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Validation of Phenotypic Response of Black Gram (*Vigna mungo* (L.) Hepper) Genotypes for Salinity Tolerance at Seedling Stage under Hydroponics System

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Salinity is an important environmental constraint that adversely affects seed germination, seedling establishment, and subsequent growth of black gram (*Vigna mungo* (L.) Hepper). Identification of genotypes capable of maintaining satisfactory growth under saline conditions is therefore important for developing suitable breeding material for salt-affected environments. A hydroponic screening for salt tolerance in black gram at the seedling stage was conducted in the Plant Breeding and Genetics Laboratory at Adhiparasakthi Agricultural College, Kalavai, Ranipet, from March to July 2026. Seedling-stage screening is simple, rapid and efficient. The present investigation was carried out using seven black gram genotypes in a completely randomized design (CRD) with two replications. Observations were recorded for seven genotypes for salinity-tolerance traits, *viz.,* germination percentage (%), root length (cm), shoot length (cm) and seedling vigour index, under different salinity levels imposed using NaCl (control and EC 4, 11 and 16 dS/m). Data were analyzed using R software version 4.6.0. Analysis of variance showed that genotype, salinity treatment and their interaction significantly affected germination, root length and shoot length. The black gram genotypes *viz.,* VBN 8 and VBN 10 performed best for most of the characters. The black gram genotypes *viz.,* PU 31 and T9 showed the poorest performance for germination percentage (%), root length (cm), shoot length (cm) and seedling vigour index at a salinity level of EC 16 dS/m. Increasing salinity stress adversely affected germination percentage (%), root length (cm), shoot length (cm) and seedling vigour index in all black gram genotypes. However, VBN 8 and VBN 10 consistently performed better under saline conditions, demonstrating greater salt tolerance. Hence, these genotypes can be used as checks for future screening and breeding programmes aimed at developing salinity-tolerant varieties of black gram.

Keywords: Black gram; hydroponics; salinity stress; seedling-stage screening; germination percentage; seedling vigour index.

1. Introduction

Black gram [*Vigna mungo* (L.) Hepper] is a self-pollinated, short-duration crop that was domesticated from *Vigna mungo var. silvestris* (Chandel *et al.,* 1984). It fixes 42 kg of nitrogen per hectare per year and reduces input costs for farmers. Black gram is a key protein source in cereal-based diets. It is easy to digest and does not cause flatulence. Black gram is cultivated on 71% of the land area in Tamil Nadu (Rajasekar *et al*., 2022). Black gram is one of the important legume crops grown throughout India. Black gram contains protein (25%), carbohydrates (60%) and fat (1.3%) and is rich in phosphoric acid. It accounts for 13% of the total pulse area and 10% of pulse production in India. It improves soil fertility by fixing atmospheric nitrogen in the soil. As a pulse crop, black gram is grown in all types of soil, ranging from sandy loam to heavy clay, except alkaline and saline soils (Sheeba *et al*., 2025). About 6% of cultivable land worldwide is affected by salinity, while around 54% of soil is affected by sodicity. Researchers classify soil salinity as primary (natural) or secondary (human-made). These two causes increase salinity by 10% per year. More than half of all arable land could become salinized by 2050 (Jamil *et al*., 2011; Parihar *et al*., 2015; Pirasteh-Anosheh *et al*., 2016; Sahab *et al.,* 2021; Shanthi *et al*., 2024). It severely harms vegetation, biodiversity and soil fertility, consequently leading to the desertification of productive arable land (Gad *et al*., 2022). Salt-affected soils are generally divided into two main types, saline and sodic soils, based on factors such as soil pH, electrical conductivity (EC) of the saturation extract, exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) (Abrol *et al*., 1980). Saline soils contain elevated concentrations of soluble salts, primarily sodium-based compounds such as chlorides, sulphates and nitrates. These soils typically have an EC above 4 dS/m, a pH below 8.5 and an ESP below 15. In contrast, sodic soils contain high amounts of exchangeable sodium, especially sodium carbonate, which leads to poor soil structure. Sodic soils usually have a pH above 8.5, an ESP greater than 15 and an EC below 4 dS/m. Salinity stress affects photosynthesis mainly by reducing leaf area, chlorophyll content and stomatal conductance, and to a lesser extent by decreasing photosystem II efficiency (Cassaniti *et al.,* 2012; Jamil *et al.,* 2006). In pulses under hydroponic conditions and salt stress, reductions in shoot length, root length, root-to-shoot ratio, development and survival were observed compared with the control (Punithavathy *et al*., 2025).

