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Genome-wide association studies of salinity tolerance in local aman rice

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Abstract

The present study aimed to identify and characterize new sources of salt tolerance among 94 rice genotypes from varied geographic origins. The genotypes were divided into five groups based on their morphological characteristics at both vegetative and reproductive stages using salinity scores from the Standard Evaluation System (SES). The experiment was designed as per CRD (Completely Randomized Design) with two sets of salinity treatments for 8 dS/meter and 12 dS/meter, respectively compared with one non-salinized control set. Using a Soil Plant Analysis Development (SPAD) meter, assessments of the apparent chlorophyll content (greenness) of the genotypes were done to comprehend the mechanism underlying their salt tolerance. To evaluate molecular genetic diversity, a panel of 1 K RiCA SNP markers was employed. Utilizing TASSEL 5.0 software, 598 filtered SNPs were used for molecular analysis. Whole-genome association studies (GWAS) were also used to investigate panicle number per plant (pn, tiller number per plant (till), SPAD value (spad), sterility (percent) (str), plant height (ph) and panicle length (pl). It is noteworthy that these characteristics oversee conveying the visible signs of salt damage in rice. Based on genotype data, diversity analysis divided the germplasm groups into four distinct clusters (I, II, III and IV). For the traits studied, thirteen significant marker-trait associations were discovered. According to the phenotypic screening, seven genotypes namely Koijuri, Asha, Kajal, Kaliboro, Hanumanjata, Akundi and Dular, are highly tolerant to salinity stress. The greenness of these genotypes was found to be more stable over time, indicating that these genotypes are more resistant to stress. Regarding their tolerance levels, the GWAS analysis produced comparable results, supporting that salinity-tolerant genotypes having minor alleles in significant SNP positions showed more greenness during the stress period. The Manhattan plot demonstrated that at the designated significant SNP position, the highly tolerant genotypes shared common alleles. These genotypes could therefore be seen as important genomic resources for accelerating the development and release of rice varieties that are tolerant to salinity.

Keywords: GWAS, Germplasm, Rice, Salt tolerance, SNP, SPAD

1. Introduction

The world population will have a sharp rise to 9.6 billion by 2050 (1). Crop productivity enhancement is a crucial issue considering stressed environments like salinity. Rice production in the southern part covering the coastal belt of the Bay of Bengal is immensely threatened by the salinity problem in Bangladesh. Global warming and the ever-rising sea levels in the coastal aquifers of the Bengal Basin have led to long-term salinization (2).

Rice (*Oryza sativa* L.) is a major food crop that feeds more than half of the world's population (3,4). Only Asia produces and consumes more than 90% of global production (5). Based on the rice production estimates from the United States Department of Agriculture (USDA) for 2019-20, Bangladesh is the third-largest rice-producing country globally (6). Besides, Bangladesh is the most densely populated country, with approximately 160 million people consuming rice as their primary food (7,8). Soil salinity is a significant constraint on food production. Every day, an area of approximately 2000 ha of irrigated cropland is lost due to varying salinity levels around the world (9,10). It is also estimated that by 2050, more than half of the arable land will be salinized (11). Rice is a glycophytic plant, and salt stress has adverse effects on rice growth and yield,

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which depend on crop stages, stress severity and duration, and the tolerance of the rice variety (12). Salt tolerance differs among different germplasms, and among different rice growth stages. Hence, this study was designed for whole life cycle salinity screening with a population of 94 germplasm including resistant and susceptible checks and was further analyzed for GWAS and simultaneously, phenotypic evaluation with salinity scoring and leaf greenness measurement as salinity visual response through SPAD scoring was done. Utilizing GWAS for stress rice breeding is minimal in Bangladesh. This is the first GWAS approach with unique local rice germplasm in Bangladesh and this study will help to redesign the salinity tolerant breeding program in Bangladesh with identified tolerant germplasm and their introgression in main stream breeding pipeline through minimizing linkage drag by QTL deployment or line augmentation (systematic backcrossing steps) system.

