PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.6870

eISSN 2348-1900

RESEARCH ARTICLE

Differential phenotypic response of the black gram genotypes under salinity stress at the tissue level

Dhamotharan Palanisamy[1], Manivannan Narayana[1*], Babu Rajendra Prasad Venugopal[2], Ganesan Kalipatty Nalliappan[1], Janaki Ponnusamy[3] & Harish Sankarasubramanian[4]

[1]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[2]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[3]Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[4]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

*Correspondence email - nm68@tnau.ac.in

Received: 23 December 2025; Accepted: 26 February 2025; Available online: Version 1.0: 24 July 2025

Cite this article: Dhamotharan P, Manivannan N, Babu RPV, Ganesan KN, Janaki P, Harish S. Differential phenotypic response of the black gram genotypes under salinity stress at the tissue level. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.6870

Abstract

Salinity stress in black gram is an important challenging issue, especially in delta regions. The independent evolving nature of the salt tolerance in each genotype is the main drawback of saline tolerance crop improvement. The previous study identified two tolerant and two susceptible black gram genotypes under salinity stress at the vegetative stage through hydroponics. In this study, these genotypes were screened for the accumulation of sodium and potassium ions and photosynthetic activity. Different parts of plants *viz.,* roots, stems and leaves were analyzed with the internal sodium and potassium ions. The tolerant genotypes showed a low level of sodium accumulation and a higher level of potassium accumulation in all plant parts compared to the susceptible genotypes. The tolerant genotypes had higher SPAD and fluorescence values, which signify the photosynthetic activity. The tolerant genotypes had higher ion homeostasis compared to the susceptible genotypes. These findings can be adopted in the salinity tolerance breeding programme in black gram.

Keywords: black gram; fluorescence; ion content; salinity tolerance; SPAD; tissue level tolerance

Introduction

Black gram is one of the proteinous grain legume crops grown for nutrition purposes (1). It has a significant role in vegetarian diets as it has nearly 24-26 % protein, 1.3 % of fat and 60 % of carbohydrates. Along with these macromolecules, minor molecules such as amino acids (leucine, arginine, valine, phenylalanine, lysine and isoleucine), elements (iron, potassium and calcium) and vitamins (A, B1, B2 and B3) are abundant (2). In Tamil Nadu, it is grown across approximately 3.41 lakh hectares, yielding a production of 1.21 lakh tonnes (3). Still, it is insufficient to meet nutritional demand in the state. To overcome this situation, it is used to grow rice fallow pulse, where salinity is a serious concern, especially during rabi season. The unavailability of saline-tolerant black gram makes it more difficult to achieve the demand (3). Identifying the potential tolerant sources and the mechanism involved in the reaction are important deciding factors in the salinity breeding program.

Salt tolerance is a continuous process and evolved independently in different species during speciation (4). The tolerance level and mechanism show significant variations among and within cultivars in many crops (5). This response of the plants is also dependent on the ontogeny of the plants and it is not correlated with stages of plant development (6). Generally, the tolerance level in non-halophytes correlated with how they restrict the toxic ions from interacting with metabolically active cells (7). However, non-halophytes showed the highest salt concentration in the tissues under moderate external salinity. The first phase of growth reduction rapidly occurs due to the osmotic effect and the second slower growth reduction occurs due to excessive salt accumulation in plants (6). The visible symptoms such as leaf injury and reduction in the photosynthetic area occur due to the second phase salt-specific effect.

The salt tolerance strategies *viz.,* osmotic tolerance, ion exclusion and ion compartmentalization or tissue tolerance are adopted by plants either alone or in combination with these strategies to combat salt stress (9). Osmotic tolerance is a long-distance signaling mechanism. Plants with an osmotic adjustment mechanism activate the accumulation of osmolytes before the inception of Na^+ accumulation in aerial parts. Ion exclusion has two phenomena: selective absorption of salts and restriction of salt ion movement. Tissue tolerance, the second phenomenon is sequestering the salt ions in membrane-bound organelles (8, 9). The complexity and involvement of numerous physio-chemical phenomena for salinity tolerance make it more cumbersome to improve the trait. Any single morphological/physiological trait has proven

fruitfulness in crop improvement for salinity as expected (10). Because the adaptive mechanism of the donors also has a great impact on the outcome of the improvement (11). Identification of the key tolerance mechanism of the donors and the interrelation of key physiological responses of the traits are inevitable (11, 12).

The acclimatization of the genotypes to salt stress is an integrative phenomenon of different organs of the plant, especially roots and leaves (11). Roots are the primary barrier to salt entering the plant system. Aerial parts such as leaves and stems are involved in direct carbon assimilation and source-sink relation (13). The photosynthetic activity of leaves and the accumulation of salts in different plant parts vary depending on age and location. These two activities are negatively related and directly affect the yield. So, it is important to elucidate the tolerance reaction of the plants (13). With this objective, the present study was made to assess the effect and intensity of salt accumulations and the mechanism of salt tolerance using the susceptible and tolerant black gram genotypes.

Materials and Methods

The experiment was conducted in a glass house laboratory at the Department of Genetics and Plant Breeding, TNAU. Two salt-tolerant genotypes of black gram *viz.,* VBG 17007 and VBG 19010 and two salt-sensitive genotypes *viz.,* ADT 3 and VBG 13003 were selected from the previous study (14). The pedigree details of these genotypes are given in Table 1. The salt-tolerant genotypes VBG 17007 and VBG 19010 showed the inclusion of the Na^+ and exclusion of the Na^+mechanism respectively. The hydroponics experiment methodology was adapted from a previous study (14). It was then supplemented with Hoagland nutrient media (15). The genotypes were screened at 13 dSm^{-1}salt stress (the critical salinity level (14)) during August 2024. The challenging environment was achieved by four instalments of salt imposition on alternative days. The plants were maintained for eight days in the final concentration at the vegetative stage. A randomized complete block design with three replications was followed for the study.