Salinity tolerance at germination and early seedling stages is particularly important in grain legumes because osmotic stress initially restricts water uptake and seedling establishment, whereas prolonged exposure can intensify ionic imbalance and oxidative injury. Therefore, early-stage screening under controlled hydroponic or solution-culture conditions is widely used to identify genotypic differences before field evaluation (Fellahi *et al*., 2024; Munns & Tester, 2008; Nadeem *et al*., 2019; Uzair *et al*., 2022; van Zelm *et al*., 2020). In black gram, previous studies have shown that increasing salinity reduces germination, radicle and plumule growth, dry matter accumulation, and seedling vigour, but the magnitude of reduction differs among genotypes. Such variation supports the use of morpho-seedling traits as practical selection criteria for identifying relatively tolerant breeding material (Gandhi *et al*., 2021; Hasan *et al.,* 2018; Kapoor & Srivastava, 2010; Priyadharshini *et al*., 2019; Shanthi et al., 2021). Additional evidence in black gram indicates that early growth traits remain responsive to salinity and can also reflect the effects of salinity-mitigating treatments (Donia *et al.,* 2020; Nagaraju *et al*., 2020; Shanti *et al.,* 2014; Singh *et al*., 2009; Win & Oo, 2017).

Comparable seed-germination evidence in mung bean (*Vigna radiata* L.) shows that increasing salinity can reduce germination and delay germination, further supporting early-stage screening within the genus (El-Kafafi *et al*., 2015; Sahoo *et al*., 2026). Recent black gram research under saline conditions further demonstrates that root development, growth, and nodulation can respond markedly to salinity, confirming that multiple seedling attributes contribute to the observed stress response (Tiwari *et al.,* 2025). A recent synthesis across *Vigna* species also emphasizes that salinity tolerance is growth-stage dependent and involves coordinated responses in germination, seedling growth, nutrient relations, and physiological stability (Suriya & Selvaraj, 2026).

Objective: The objective of the present study was to evaluate the phenotypic response of seven black gram genotypes to salinity at the seedling stage under hydroponic conditions using germination percentage, root length, shoot length, and seedling vigour index, and to identify comparatively tolerant genotypes for subsequent screening and breeding work.

2. Materials and Methods

The present investigation was carried out using seven black gram genotypes in a Completely Randomized Block Design (CRBD) with two replications. These genotypes were collected from the ADA office, Vellore. The evaluation of the phenotypic response of black gram genotypes for salinity tolerance at the seedling stage under a hydroponic system (Kumar *et al.,* 2024; Monisha *et al.,* 2023) was conducted in the Plant Breeding and Genetics Laboratory at Adhiparasakthi Agricultural College, Kalavai, Ranipet, from March to July 2026 (Table 1). Observations were recorded for seven genotypes for salinity-tolerance traits, *viz.,* germination percentage (%), root length (cm), shoot length (cm) and seedling vigour index, under different salinity levels imposed using NaCl (control and EC 4, 11 and 16 dS/m). All experiments were conducted using a randomized complete block design with two replications.