The use of landraces has grown in recent years for their novel genetic architecture. The study of genetic diversity, population structure, linkage disequilibrium, genome-wide association studies (GWAS), QTL mapping, fine mapping, and gene cloning opens a new avenue in the integration of genomics and phenomics. It is essential to combine beneficial techniques such as GWAS and QTL blending with specific tolerance traits such as drought, salinity, flooding, disease, and insect resistance (13,14). The GWAS is one of the most popular methods for deciphering the genetic architectures of complex traits in crops. GWAS has proven to be an effective tool for identifying the genes responsible for complex biotic and abiotic stresses. Genome-level profiling of rice germplasm collections is an important first step in identifying divergent parents for effective use in rice breeding programs (15,16). This was the first major effort in Bangladesh to identify salt tolerance using a genome-wide association study, which used a panel of 92 local coastal rice germplasm collections and two standard salt checks. Based on morphological expression, the genotypes were screened for salinity tolerance. For whole-genome genotyping, all 94 rice genotypes were analyzed using 1 K RiCA panel SNP markers.

2. Materials and Methods

2.1 Experiment location

The experiment was carried out from June 2019 to November 2019 at the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. The investigation site was 34 meters above sea level and located at 24°03'79" North latitude and 90°39'63" East longitude (17). The average daytime and nighttime temperatures were 30 and 26 °C, respectively, with a relative humidity of 60 percent (18) . The Bangladesh Rice Research Institute (BRRI), Gazipur (23°99'00" North latitude 90°40'63" East longitude) Molecular Laboratory of Plant Breeding Division, prepared samples for the SNP panel (19). Experimental soil was prepared with a standard dose of fertilizer used in Bangladesh.

2.2 Experimental materials

In this investigation, 94 rice germplasm samples from various genetic backgrounds were employed, together with the widely used salinity-tolerant checks BRRI dhan67 and BRRI dhan73, and the two sensitive local checks, Barisail and Vobanibhog (Table S1). Sixty-seven genotypes were obtained from the BRRI's Genetic Resource and Seed Division. The remaining 27 genotypes were collected locally in Satkhira.

2.3 Experimental design

A completely randomized design (CRD), with one control set and two treatments, was used in the experiment. Treatment sets 1 and 2 were exposed to salinity levels of 8 dS/meter and 12 dS/meter, respectively, while the control set received no salinity exposure. The control and treatments were replicated three times.

2.4 Planting tray set up

A total of 846 pipes were used in the experiment to accommodate the replicated treatments, which included 846 plants with two treatments. The pipes were tied to the net at one end and poured with the soil after the soil was mixed with dry cow dung. In accordance with the instructions, an equal amount of fertilizer was applied to each pipe in the tray. Each pipe received an application of the prescribed fertilizer dose. In each pipe, chemical fertilizers such as urea (33.76 g), triple super phosphate (TSP, 36 g), muriate of potash (MoP, 56 g) and zinc sulphate ($ZnSO_4$, 41 g) were applied (453 mg mixture of all fertilizer in each pipe). Three separate applications of urea were made in three installments 15, 30 and 45 days after sowing in the pipe.

2.5 Sowing of pre-germinated seeds

To break dormancy, the seeds were dried in an oven for 72 hours at 48^0C. The fungicide Vitavax-200 (Syngenta) was used to sterilize the surface before it was rinsed with distilled water several times. After sterilizing the seeds, they were placed in petri dishes with wet moistened filter paper and incubated at 30^0C for 48 hours to encourage germination. The pre-germinated seedlings were planted in trays on 27/07/2019.

2.6 Agronomic management

To avoid rain, excess sunshine, and bird damage the experiment site was protected by polythene cover. This cover was removed daily to ensure optimal conditions for temperature and relative humidity. Weeding and gap-filling were among the necessary agronomic practices carried out. There were no herbicide applications. Weeding was carried out manually at regular intervals.

2.7 Preparation and application of saline solution

Water was collected from the Bay of Bengal and diluted according to study requirements to stress the plants with salinity. To prepare the saline solution, seawater was diluted to 8 and 12dS/m, respectively. Further, to avoid plant cellular osmotic shock, a dose of 4 and 6 dS/m saline water was applied one week before the final dosage on 18/08/2019 and 21/08/2019, on 28/08/2019, the plants began receiving treatments with 8 and 12 dS/m saline water. Simultaneous experiments were also carried out with a set of controls (no saline). Every day, the soil around the pipe was tested for salinity. According to Gregorio et al. (20) , the salinity response was scored using the SES (Standard Evaluation System) method to assess the visual symptoms of salt injury during the vegetative phase (Supplementary: Table S2). At the same time, reproductive phase salinity stress was scored using Table S3, and it was a unique assessment developed based on the stress state condition of

the plants (Table S3).