Preparations of the samples

The roots, stems and leaves of each genotype were separated and each plant's parts were segmented into upper, middle and bottom portions (Fig. 1). The upper tender leaves and 1st trifoliate leaves were designated as the upper portion of the leaves. 2nd trifoliate leaves were designated as the middle portion. The lower leaves were designated as the bottom category. The stem portion between the trifoliate leaves and lower leaves was designated as the upper portion of the stem. Then the stem above the collar regions was designated as the lower portion of the stem and the remaining portion was designated as the middle portion of the stem. Roots were separated into three portions *viz.,* upper, middle and bottom portions. SPAD and fluorescence values were obtained in these three designated portions of the leaves to observe chlorophyll activity.

Table 1. Genotypes used for the study and its pedigree

Genotypes	Pedigree	Remarks
ADT 3	PLS from Tirunelveli Local	Saline susceptible; Variety developed from TRRI, Aduthurai, Tamil Nadu. Recommended for rice fallow situations of Tamil Nadu
VBG 13003	KU 2016 × VBN 3	Saline susceptible; Advanced culture developed from NPRC, Vamban
VBG 17007	VBN 5 × MDU 1	Saline tolerant; Advanced culture developed from NPRC, Vamban
VBG 19010	BDR 1 × *Vigna mungo* var *sylvestris*	Saline tolerant; Advanced culture developed from NPRC, Vamban

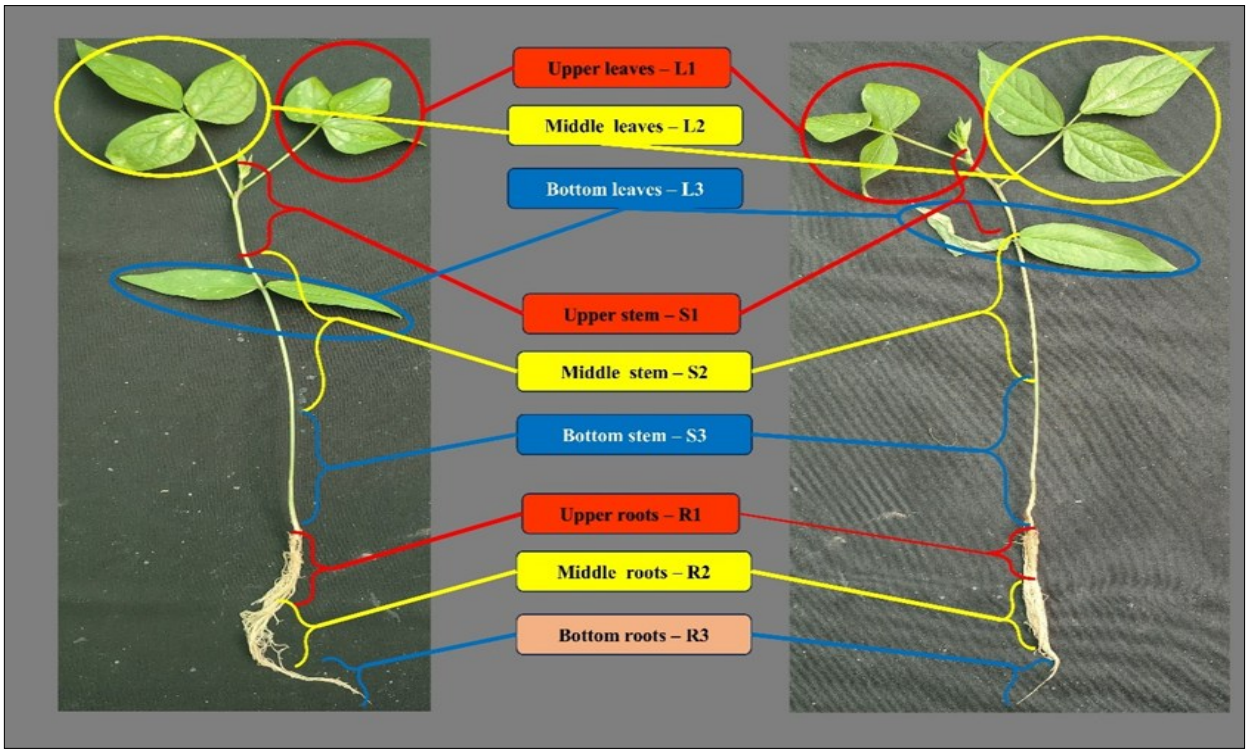

Fig. 1. Differential sections of the root, stem and leaves of the four genotypes.

Digestion and element analysis

The upper, middle and lower portions of the root, shoot and leaves were collected and dried separately in a hot air oven at 70 °C for three days. Samples were well pulverized and digested in a triacid mixture (9 parts nitric acid, 2 parts sulfuric acid and 1 part perchloric acid) in the sand bath digester at 95 °C temperature. Element analysis was carried out in a Flame photometer (Systronics Flame Photometer, model: 128, Systronics India Pvt. Ltd., Gujarat, India). Standard curves were prepared using NaCl and KCl (Sigma Aldrich) and used for calibration.

Observations recorded

The sodium (Na) and potassium (K) content in the upper, middle and lower portions of the root (UR_Na, UR_K, MR_Na, MR_K, LR_Na, LR_K), stem (US_Na, US_K, MS_Na, MS_K, LS_Na, LS_K) and leaves (UL_Na, UL_K, ML_Na, ML_K, LL_Na, LL_K) were measured. SPAD (Soil Plant Analysis Development) and fluorescence values of the upper (USPAD, UL_fluorescence), middle (MSPAD, ML_fluorescence) and lower (LSPAD, LL_fluorescence) compound leaves were recorded using SPAD and fluorescence meter. The wilting percentage was also recorded for each genotype.

Data analysis

The ANOVA analysis was carried out in the STAR software (version 2.0.1) (16). R packages such as 'ggcorrplot', 'FactoMineR' and 'factoextra' were utilized to analyse correlation and PCA (17, 18).

Results

Generally, plants showed significant variation in salt-responsive traits under salinity stress. Based on the significant variation in ion accumulation and photosynthetic activity, it can be deciphered that there are more potential genotypes tolerant to stress.

ANOVA

All characters showed significance except for the lower root potassium content and lower leaf SPAD value (Table 2). The lower root potassium content, lower leaf SPAD value and homeostasis in the middle root and shoot part were eliminated for further mean comparison analysis and other multivariate analyses.