Table 1. List of black gram genotypes

S. No	Black gram Genotypes	Origin	Duration	Special features
1	VBN 6	NPRC, Vamban, TN, India	65–70 days	High-yielding, short-duration, Susceptible check to salinity.
2	VBN 8	NPRC, Vamban, TN, India	65-70 days	Moderate tolerance to salinity stress, highly resistance to YMV
3	VBN 9	NPRC, Vamban, TN, India	70-75 days	Susceptible to salinity, High yielding, Suitable for Rice-fallow pulses.
4	VBN 10	NPRC, Vamban, TN, India	70-75 days	Moderate tolerance to salinity, non-hairy pods, Synchronized maturity
5	VBN 11	NPRC, Vamban, TN, India	70-75 days	Susceptible to salinity stress, Highly Resistant to MYMV, moderately resistant to powdery mildew.
6	PU-31	G.B. Pant University of Agriculture and Technology, Pantnagar, India	75-85 days	Susceptible to salinity stress, short duration, moderately resistant to YMV
7	T-9	Local selection at Bareilly, UP, India	65-75 days	Susceptible to salinity, moderately resistant to powdery mildew

2.1 Screening Protocol

The screening used the IRRI protocol for the experimental procedure and nutrient preparation (Gregorio *et al.,* 1997; Punithavathy *et al*., 2025). Styrofoam sheets with dimensions of 22 × 22 × 3 cm were used to create the experimental setup. One hundred and forty identical holes were drilled at uniform spacing (Fig. 1). Sterilized seeds were germinated using the Petri plate method for three days. Healthy seedlings were then transferred to the Styrofoam sheets placed on Yoshida nutrient solution. One seedling was placed in each hole without damaging the roots. A total of 20 seedlings per genotype per replication were maintained. Eighteen days after sowing, the true leaf emerged (the three-leaf stage). Salt stress was imposed in the Yoshida nutrient solution using different concentrations of NaCl. Initial screening started at an electrical conductivity (EC) level of 4 dS/m, and EC was increased to 11 and 16 dS/m. The study adopted three EC levels of 4, 11 and 16 dS/m to determine a critical level.

2.2 Data Collection

On the 7th day after salinity treatment, the following characters were measured: germination percentage, shoot length (cm), root length (cm) and seedling vigour index.

2.3 Statistical Analysis

Analysis of variance was carried out for each black gram genotype. Data were analyzed using R software version 4.6.0.

Scoring visual symptoms of salinity stress: Reduced leaf area, whitening of lower leaves, leaf-tip death and leaf rolling were recorded. The salinity-screening technique is based on the ability of seedlings to grow in salinized nutrient solutions. Standard genotypes are included in each test tank for comparison. Scoring is relative and is carried out according to the IRRI standard evaluation system, with a score of 1 indicating tolerance and 9 indicating sensitivity. Scoring is carried out on or around day 12 of salt treatment. At this stage, sensitive seedlings begin to die, whereas intermediate genotypes show varying degrees of tolerance (Table 2).

Table 2. Salinity scoring using IRRI Classification

Score	Tolerance Category	Plant Symptoms	Varieties
1	Highly Tolerant	Normal growth, no visible salt injury	VBN 8 & VBN 10
3	Tolerant	Nearly normal growth, but some leaf-tip discoloration or slight rolling.	VBN 6
5	Moderately tolerant	Growth severely retarded, most leaves rolled and starting to dry.	VBN 11
7	Susceptible	Complete cessation of growth, most leaves dried, few new leaves.	VBN 9 & PU31
9	Highly susceptible	Almost all plants dead or dying	T9

3. Results and Discussion

Analysis of variance indicated significant variation among the genotypes for all traits studied for salinity tolerance. The experiment followed a Completely Randomized Block Design (CRBD) with two replications. The ANOVA and treatment means were compared using Tukey's honestly significant difference (HSD) test at the 5% significance level. Analysis of variance showed that genotype, salinity treatment and their interaction significantly affected germination, root length and shoot length. For seedling vigour, genotype and treatment effects were significant, while the genotype × treatment interaction was non-significant (Tables 3, 3a, 3b, 3c and 3d). The significant genotype, treatment and genotype × treatment effects for germination, root length and shoot length indicate that salt response was not uniform across the tested materials. This pattern is consistent with the concept that salinity tolerance is a genotype-dependent and trait-dependent response governed by osmotic adjustment, ion exclusion, tissue tolerance and maintenance of growth under stress (Munns & James, 2003; Munns & Tester, 2008; van Zelm *et al.,* 2020).