2.8 Data collection

For GWAS, data from all samples were collected and an average value was used. Data were collected on the number of tillers per plant, plant height (cm), panicle length (cm), panicle number and sterility percentage in accordance with IRRI guidelines (21) .

2.9 Sample preparation for molecular analysis

Leaf samples from each genotype were collected and stored in ultra-low temperatures at minus 80°C. During sample preparation, each sample was punched and inserted into the specific well of a 96-well plate according to the desired layout. Each tube was loaded with two leaf punches for each entry to ensure that the DNA sample was uniform. The sample plate was then oven-dried at 50^0C for 24 hours before being wrapped in a plastic zipper bag and sent for the 1 k RiCA panel (SNP Marker) test. Genotyping was done for 94 rice genotypes using 995 SNP markers at the Agriplex genomic, Cedar Avenue, Suite 250, Cleveland, 011444106, USA.

2.10 Statistical analysis and methodology for GWAS

Tassel 5.0 software (22) was used for genotypic data analysis. The General Linear Model (GLM) was used to map associations, including covariates. An adjusted Bonferroni correction method was used to determine the threshold level for significant SNP. Therefore, instead of α/ n, 1/n was used (22) . As a result of 1/ Total number of polymorphic markers =X and $-\log_{10}$ (X) = Y, the value of Y was considered the threshold level of Manhattan plots investigated in the research to determine the associated significant SNP position. Data filtering and imputation processes were used to eliminate monomorphic sites. Only 598 1K RiCA informative SNPs and 92 genotypes were considered after filtering and data quality control. The FILLIN method was used to impute missing genotype information when the number of considered high linkage disequilibrium (LD) sites was 30, the number of nearest neighbors was 10, and the maximum distance between location and LD was 10,000,000.

The threshold value of 4.07 was calculated as follows:

$0.01/ 598 = 1.67\times 10^{-5}$, and $-\log_{10} (1.67\times 10^{-5}) = 4.07$ (at 1 percent level of significance).

After the filtering, the number of SNP markers in this process was 598.

The genome-wide association study was visualized using the Manhattan plots for the studied traits. The threshold level was determined using the adjusted Bonferroni correction method of 1/n, according to Zhao et al. (23). The threshold value of 2.78 was calculated as follows:

$1/ 598 =0.00167$, and $-\log_{10} (0.00167) = 2.78$

3. Results

3.1 Salinity screening results of the germplasm

The experiments were conducted at salinities of 8 dS/m and 12 dS/m, respectively. For plants grown at 8 and 12 dS/m, the SES score was calculated to estimate the level of salinity tolerance. Figure 1 shows the SES score-wise distribution for responses to salt stress in the four different stressed conditions at 8 dS/m and 12 dS/m of salinity during the vegetative and reproductive phases of the studied genotypes.

Plants that grew at a similar rate as control plants while showing no signs of leaf disease were referred to as "high tolerance." Tolerant plants grew to the same size as the average, but the tips of the leaves were white and curled in them. Plants in the moderately tolerant group had severely slowed growth and folded leaves. Some plants have completely stopped growing and died as a result of the condition. Finally, the dead are classified as highly susceptible. Eighteen genotypes demonstrated a high level of tolerance to salinity, 24 were moderately tolerant, 37 were susceptible and four highly susceptible genotypes were found at 8 dS/m during the vegetative phase (Figure 1a, Table S2, Table S4). Figure 1c shows salinity stress in the vegetative phase at 12 dS/m salinity, as shown by the SES score. As a result, five genotypes investigated were found to be very tolerant; five were found to be tolerant, 15 genotypes are moderately tolerant, 17 susceptible, and 52 highly susceptible (Table S2, Table S6).

In the reproductive SES screening the highly salt tolerance genotypes had 70% fertile spikelets. Moderately tolerant genotypes had reduced plant height, delayed heading, shorter panicle length, and low spikelet fertility (30%). Genotypes with broken and empty spikelets were susceptible. Highly susceptible genotypes had no heading and died slowly. At 8 dS/m, seven genotypes demonstrated high salinity tolerance; 18 showed tolerance; 33 were moderately tolerant; 20 were susceptible and 10 genotypes were highly susceptible (Figure 1b, Table S3, Table S5).