Mean comparison

Sodium ions accumulation

In the upper, middle and lower root regions, saline-sensitive genotype ADT 3 and VBG 13003 showed a higher sodium accumulation than saline-tolerant genotypes VBG 17007 and VBG 19010. For the middle and lower stem region, saline-sensitive genotypes exhibited higher sodium accumulation than the saline-tolerant genotypes. However, in the case of the upper stem region, the difference between these two categories was not evident. The saline tolerant genotype VBG 19010 had on par sodium accumulation with saline sensitive genotypes ADT 3 and VBG 13003. Another saline tolerant genotype VBG 17007 had a lower level of sodium accumulation in the upper stem portion than saline sensitive genotypes (Table 3). Both saline tolerant genotypes, VBG 17007 and VBG

Table 2. Analysis of variance for the morphological traits of four black gram genotypes under the salinity level of 13 dSm^{-1}

Characters	Replication	Genotype	Error
UR_Na	63.09	15059.66**	580.87
MR_Na	60.80	6189.12**	810.76
LR_Na	164.97	4891.44**	720.61
US_Na	133.37	3478.44*	1152.79
MS_Na	806.63	6687.96**	1444.43
LS_Na	59.42	14009.08**	1436.29
UL_Na	192.67	48532.50**	701.89
ML_Na	326.74	19185.52**	2253.56
LL_Na	300.66	27316.69**	1626.98
UR_K	200.21	1752.08**	190.49
MR_K	396.42	1108.84*	438.65
LR_K	290.58	309.78	180.68
US_K	352.42	7254.38**	321.65
MS_K	404.95	4761.64**	909.65
LS_K	37.08	4949.88**	312.53
UL_K	195.07	17064.42**	1008.55
ML_K	408.33	11299.64**	555.31
LL_K	119.33	3268.58**	537.84
USPAD	16.53	218.11*	74.70
MSPAD	132.31*	121.56*	46.13
LSPAD	60.46	53.42	136.14
UL_fluorescence	0.01	0.02*	0.01
ML_fluorescence	0.01*	0.04*	0.01
LL_fluorescence	0.03**	0.02**	0.00
UR_K/Na	0.01	1.71**	0.37
MR_K/Na	0.33	0.87	0.46
LR_K/Na	0.04	0.17**	0.03
US_K/Na	0.07	4.22**	0.46
MS_K/Na	0.24	2.61	1.66
LS_K/Na	0.01	1.28**	0.04
UL_K/Na	0.08	4.60**	0.45
ML_K/Na	0.04	1.05**	0.10
LL_K/Na	0.01	0.34**	0.05

*, ** significant at 5 % and 1 % level probability

Table 3. Mean performance of four genotypes for nutritional and photosynthetic traits under the salinity level of 13 dSm^{-1}

Characters	Saline sensitive		Saline tolerant		CD
	ADT 3	VBG 13003	VBG 17007	VBG 19010	
UR_Na	130.59	120.50	49.25	62.01	15.75
MR_Na	102.93	113.22	55.59	75.37	18.60
LR_Na	121.39	124.53	73.67	103.70	17.54
US_Na	99.41	80.04	51.57	78.42	22.18
MS_Na	115.19	111.15	66.23	65.86	24.83
LS_Na	155.13	146.79	87.71	78.66	24.76
UL_Na	193.99	210.24	84.73	67.35	17.31
ML_Na	197.03	207.12	130.36	115.82	31.01
LL_Na	238.36	228.36	160.70	123.23	26.35
UR_K	41.02	38.81	46.32	69.23	9.02
MR_K	44.55	43.37	49.29	67.34	13.68
LR_K	37.77	44.05	45.95	52.01	-
US_K	35.64	52.73	98.31	82.85	11.72
MS_K	30.19	47.93	80.85	71.82	19.70
LS_K	27.95	36.85	77.50	65.85	11.55
UL_K	33.04	22.55	110.31	93.49	20.75
ML_K	31.43	27.05	98.37	79.87	15.40
LL_K	38.01	30.8	69.93	63.45	15.15
UR_K/Na	0.32	0.32	0.97	1.15	0.40
MR_K/Na	0.43	0.38	0.96	0.92	-
LR_K/Na	0.32	0.36	0.62	0.50	0.11
US_K/Na	0.36	0.66	1.93	1.12	0.44
MS_K/Na	0.26	0.44	1.27	1.28	-
LS_K/Na	0.18	0.25	0.90	0.84	0.14
UL_K/Na	0.17	0.11	1.32	1.43	0.44
ML_K/Na	0.16	0.13	0.76	0.71	0.20
LL_K/Na	0.16	0.14	0.45	0.52	0.14
USPAD	23.40	25.60	32.50	33.23	5.65
MSPAD	30.20	31.40	35.70	38.07	4.44
LSPAD	35.60	34.80	37.90	40.20	-
UL_fluorescence	0.42	0.50	0.51	0.54	0.06
ML_fluorescence	0.51	0.54	0.62	0.65	0.06
LL_fluorescence	0.47	0.51	0.57	0.58	0.03

U, M and L (first letter) - refer to Upper, Middle and lower. R, S and L (second letter) – refer to Root, Stem and Leaves. Na and K are sodium and potassium

19010, exhibited a trend of lower sodium accumulation in upper, middle and lower leaves than the saline sensitive genotypes.

Potassium ions accumulation

Saline-tolerant genotype VBG 19010 had higher potassium ion accumulation in upper, middle and lower roots than the saline-sensitive genotypes. However, saline tolerant genotype VBG 17007 had a similar level of potassium accumulation with saline sensitive genotypes in all levels of roots. All genotypes showed the highest potassium accumulation in the upper stem, with a decreasing trend from the upper to the lower stem. In general, the saline-tolerant genotypes had higher potassium accumulation than the sensitive genotypes at upper, middle and lower stem and leaf areas.

SPAD and fluorescence

The tolerant genotypes had significantly higher SPAD and fluorescence values in leaves over susceptible genotypes. VBG 19010 recorded higher SPAD and Fluorescence values than VBG 17007. VBG 13003 recorded higher values for SPAD and fluorescence over ADT 3. Invariably, all genotypes were witnessed with maximum SPAD and Fluorescence values in the middle compound leaves.

