multi-trait genotype-ideotype distance index (MGIDI) of soybean genotypes for seedling quality and vigour parameters under both.

highlighting varieties such as IC-81655, DS-76-35-4, Pusa - 12, Pusa-9712, PS-1347, Himso-1681, DS-9703 and EC-14427 under control conditions and Pusa-9712, PS-1347, HIMSO-1574, Pusa-12, J-231, DS-76-37-2, EC-34141 and PS-1503 under salt stress (Fig 10). Genotypes near the cut-off threshold also displayed potential for tolerance and for further evaluation.

CONCLUSION

The present study standardised a reliable protocol for salinity stress evaluation in soybean, with the top-of-paper method using alternate-day application of 2.5 ml NaCl solution. Salinity stress significantly reduced germination, seedling length, vigour indices and biomass traits, with greater reduction at higher salinity levels. The Salt Tolerance Index (STI) emerged as a robust criterion for categorising genotypes into highly tolerant, moderately tolerant and highly susceptible groups. Black-seeded accessions and released varieties correlated strongly with STI at 6 dS/m,

while yellow-seeded accessions showed significant associations at 3 dS/m and brown-seeded accessions showed no consistent response. Multivariate selection using MGIDI improved efficiency in identifying superior genotypes under saline conditions. Overall, the study demonstrated wide genetic variability for salinity tolerance in soybean. The identified tolerant genotypes provide valuable material for breeding programs. This integrated approach offers a practical framework for improving soybean resilience under saline environments.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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