At 12 ds/m, five genotypes showed high tolerance in this study. It is interesting to note that five of these accessions showed similar salinity tolerance in the initial treatment of 8 dS/m, suggesting new possible donors that are tolerant to salt stress. The tolerant group had four genotypes, 14 moderately tolerant genotypes, 10 susceptible genotypes and 61 highly susceptible genotypes (Figure 1d, Table S3 and Table S7). The symptoms and responses of plants to salt stress during both the vegetative and reproductive stages are depicted in Figure 2.

3.2 Genetic distance among the tested germplasm

Genotype data from TASSEL 5.0 is summarized (Table S8) (22). The genetic distance is calculated by comparing the allele frequencies between two genotypes. An estimated genetic distance of 598 1K RiCA SNP markers was used to calculate the IBS genetic distance between genotypes. The genetic difference between L-69 and BRRI dhan67 and BRRI dhan67 and Maitchal was the greatest at

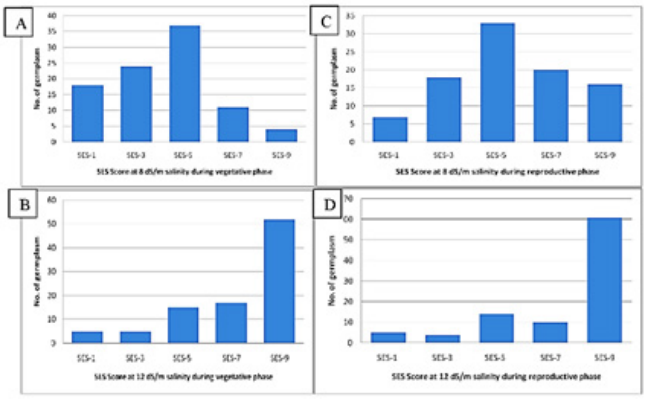

Fig. 1. SES score-wise distribution of the studied germplasm at two different salinity levels during the vegetative and reproductive phases.

0.61. BRRI dhan67 with nonakhorchi, jolpaira, chikirampatnai, and BRRI dhan73 with Gunshi obtained a distance of 0.60, which was the second-highest distance.

3.3 Diversity analysis by molecular SNP markers

In TASSEL 5.0, a circular alpha (Figure 3) was c to represent the population's genetic diversity based on simple parsimony substitution models. Exploration of genetic diversity of accessions from various geographic areas was expected to have a significant impact on native rice germplasm conservation and utilization programs. Four major clusters were formed. Based on genotype data, the alpha root matrix classified the 94 rice samples into four main clusters. These four categories were labelled I, II, III, and IV. The color dark red denotes cluster 1. Cluster II is represented by the colour blue, cluster III by the colour green, and Cluster IV by colour pink. Group I contained 58 accessions, Group II contained 12, Group III contained 17, and Group IV contained 7 accessions. Group I was subdivided into three sections: Ia, Ib and Ic. These three subgroups were made up of 10, 23 and 25 genotypes. One accession, Lamba-vojon, was observed scattered in a dendrogram.

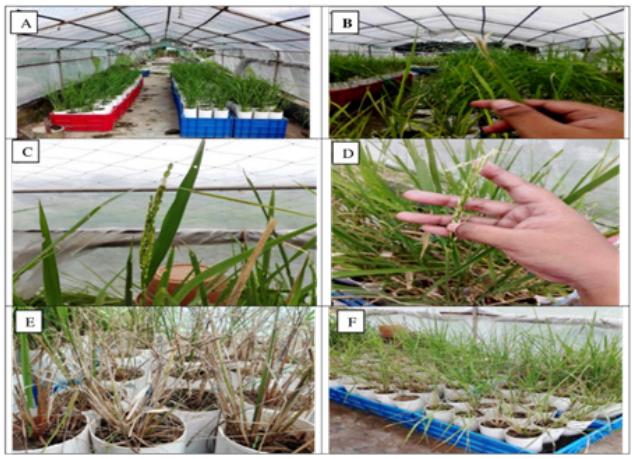

Fig. 2. (A) Stunted plants (B) Burning leaf tips (C) Tolerant germplasm showing flowering (D) Susceptible germplasm showing sterility (E) Rapid death of susceptible plants at 12dS/m at 80 DAS (Days after seeding) and (F) 8dS/m at 80 DAS.