Ion homeostasis

The genotypes *viz.,* VBG 17007 and VBG 19010 witnessed higher ion homeostasis rates in all portions of the plants. Upper shoot regions had overall higher homeostasis rates and a very low rate was recorded for the leaves of VBG 13003 (Fig. 2).

Correlation

Sodium content in the upper root region, middle and lower stem and all regions of the leaves were positively correlated with wilting percentage (WP) (Fig. 3). WP was negatively correlated with potassium content in all stem and leaf segments. Upper and middle leave SPAD values were negatively correlated with WP. The middle and lower leaf fluorescence values were negatively associated with WP.

The upper root region's sodium content was positively related to the middle and lower stem sodium content. It was negatively correlated with the majority of the potassium contents. It negatively correlated with the upper leaves SPAD value and the lower leaves fluorescence value. Middle and lower root sodium content were related to each other. Middle and lower root sodium content was not correlated with any other traits. Middle-root sodium was negatively correlated with potassium content in the leaves. Upper stem sodium content was almost not correlated with any other traits. Middle and lower stem sodium content and all regions of leaf sodium were correlated to each other. Middle stem sodium content was negatively correlated with potassium content in stem and leaves. SPAD values of the upper and middle leaves were negatively correlated with stem and leaf sodium content in all portions.

Upper and middle root potassium content were not correlated with any other traits. Potassium content in stems and leaves was correlated among them. SPAD and fluorescence values were correlated with each other. Leaf

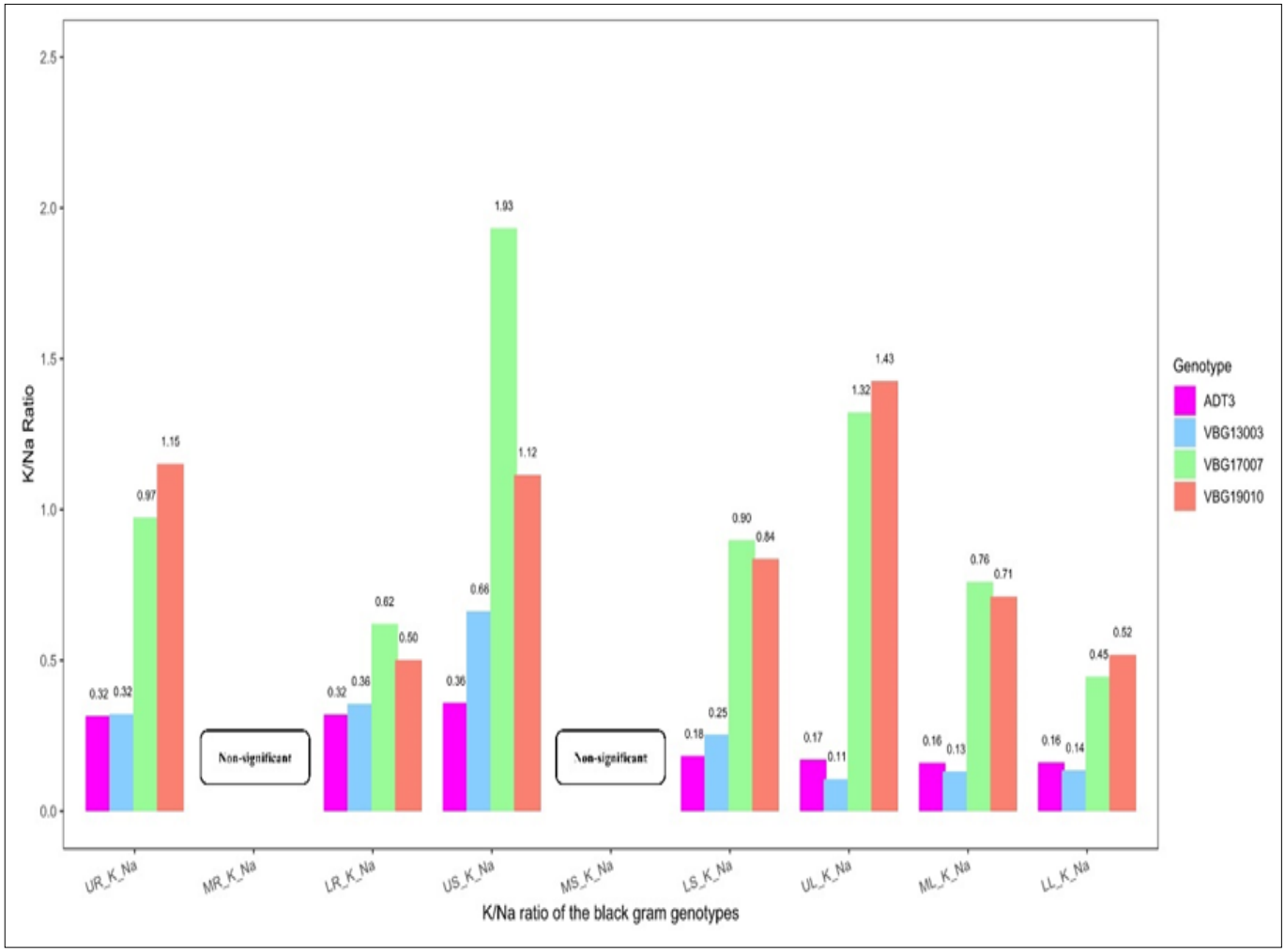

Fig. 2. Potassium to sodium ratio of the four black gram genotypes under the salinity level of 13 dSm^{-1}.