The non-significant genotype x treatment interaction for seedling vigour index suggests that, although genotypes and salinity levels differed in their main effects, the relative response pattern for this composite trait was more stable than for individual growth traits. Composite indices should therefore be interpreted together with germination, root and shoot measurements to avoid over-reliance on a single parameter (Abdul-Baki & Anderson, 1973; Chen *et al.,* 2012; Priyadharshini *et al.,* 2019; Shanthi *et al*., 2021).

Table 3. Analysis of variance (ANOVA) for seedling traits of black gram

Table 3a. Germination percentage

Source	Degree of freedom	Sum of Square	Mean Square	F value	Pr(>F)
Variety	6	11761	1960	599.83	< 2e-16***
Treatment	3	11359	3786	1158.66	< 2e-16***
Var: Trt	18	787	44	13.38	2.5e-09***
Error	28	92	3		

Table 3b. Root length

Source	Degree of freedom	Sum of Square	Mean Square	F value	Pr(>F)
Variety	6	37.71	6.29	510.12	< 2e-16***
Treatment	3	282.24	94.08	7635.41	< 2e-16***
Var: Trt	18	4.85	0.27	21.88	6.17e-12***
Error	28	0.34	0.01		

Table 3c. Shoot length

Source	Degree of freedom	Sum of Square	Mean Square	F value	Pr(>F)
Variety	6	40.97	6.83	588.31	< 2e-16***
Treatment	3	267.20	89.07	7673.33	< 2e-16***
Var: Trt	18	2.77	0.15	13.27	2.75e-09***
Error	28	0.33	0.01		

Table 3d. Seedling vigour index

Source	Degree of freedom	Sum of Square	Mean Square	F value	Pr(>F)
Variety	6	1988576	331429	18.429	0.00***
Treatment	3	5679507	1893169	105.269	0.00***
Var: Trt	18	506577	28143	1.565	0.14^{NS}
Error	28	5035557	17984		

3.1 Germination Percentage

Germination decreased progressively with increasing salt concentration in all genotypes. Under control conditions, VBN10 (96.5%) and VBN8 (95.5%) showed the highest germination. At the highest salinity level, EC 16 dS/m, VBN8 (62.5%) and VBN10 (61.0%) maintained the highest germination, indicating better salt tolerance. T9 (27.0%) and PU31 (31.5%) showed the lowest germination under salt stress (Fig. 1 and Table 4).

Fig. 1. Germination response observed in seven black gram genotypes under different electrical conductivity (EC) levels

Table 4. Effect of different levels of salt stress on Germination percentage (%) of black gram genotypes

Genotypes	Germination percentage (%)			
	Control	EC 4 ds/m	EC 11 ds/m	EC 16 ds/m
VBN6	87.5	66.5	54.5	41
VBN8	95.5	85.5	78.5	62.5
VBN9	78.5	52.5	49	32.5
VBN10	96.5	89.5	76.5	61
VBN11	84.5	74.5	63.5	45
PU31	69.5	54	46	31.5
T9	60	34.5	29.5	27
Grand mean	81.71	65.29	56.79	42.93
Sed	2.61	3.93	3.36	2.43
CD	5.21	7.86	6.71	4.86

The reduction in germination with increasing EC is expected because NaCl lowers the external water potential and delays or restricts seed imbibition, while sodium and chloride accumulation may further disturb embryo metabolism. Similar reductions in black gram germination under increasing salinity have been reported in genotype-screening studies, confirming that germination percentage is a useful early indicator of salt stress response (Mariyappillai & Kulanthaivel, 2024; Gandhi *et al.,* 2021; Jamil *et al.,* 2006; Kamaleshwaran *et al.,* 2023; Priyadharshini *et al.,* 2019).