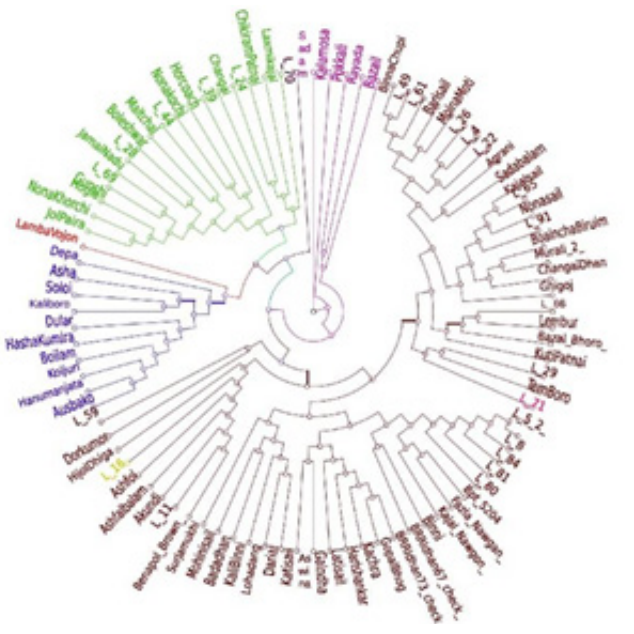

Fig. 3. Relatedness of the genotypes based on 1k RiCa SNP marker data by alpha (rooted) matrix.

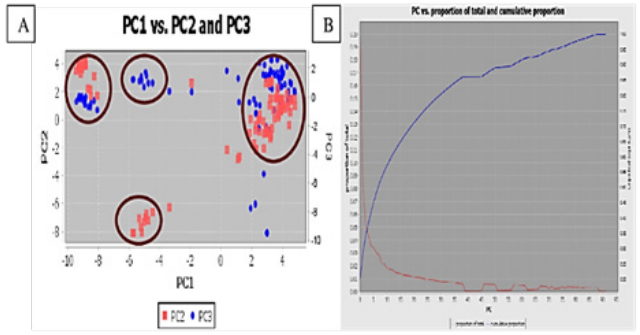

Fig. 4. (A) Four significant clusters were obtained from the principal component analysis (B) A scree plot to show the proportion of explained variance by PC.

3.4 Principal component analysis (PCA)

Using a 2D scatter plot of PCA and 598 filtered SNPs, four significant clusters were clearly distinguished among the rice populations (Figure 4.A), consistent with phylogenetic tree analysis results. The circle represented rice genotypes from these clusters. The principal component analysis revealed three main components that accounted for 77.2 percent of the total variance observed. This cumulative variance value breakdown revealed contributions of 41.5 percent, 21.7 percent, and 14.0 percent for PCA1, PCA2 and PCA3, respectively (Table S9). The scree plot in Figure 4.B displayed PC's proportion of explained variance. The first five principal components account for more than 40% of the total variance.

3.5 Association analysis by the general linear model (GLM)

TASSEL employs a fixed-effects linear model to examine the relationship between genotypes and phenotypes. This analysis considers population structure by utilizing covariates that indicate the degree of membership. The significant threshold for $-\log_{10}$ P-value is 4.07 at 1% level of significance, according to the Bonferroni correction method. This means that SNPs with a pre-specified value of 4.07 or higher are assumed to be associated with corresponding traits. It demonstrates a significant relationship between genotypic and phenotypic traits.

For the trait sterility (percent), seven significant associations with genotypic data were discovered (Figure 5.A). As a result, the presence of favourable alleles at the specified position was assumed to be responsible for determining sterility (percent) per plant as a result of salinity stress. Significant SNP were discovered at 14489210 bp on chromosome 12, 340012 bp on chromosome 10, 29056693 bp on chromosome 6, 7563923 bp on chromosome 1, 4019997 bp on chromosome 10, 41020155 bp on chromosome 1 and 15140411 bp on chromosome 12. The values of $-\log_{10}$(P) were 5.503, 3.755, 3.651, 3.433, 3.137, 3.088 and 2.970, respectively.