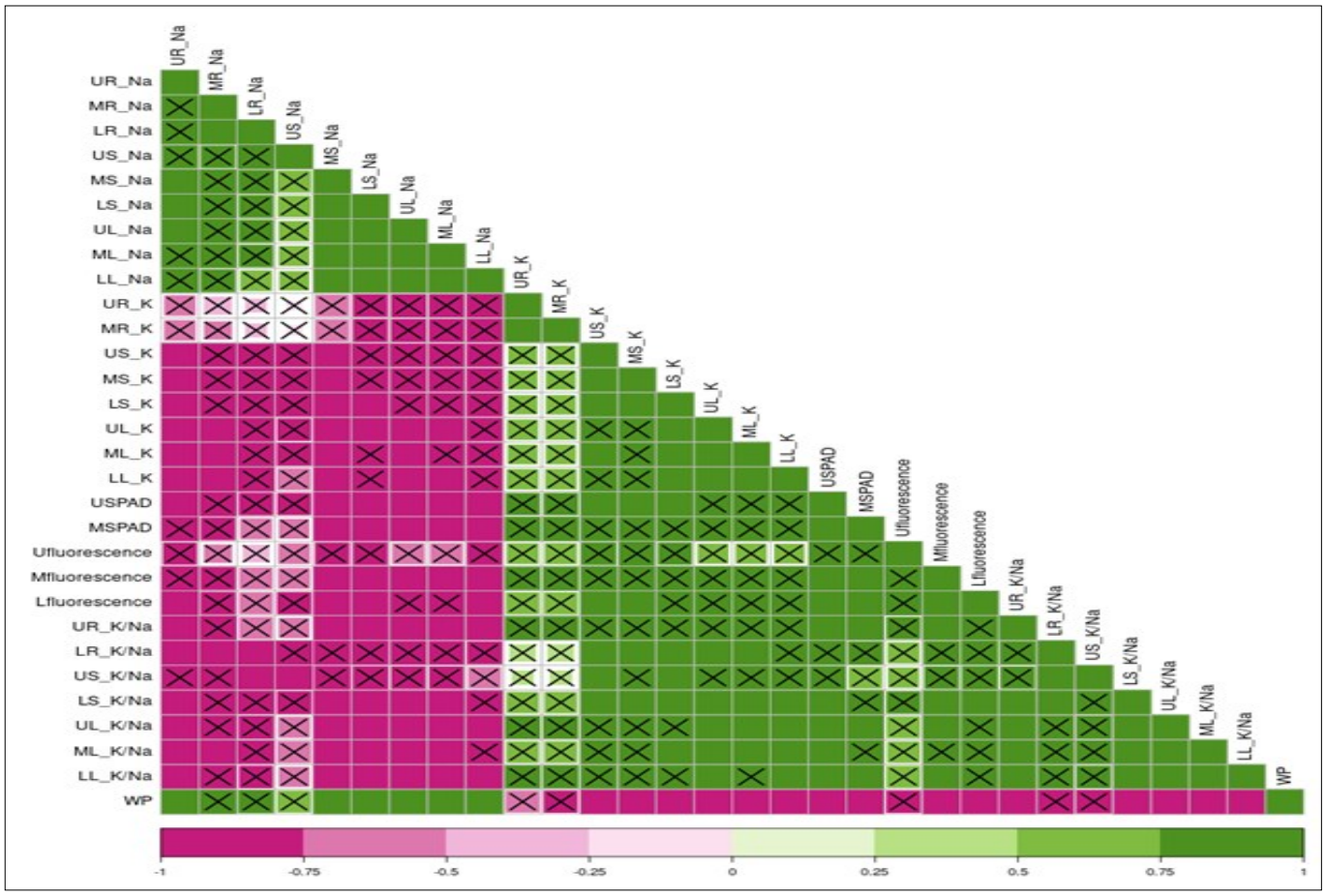

Fig. 3. The correlogram of the salt responsive traits (P=0.05) and cross mark refers to insignificance.

homoeostasis traits were negatively correlated with wilting percentage. It also negatively correlated with almost all the sodium contents in all parts of the and positively correlated with potassium contents compared to other homeostasis traits in stem and leaves. With leaf chlorophyll parameters it was positively correlated.

PCA analysis

Principal component analysis is a singular value decomposition technique, used to extract the key traits (Table 4 & Fig. 4). Three principal components combined explained 100 % of the variation and all three had eigenvalue greater than one. The first principal component explains 88.56 % and sodium content -related traits had the highest negative loadings along with wilting percentage in the component. Potassium content and chlorophyll-related traits had positive loadings. The second principal component explains 8.35 % and potassium content in the upper root portion had the highest loadings followed by middle root potassium content. The third component explains 3.09 % of the available variation. It had given higher weightage to the upper leaf fluorescence value. In PC_{1-2}, the traits were classified into two clusters, one with sodium content in all the segments of the plant and another with potassium and chlorophyll traits. The susceptible and tolerant genotypes were also distinguished with positively and negatively contributing characteristics respectively.

Discussion

The salt tolerance mechanisms of the genotypes were elucidated in previous studies. The salt-tolerant genotype VBG 17007 exhibited a strategy of sodium ion (Na^+) accumulation, sequestering Na^+ ions within the leaf tissues. In contrast, the genotype VBG 19010 adhered to the sodium exclusion mechanism, effectively limiting the translocation of Na^+ from the roots to the aerial parts of the plant. Given that the roots are the first organ to encounter saline stress, the accumulation patterns of sodium across different root regions were assessed. Salt-susceptible genotypes exhibited a significantly higher sodium accumulation in three distinct root regions compared to the tolerant genotypes. Specifically, the genotype ADT 3 demonstrated the highest sodium accumulation in the upper root regions, while VBG 13003 exhibited greater accumulation in the lower root regions. Despite similar overall sodium accumulation levels in the roots of these genotypes, the distribution of sodium across root regions differed. Notably, VBG 17007 showed lower sodium accumulation in the roots relative to VBG 19010, with both tolerant genotypes accumulating the highest concentrations of sodium in the lower root region. However, VBG 19010 displayed a more pronounced sodium accumulation in the roots compared to VBG 17007, suggesting that its tolerance response involves both the sequestration of sodium ions within the roots and the exclusion of sodium ions from the aerial tissues (19). The genotype VBG 17007 accumulated fewer sodium ions in the roots, indicating that it effectively restricts the entry of sodium ions into this organ. Furthermore, this genotype demonstrated moderate accumulation of potassium in the roots, suggesting a selective absorption mechanism that favours potassium over sodium ions. This preferential cation uptake may contribute to the genotype's ability to maintain ionic homeostasis and mitigate the detrimental effects of salinity stress, enhancing its salt tolerance (19, 20).