The relatively higher germination of VBN8 and VBN10 at EC 16 dS/m suggests better capacity to maintain early establishment under saline conditions. However, this response should be considered as seedling-stage tolerance only and should be validated under pot and field conditions before recommending these genotypes for salt-affected production environments (Shanthi *et al.,* 2021; Rajasekar *et al.,* 2022; van Zelm *et al.,* 2020).

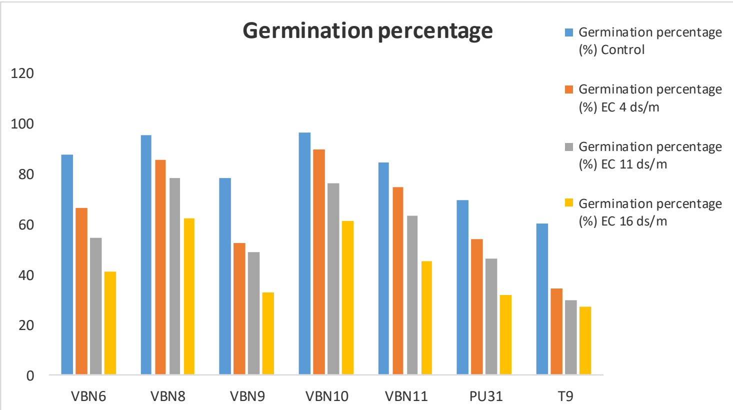

Fig. 2. Graph showing Germination percentage of black gram genotypes under different salt-stress levels

3.2 Root Length

Root length was significantly reduced as salinity increased. VBN8 and VBN10 recorded the longest roots at all salinity levels. At EC 16 dS/m, VBN8 and VBN10 maintained a root length of 3.25 cm, whereas T9 (1.15 cm) and PU31 (1.3 cm) recorded the shortest root lengths (Table 5 and Fig. 3). Root growth is often one of the earliest morphological traits affected by salinity because root cells are directly exposed to the saline medium and must sustain water uptake under osmotic stress. Maintenance of comparatively longer roots in VBN8 and VBN10 may indicate better early root growth stability, which is advantageous for water and nutrient acquisition under stress (Munns & Tester, 2008; Nadeem *et al*., 2019; Shanthi *et al.,* 2021).

Table 5. Effect of different levels of salt stress on Root Length (cm) of black gram genotypes

Genotypes	Root Length (cm)			
	Control	EC 4 ds/m	EC 11 ds/m	EC 16 ds/m
VBN6	8.3	7.05	4.65	2.2
VBN8	9.75	8.35	6.25	3.25
VBN9	7.25	6.25	4.35	1.85
VBN10	9.15	7.8	5.65	3.25
VBN11	7.9	6.8	5	1.95
PU31	7.5	7.2	3.9	1.3
T9	7.4	5.6	3.75	1.15
Grand mean	8.04	7.01	4.79	2.14
Sed	0.17	0.20	0.18	0.15
CD	0.34	0.39	0.36	0.30

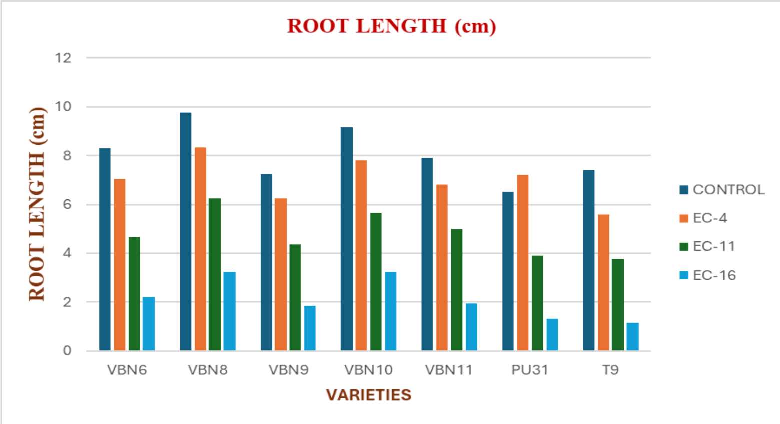

Fig. 3. Graph showing Effect of different levels of salt stress on root length (cm) of black gram genotypes