For the trait plant height (PH), three significant associations with genotypic data were discovered (Figure 5.B). The beneficial alleles were thought to reduce plant height due to salinity stress. A significant SNP was discovered on chromosome 12 at 14489210 bp and at 15140411 bp, on chromosome 1 at 6130240 bp. The value of $-\log_{10}$ (P) values were 4.637, 3.097 and 2.803, respectively.

For the trait, tiller number per plant (till) (Figure 5. C), two significant associations in chromosome 11 at 9561302 bp and in chromosome 4 at 4456304 bp were identified that could be responsible for the reduction in tiller number per plant due to salinity stress. The $-\log_{10}(P)$ values were 3.257 and 3.023, respectively.

For the trait panicle length, one significant association with genotypic data was found (Figure 5. D). Favourable alleles at the specified position were assumed to be responsible for panicle length reduction due to salinity stress. A significant SNP was discovered on chromosome 12 at 14489210 bp, with a $\log_{10}(P)$ value of 4.223.

For the trait panicle number per plant (pn), two significant associations with genotypic data were found (Figure 5. E). Due to salinity stress, the specified position may harbour favourable alleles responsible for panicle number regulation per plant. Significant SNPs were discovered on chromosome 12 at 14489210 bp and on chromosome 1 at 7563923 bp. The $-\log_{10}(P)$ were 3.480 and 3.138, respectively.

Three significant associations with the genotypic data were identified for the trait SPAD value. It was assumed that salinity level fluctuation was responsible for SPAD value regulation or chlorophyll content balancing (Figure 5. F). Significant SNPs were discovered on chromosome 4 at 19353709 bp, chromosome 7 at 18689329 bp and chromosome 5 at 2045112 bp. The values of $-\log_{10}(P)$ values were 3.622, 2.843 and 2.787, respectively.

3.6 SNP investigation based on the genome-wide association study

A total of thirteen different marker-trait association (MTA) were identified from the association study for the six traits studied in the Manhattan plot. A significant SNP was discovered at 14489210 bp for four other features: panicle number, sterility, plant height, and panicle length. For two traits, panicle number and sterility, a significant SNP position at 756393 bp was discovered. For two traits, sterility and plant height, a significant SNP position at 15140411 bp was discovered (Table 1 and Table S10).

Notably, the minor allele was thought to be a favourable allele because genotypes that showed tolerance to salinity based on the screening results were found to have a minor allele in the majority of cases in the genome-wide association study.

With an SES of 8 dS/m salinity stress during the reproductive phase, it was discovered that seven genotypes were highly saline tolerant and best-performing genotypes (Table S5). Hanumanjata had minor alleles (favourable alleles for salt tolerancel) at 14489210 bp, 2045112 bp, 4019997 bp and 6130240 bp, according to germplasm analysis. Koijuri, Asha and Dular shared a minor allele at 2045112 bp, 4019997 bp and 6130240 bp. Kutipatnai and Akundi both possessed a minor allele at 9561302 bp. After analyzing the Table, it was assumed that minor alleles at 4019997 bp, 2045112 bp, 6130240 bp, 3400212 bp, 14489210 bp and 4456304 bp were responsible for demonstrating tolerance to salinity stress (Table S10).

There were also several 18 tolerant and 33 moderately susceptible genotypes (Table S5). Susceptible checks BRRI dhan67 and BRRI dhan73 had minor alleles at 14489210 bp, 4456304 bp, 4019997 bp and 15140411 bp. Because of their matching tendencies on the tolerant genotypes, the minor allele was thought to be a favourable allele (Tables S11 and S12). The population containing favourable alleles was listed in the germplasm studied (Table S13).

3.7 Q/Q plot for the association study

The Q-Q plot was used to compare the number and magnitude of observed associations between genotyped single nucleotide polymorphisms (SNPs) and the traits under study to the null hypothesis of no association. The Q-Q plot can be used to describe how closely the observed distribution of the test statistic matches the expected (null) distribution. A p-value is statistically significant if it deviates from the expected distribution.

In Figure 6, the observed association statistics ($-\log_{10}$ P values) calculated from genotype and traits are ranked from smallest to largest on the Y-axis and plotted against the distribution expected under the null hypothesis of no association on the X-axis. Inflation from the identity line implies that the assumed distribution is responsible for a true association. The grey line represents the expected null distribution, while the red line represents the observed distribution.