Table 4. Principal components of salt responsive traits on black gram genotypes under the salinity level of 13 dSm^{-1}

Variables	PC1	PC2	PC3
UR_Na	-0.19	0.07	-0.01
MR_Na	-0.18	0.17	0.27
LR_Na	-0.17	0.31	0.18
US_Na	-0.15	0.36	-0.30
MS_Na	-0.19	-0.02	0.02
LS_Na	-0.19	-0.07	-0.02
UL_Na	-0.19	-0.08	0.17
ML_Na	-0.19	-0.10	0.16
LL_Na	-0.19	-0.19	-0.03
UR_K	0.14	0.44	-0.06
MR_K	0.14	0.43	-0.04
US_K	0.19	-0.14	0.15
MS_K	0.19	-0.10	0.23
LS_K	0.19	-0.12	0.06
UL_K	0.19	-0.08	-0.20
ML_K	0.19	-0.11	-0.15
LL_K	0.19	-0.06	-0.25
USPAD	0.19	0.04	0.11
MSPAD	0.19	0.17	0.09
Ufluorescence	0.15	0.13	0.62
Mfluorescence	0.19	0.12	0.13
Lfluorescence	0.19	0.05	0.25
UR_K.Na	0.19	0.12	-0.06
LR__K.Na	0.18	-0.22	0.01
US__K.Na	0.17	-0.30	0.07
LS__K.Na	0.19	-0.03	-0.01
UL__K.Na	0.19	0.06	-0.11
ML__K.Na	0.19	-0.02	-0.13
LL_K.Na	0.19	0.11	-0.11
WP	-0.19	-0.03	0.00
Eigenvalue	26.57	2.51	0.93
Percentage of variance	**88.56**	**8.35**	**3.09**
Cumulative percentage of variance	88.56	96.91	**100.00**

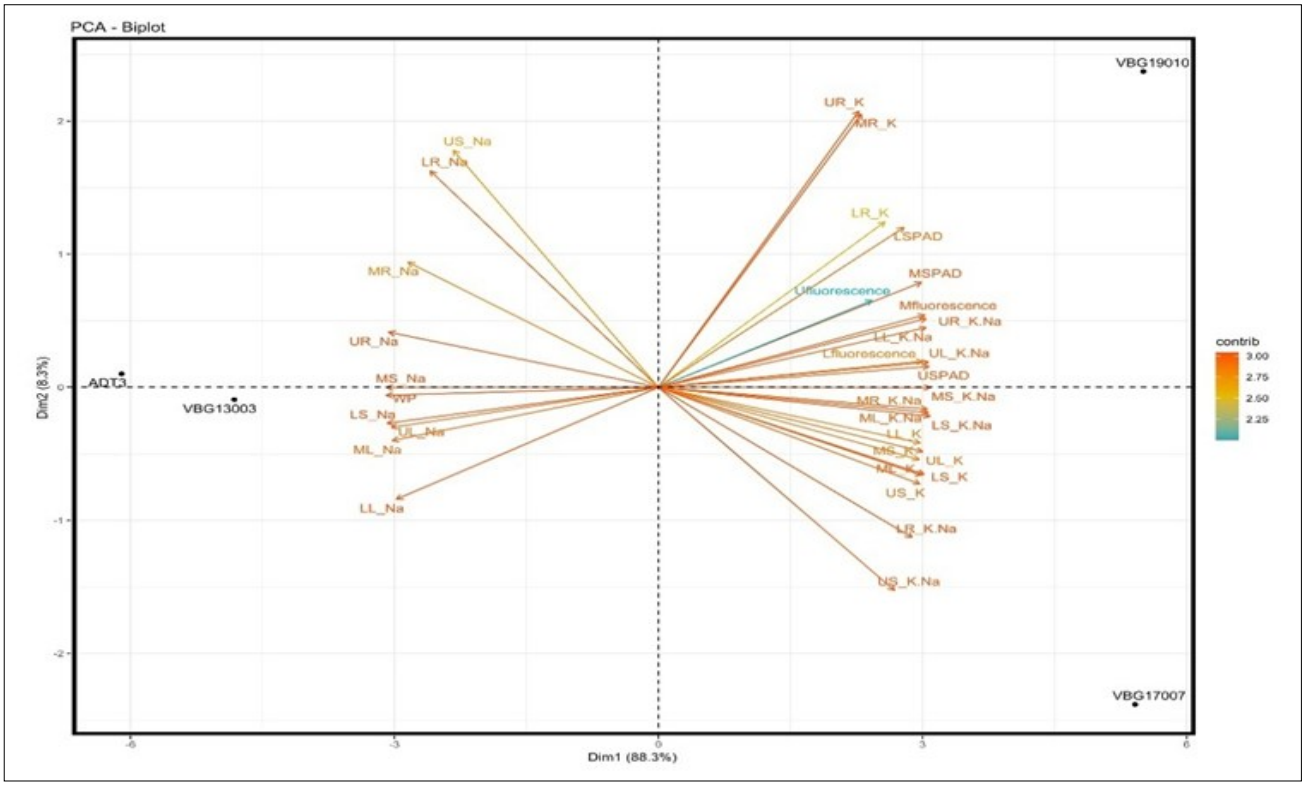

Fig. 4. Principal component analysis of black gram genotypes under the salinity level of 13 dSm^{-1}.