The strong reduction in root length observed in PU31 and T9 at EC 16 dS/m agrees with earlier reports that radicle length is a sensitive screening trait in black gram under salinity. Because root response can differ from shoot response, root length should be evaluated with shoot length and seedling vigour index for more reliable genotype classification (Priyadharshini *et al.,* 2019; Gandhi *et al.,* 2021; Munns & James, 2003).

3.3 Shoot Length

Shoot length declined with increasing salt stress. VBN10 and VBN8 exhibited better shoot growth than the other genotypes under saline conditions. At EC 16 dS/m, VBN10 had the greatest shoot length (3.25 cm), while T9 (1.55 cm) and PU31 (1.15 cm) showed the lowest shoot lengths (Table 6 and Fig. 4).

Table 6. Effect of different levels of salt stress on Shoot Length (cm) of Black gram genotypes

Genotypes	Shoot length (cm)			
	Control	EC 4 ds/m	EC 11 ds/m	EC 16 ds/m
VBN6	8.15	7.2	4.65	2.2
VBN8	9.4	7.95	5.95	2.75
VBN9	6.9	5.75	3.95	1.85
VBN10	9.15	7.8	5.65	3.25
VBN11	8	6.9	4.85	2.3
PU31	6.65	5.25	3.75	1.15
T9	7.35	5.45	3.9	1.55
Grand mean	7.04	6.61	4.67	2.15
Sed	0.13	0.17	0.13	0.12
CD	0.26	0.34	0.25	0.24

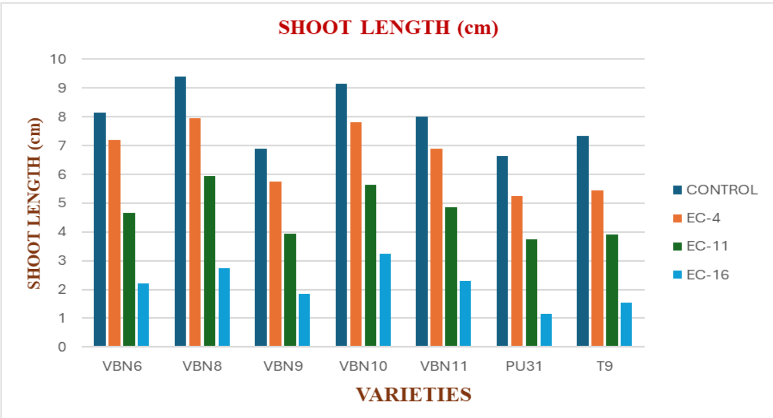

Fig. 4. Graph showing Effect of different levels of salt stress on shoot length (cm) of black gram genotypes

The decline in shoot length under higher EC levels may be attributed to reduced cell expansion, restricted water uptake and altered hormonal balance under salt stress. Shoot growth reduction under NaCl stress has been reported in black gram and other grain legumes, indicating that shoot length is a useful but stress-sensitive trait during early screening (Mariyappillai & Kulanthaivel, 2024; Gandhi *et al.,* 2021; Nadeem *et al.,* 2019). The superior shoot growth of VBN10 and VBN8 under EC 16 dS/m indicates comparatively better maintenance of aerial growth under saline conditions.

3.4 Seedling Vigour Index

Seedling vigour index decreased markedly with increasing salinity. VBN8 and VBN10 maintained the highest vigour indices across all stress levels. Based on the standard seedling vigour index calculation using germination percentage and seedling length, VBN10 (396.50) and VBN8 (375.00) retained superior vigour at EC 16 dS/m, whereas T9 (72.90) and PU31 (77.18) showed the lowest values (Table 7 and Fig. 5). Seedling vigour index integrates germination and seedling growth and is therefore a useful composite measure for comparing early establishment under stress. The higher vigour index of VBN8 and VBN10 is consistent with their comparatively better germination, root length and shoot length across salinity levels (Abdul-Baki & Anderson, 1973; Priyadharshini *et al.,* 2019; Shanthi *et al.,* 2021).