The sharp increase above 2.78 is due to a strong association of the traits under study with salinity. This Q/Q plot displayed the attributes panicle number per plant (pn),

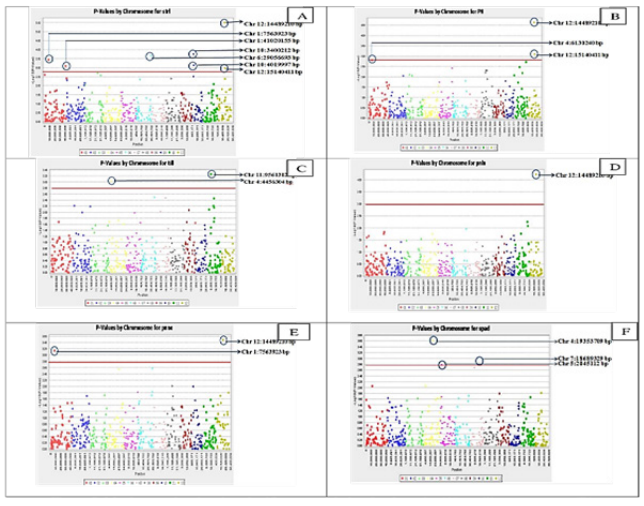

Fig. 5. Manhattan plot showing significant association for the traits (A) sterility percentage (B) plant height (C) tiller number (D) panicle length (E) panicle number (F) SPAD value with the 1k SNP molecular data.

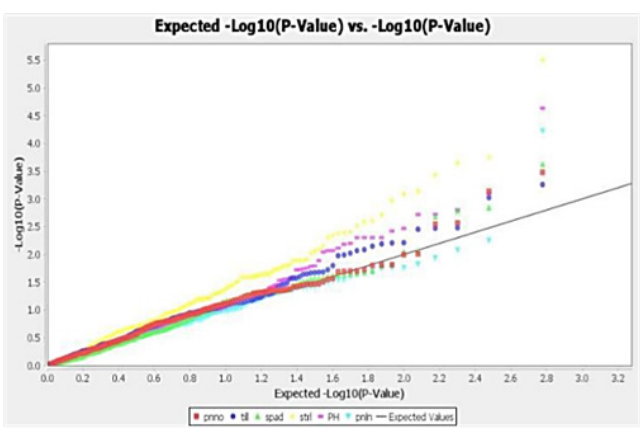

Fig. 6. Q/Q plots of association mapping for the studied traits using the GLM model.

Table 1. Trait-wise significant SNP investigation.

SL.No.	Traits responsive to salinity	value of $-\log_{10}(P)$ (above threshold)	Significant SNP Position (base pair)	Chromosome No.	Site No.	Allele (%)	
						Major allele	Minor allele
1	Panicle Number/ plant	3.480	14489210	12	574	C-73%	A-27%
		3.138	7563923	1	19	G-66%	A-34%
2	Tiller number per plant	3.257	9561302	11	513	G-58%	T-42%
		3.023	4456304	4	188	T-83%	C-17%
3	SPAD Value	3.622	19353709	4	216	A-89%	G-11%
		2.843	18689329	7	348	G-87%	T-13%
		2.787	2045112	5	242	G-70%	T-30%
4	Spikelet Sterility (%)	5.503	14489210	12	574	C-73%	A-27%
		3.755	3400212	10	461	T-83%	C-17%
		3.651	29056693	6	332	A-78%	G-22%
		3.433	7563923	1	19	G-66%	A-34%
		3.137	4019997	10	462	C-75%	T-25%
		3.088	41020155	1	66	C-70%	T-30%
		2.970	15140411	12	577	A-87%	T-13%
5	Plant Height	4.637	14489210	12	574	A-27%	C-73%
		3.097	15140411	12	577	A-87%	T-13%
		2.803	6130240	1	16	T-68%	G-32%
6	Panicle Length	4.223	14489210	12	574	C-73%	A-27%

Table 2. List of functional genes locating the identified MTA.

SL. No.	SNP (bp)	Chromosome	Gene (Name)	Range of Gene
1	6130240	1	BGIOSGA002167	6129869-6134479
2	4456304	4	BGIOSGA015573	4451144-4462651
3	18689329	7	BGIOSGA025843	18686028-18689946

tiller number per plant (till), SPAD value (spad), sterility (percent) (str), plant height (PH) and panicle length (pl). The thirteen MTA were searched in the Gramene database at https://www.gramene.org/, and only three MTA were found within the range of functional genes (Table 2).