Both ADT 3 and VBG 13003 exhibited higher sodium accumulation in the lower stem regions. A gradient of decreasing sodium accumulation was observed from the lower to the higher stem regions in the genotypes ADT 3, VBG 13003 and VBG 17007 (20). Notably, the salt-susceptible genotypes accumulated significantly more sodium than their salt-tolerant counterparts. VBG 17007 demonstrated a higher sodium concentration in the stem compared to the roots, while the reverse trend was evident in the genotype VBG 19010, which exhibited greater sodium accumulation in the roots. In all genotypes, the bottom portion of the roots consistently displayed the highest levels of sodium accumulation. The salt-tolerant genotype VBG 17007 exhibited a notable pattern where sodium ion accumulation was predominantly restricted to the lower regions of the stem, with effective regulation of sodium movement into the aerial parts of the plant. This suggests that VBG 17007's tolerance mechanism includes both limiting sodium uptake in the roots and restricting its upward movement into the stems and leaves (20). The interaction of sodium ions with tissue and the tolerance responses were discussed (16). All four genotypes showed a higher rate of sodium accumulation in lower leaves. Similar results were also recorded in wild *Vigna* genotypes (22). The susceptible genotypes accumulated sodium ions equally in the upper and middle leaves (23). In the salt-tolerant genotypes, a gradient of decreasing sodium accumulation was observed from the lower to the upper portions of the leaves. This distribution pattern indicates localized sequestration of sodium and chloride ions in the lower leaves, which may serve as a strategy to minimize ion toxicity in the more metabolically active upper leaves. Specifically, the lower leaves of *Vigna unguiculata* exhibited higher concentrations of both sodium and chloride ions, suggesting that these regions function as storage sites for excess salts, thereby protecting the plant's upper aerial tissues from high ionic stress. This differential ion distribution is likely part of the plant's adaptive mechanism to cope with salinity by compartmentalizing toxic ions in less critical areas of the plant (11, 26). The low rate of sodium accumulation in the upper leaves (2). The effect of sodium ions accumulation in the leaves and the dry matter production reported (18). However, these tolerant genotypes exhibited differential accumulation of potassium in roots. Relatively VBG 17007 accumulated more sodium ions in all three leaf portions over VBG 19010. This may be the reason for the Na^+ inclusion principle of the VBG 17007 and the Na^+ exclusion principle of the VBG 19010. Similar results were observed in the *V. leutola* (17). The lower leaves of all the genotypes witnessed increased sodium accumulation, which ultimately withered off from the plants and eliminated the sodium ions from the plant system. To counteract the deleterious effect of the higher sodium accumulation in the leaves and roots portion of the respective genotypes, they accumulated higher potassium in the respective tissues (5, 22). This result was accorded with the ion homeostasis rate of the tolerant genotype (21, 24) and with the correlation analysis. The salt-tolerant genotypes were observed with higher ion homeostasis ratio than the susceptible genotypes in all segments of the plant (19).

The salt-tolerant genotypes exhibited higher SPAD values in the upper and middle compound leaves, indicating greater chlorophyll content and, consequently, higher photosynthetic potential in these leaf regions. Among the four genotypes, the middle compound leaves consistently showed higher photosynthetic activity than the upper leaves. This suggests that the middle leaves may play a more prominent role in sustaining photosynthetic efficiency under saline conditions. Furthermore, the relative chlorophyll content was significantly higher in the salt-adapted genotypes compared to the unadapted genotypes of *V. unguiculata*. This difference underscores the enhanced ability of salt-adapted genotypes to maintain chlorophyll integrity and photosynthetic capacity,

despite the osmotic and ionic stresses imposed by salinity. Thus, these genotypes likely exhibit more efficient mechanisms for chloroplast protection and photosynthetic regulation in response to salt stress (17). It was observed that the salt-adapted genotypes retained their chlorophyll content for a longer duration compared to the unadapted genotypes, indicating a more effective mechanism for chlorophyll preservation under saline stress. The genotype VBG 19010 exhibited higher photosynthetic activity in the middle leaves compared to VBG 17007, suggesting that VBG 17007 may have a higher concentration of sodium ions (Na^+) in its aerial parts, which could inhibit photosynthetic efficiency in these tissues. A similar trend was observed in fluorescence values, where VBG 19010 displayed more favourable fluorescence characteristics, further supporting the higher photosynthetic activity observed in its middle leaves. These findings suggest that the increased photosynthetic activity and leaf biomass in the tolerant genotypes are closely linked to their salinity tolerance responses, with the plants effectively maintaining cellular function and metabolic processes despite the ionic stress imposed by high salinity (13, 25). The importance of the activity of photosynthetic apparatus under salinity was also reported (20). Based on the correlation and principal component analysis, potassium accumulation and increased photosynthetic apparatus activity were indelible characteristics of the tolerant genotypes.

Conclusion

The tolerant genotype VBG 19010 exhibited sodium accumulation in the root regions and prevented the translocation of sodium ions, which is otherwise termed as Na^+ exclusion principle. VBG 17007 showed less accumulation of sodium in the roots and simultaneously accumulated more in lower leaves. Eventually, it withered off and was eliminated from the plant. Similarly, it shows the selective absorption of potassium ions over sodium ions. So, it can be concluded that VBG 17007 had the Na^+ inclusion and VBG 19010 had the Na^+ exclusion principle for salt stress.

Future perspective

The present study evaluated selected black gram genotypes for their tolerance level at the tissue level. These genotypes further can be utilized to decipher the molecular mechanism of salt tolerance. These genotypes were further evaluated for genomic and transcriptomic aspects to find the genetic basis of trait expression. Based on this, the KASP/PACE marker can be developed for further crop improvement programs through MAS/MABC.

Acknowledgements

The first author acknowledges the Council of Scientific and Industrial Research for providing a CSIR-SRF grant (File No: 09/0641(14045)/2022-EMR-I).