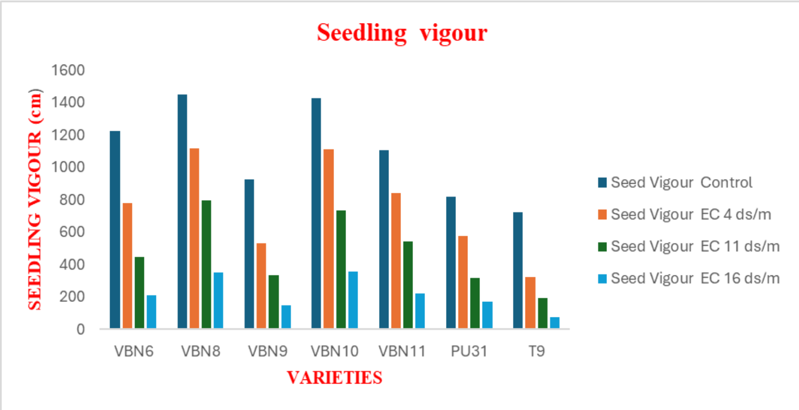

Fig. 5. Graph showing Effect of different levels of salt stress on seedling vigour index of black gram genotypes

Table 7. Effect of different levels of salt stress on seedling vigour index of black gram genotypes

Genotypes	Seedling vigour index			
	Control	EC 4 dS/m	EC 11 dS/m	EC 16 dS/m
VBN6	1439.38	947.62	506.85	180.40
VBN8	1828.82	1393.65	957.70	375.00
VBN9	1110.78	630.00	406.70	120.25
VBN10	1765.95	1396.20	864.45	396.50
VBN11	1343.55	1020.65	625.48	191.25
PU31	983.43	672.30	351.90	77.18
T9	885.00	381.23	225.68	72.90
Grand mean	1336.70	920.24	562.68	201.92
Sed	52.03	56.57	42.97	19.74
CD	104.06	113.14	85.94	39.48

The lower vigour indices of T9 and PU31 indicate poor early establishment under high EC, which may reduce their suitability as tolerant checks in future screening. Because seedling vigour is influenced by multiple component traits, future evaluation should include physiological traits such as ion accumulation, relative water content and chlorophyll stability to strengthen genotype classification (Acosta-Motos *et al.,* 2017; Nadeem *et al*., 2019; Palanisamy et al., 2026).

4. Conclusion

The black gram genotypes *viz.,* VBN 8 and VBN 10 performed best for most traits, namely germination percentage (%), root length (cm), shoot length (cm) and seedling vigour index, at the critical salinity level of EC 16 dS/m. The black gram genotypes *viz.,* PU 31 and T9 showed the poorest performance for germination percentage (%), root length (cm), shoot length (cm) and seedling vigour index at EC 16 dS/m. Increasing salinity stress adversely affected germination percentage (%), root length (cm), shoot length (cm) and seedling vigour index in all black gram genotypes. However, VBN 8 and VBN 10 consistently performed better under saline conditions, demonstrating greater salt tolerance. Hence, these genotypes can be used as checks in future screening and breeding programmes to develop salinity-tolerant varieties of black gram.

5. Limitation

This study was limited to seven black gram genotypes evaluated at the seedling stage under controlled hydroponic conditions. Salinity response was assessed mainly through germination percentage, root length, shoot length and seedling vigour index. Physiological, biochemical and ion-accumulation traits were not measured. Because the evaluation was restricted to early growth under controlled conditions, the observed tolerance of VBN 8 and VBN 10 should be validated under pot and field conditions.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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