Authors' contributions

DP performed the field experiments, measurements and data analysis and drafted the manuscript. MN supervised and worked on the manuscript. All authors were involved in planning and provided critical feedback on the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Kaewwongwal A, Kongjaimun A, Somta P, Chankaew S, Yimram T, Srinives P. Genetic diversity of the black gram [*Vigna mungo* (L.) Hepper] gene pool as revealed by SSR markers. Breed Sci [Internet]. 2015;65(2):127–37. https://doi.org/10.1270/jsbbs.65.127
2. Nair RM, Chaudhari S, Devi N, Shivanna A, Gowda A, Boddepalli VN, et al. Genetics, genomics and breeding of black gram [*Vigna mungo* (L.) Hepper]. Front Plant Sci [Internet]. 2024;14:1273363. https://doi.org/10.3389/fpls.2023.1273363
3. Shanthi P, Ramesh P, Parameshwaran M, Umadevi M, Sakaravarthy KS, Vivekananthan T. Morphological and yield attribute of black gram genotypes under different salinity stress conditions. Indian J Agric Res [Internet]. 2021;1. https://doi.org/10.18805/IJARe.A-5697
4. Iseki K, Takahashi Y, Muto C, Naito K, Tomooka N. Diversity and evolution of salt tolerance in the genus *Vigna*. In; Mondal TK, editor. PLoS One [Internet]. 2016;11(10):e0164711. https://doi.org/10.1371/journal.pone.0164711
5. Noda Y, Sugita R, Hirose A, Kawachi N, Tanoi K, Furukawa J, et al. Diversity of Na^+ allocation in salt-tolerant species of the genus *Vigna*. Breed Sci [Internet]. 2022;72(4):22012. https://doi.org/10.1270/jsbbs.22012
6. Naveed SA, Zhang F, Zhang J, Zheng TQ, Meng LJ, Pang YL, et al. Identification of QTN and candidate genes for salinity tolerance at the germination and seedling stages in rice by genome-wide association analyses. Sci Rep [Internet]. 2018;8(1):6505. https://doi.org/10.1038/s41598-018-24946-3
7. Julkowska MM, Hoefsloot HCJ, Mol S, Feron R, de Boer GJ, Haring MA, et al. Capturing *Arabidopsis* root architecture dynamics with root-fit reveals diversity in responses to salinity. Plant Physiol [Internet]. 2014;166(3):1387–402. https://doi.org/10.1104/pp.114.248963
8. Munns R. Genes and salt tolerance: bringing them together. New Phytol. [Internet]. 2005;167(3):645–63. https://doi.org/10.1111/j.1469-8137.2005.01487.x
9. Roy SJ, Negrão S, Tester M. Salt resistant crop plants. Curr Opin Biotechnol [Internet]. 2014;26:115–24. https://doi.org/10.1016/j.copbio.2013.12.004
10. Jackson P, Robertson M, Cooper M, Hammer G. The role of physiological understanding in plant breeding; from a breeding perspective. Field Crops Res [Internet]. 1996;49(1):11–37. https://doi.org/10.1016/S0378-4290(96)01012-X
11. Munns R. Comparative physiology of salt and water stress. Plant Cell Environ [Internet]. 2002;25(2):239–50. https://doi.org/10.1046/j.0016-8025.2001.00808.x
12. Zhu JK. Plant salt tolerance. Trends Plant Sci [Internet]. 2001;6(2):66–71. https://doi.org/10.1016/S1360-1385(00)01838-0
13. Lacerda CF, Júnior AJO, Filho LCA, Oliveira TS de, Guimarães

FVA, Gomes-Filho E, et al. Morpho-physiological responses of cowpea leaves to salt stress. Braz J Plant Physiol [Internet]. 2006;18(4):455–65. https://doi.org/10.1590/S1677-04202006000400003

14. Punithavathy. Variability analysis for yield, yield components and salinity tolerance in black gram [*Vigna mungo* (L.) Hepper]. [Coimbatore]: Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University; 2020
15. Hoagland DR, Snyder WC. Nutrition of strawberry plant under controlled conditions:(a) effects of deficiencies of boron and certain other elements: (b) susceptibility to injury from sodium salts. In: Proceedings of the American Society of Horticultural Sci; 1933. p. 288–94
16. IRRI. STAR - Statistical Tool for Agricultural Research [Internet]. LosBaños, Laguna, Philippines: Biometrics and Breeding Informatics, PBGB Division, International Rice Research Institute; 2016 [cited 2024 Nov 27]. Available from: https://bbi.irri.org/products
17. Lê S, Josse J, Husson F. FactoMineR: An R package for multivariate analysis. J Stat Softw [Internet]. 2008;25(1):1–18. https://doi.org/10.18637/jss.v025.i01
18. Kassambara A. ggcorrplot: Visualization of a correlation matrix using "ggplot2" [Internet]. Vol. 3, CRAN: Contributed Packages; 2016. p. 908. https://doi.org/10.32614/CRAN.package.ggcorrplot
19. Zhang JL, Flowers TJ, Wang SM. Mechanisms of sodium uptake by roots of higher plants. Plant Soil. 2010;326(1–2):45–60. https://doi.org/10.1007/s11104-009-0076-0
20. Warwick NWM, Halloran GM. Accumulation and excretion of sodium, potassium and chloride from leaves of two accessions of *Diplachne fusca* (L.) Beauv. New Phytol. 1992;121(1):53–61. https://doi.org/10.1111/j.1469-8137.1992.tb01092.x
21. Schachtman D, Liu W. Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. Trends Plant Sci [Internet]. 1999;4(7):281–87. https://doi.org/10.1016/S1360-1385(99)01428-4
22. Wang F, Iki Y, Tanoi K, Naito K. Phenotypic responses in the root of salt-tolerant accessions of *Vigna marina* and *Vigna luteola* under salt stress. Genet Resour Crop Evol [Internet]. 2024;71(6):2631–40. https://doi.org/10.1007/s10722-023-01794-3
23. Iwahashi M, Tachibana Y, Ohta Y. Accumulation of calcium, magnesium, potassium and sodium with growth of individual leaves, petioles and stems of cucumber plants. Soil Sci Plant Nutr. 1982;28(4):441–49. https://doi.org/10.1080/00380768.1982.10432384
24. Poustini K, Siosemardeh A. Ion distribution in wheat cultivars in response to salinity stress. Field Crops Res [Internet]. 2004;85(2–3):125–33. https://doi.org/10.1016/S0378-4290(03)00157-6
25. de Lacerda CF, Cambraia J, Oliva MA, Ruiz HA, Prisco JT. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environ Exp Bot [Internet]. 2003;49(2):107–20. https://doi.org/10.1016/S0098-8472(02)00064-3
26. Nandy (Datta) P, Das S, Ghose M, Spooner-Hart R. Effects of salinity on photosynthesis, leaf anatomy, ion accumulation and photosynthetic nitrogen use efficiency in five Indian mangroves. Wetl Ecol Manag [Internet]. 2007;15(4):347–57. https://doi.org/10.1007/s11273-007-9036-8

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.