4.Discussion

The tested germplasm was divided into five groups based on Standard Evaluation System (SES) scores of 8 and 12 dS/m of salinity for both the vegetative and reproductive phases. They were highly tolerant, tolerant, moderately tolerant, susceptible, and highly susceptible. The rapidly decreasing trend of SPAD readings in susceptible genotypes indicates that their chlorophyll content is rapidly depleting. The SPAD chlorophyll meter is useful for quickly estimating the chlorophyll content of crops (24) . Several studies have found that salinity stress has a negative impact on the vegetative and reproductive stages of rice crops (25) . By maintaining chlorophyll levels on leaves, the salt-tolerant genotypes were able to minimize salt injury. Salinity stress reduces plant greenness or chlorophyll concentration (26) . As a result, genotypes with tolerant genes experience minor salinity injuries than susceptible genotypes. Moderately tolerant genotypes experienced severe growth retardation and rolled leaves. During salinity stress, the inhibitory accumulation of ions of various salts on the biosynthesis of different chlorophyll fractions may reduce chlorophyll concentration in the leaves (26) . Salinity stress also has an impact on rice reproductive performance, specifically spikelet number, spikelet fertility, and plant productivity (27). Salinity reduces spikelet fertility by up to 70% in rice as reported by Dooki et al. (28).

Exploration of genetic diversity of accessions from various geographic areas was expected to have a significant impact on native rice germplasm conservation and utilization programmes (29). The genetic distance between two germplasms is measured by the difference in the allele frequency (30). Molecular tools enable the study of genetic distances between genotypes, allowing for more effective crop improvement programmes. The genetic distance between genotypes was calculated using filtered 598 1K RiCA SNP markers. The number of shared genes that were identical by state was used to detect a linkage between the marker loci and the targeted gene (31). TASSEL 5.0 genotypic data revealed the greatest genetic distance (0.61) between the genotypes L-69 with BRRI dhan67 and BRRI dhan67 with Maitchal. These genotypes could be used in future breeding to create recombinant stress-tolerant lines.

Circular alpha was built in TASSEL 5.0. to represent genetic diversity within the population and divided 94 rice accession into four groups, each of which was further subdivided into sub-groups. The accession Lamba-vojon stands apart from the other accessions in the dendrogram. Using principal component analysis (PCA) on 598 filtered SNPs, four significant clusters were clearly distinguished among rice populations, correlating with phylogenetic tree results. The distribution pattern for the entire genome was visualized using graphs generated as linkage disequilibrium (LD) heatmaps with 598 filtered SNP markers. LD calculated r2 and suggested the degree of association (32). Lower LD values approaching 0 (zero) indicate lower linkage disequilibrium, which implies no linkage. Higher LD values near 1.0 indicate that higher levels of linkage disequilibrium are more likely to be inherited together (32) .

According to the Bonferroni correction method, the significant threshold for the –log10 P value is 4.07 at the 1% level of significance. This means that SNPs with a predetermined deal of 4.07 or higher are assumed to be associated with corresponding traits (33). Following the current Bonferroni correction method, the trait sterility (percent) showed a significant association, while other attributes showed a non-significant association. Seven significant associations with genotypic data were found for the trait sterility (percent). As a result, the appearance of favourable alleles at the specified position was assumed to be responsible for determining sterility (percent) per plant because of salinity stress (33).

Minor alleles at 14489210 bp, 4456304 bp, 4019997 bp, and 15140411 bp were found in the tolerant checks BRRI dhan67 and BRRI dhan73, which were thought to be favorable alleles due to their matching tendencies on the tolerant genotypes. Hanumanjata, Koijuri, Asha and Dular all had minor alleles that were similar or shared at 4019997 bp, 2045112 bp, 6130240 bp, 3400212 14489210 bp, 4456304 bp. Minor alleles were assumed to be responsible for tolerance to salinity stress in the results. Because the genotypes were found to have a minor allele in most cases in genome-wide association, those tested positive for salinity tolerance. SALTOL-AUS and SALTOL-ARO are the two QTLs responsible for salinity tolerance, and their favourable alleles have previously been reported (34,35). For the six traits studied in the GWAS, a total of 13 different marker-trait association (MTA) were discovered. A significant SNP was discovered at 14489210 bp for four other features: panicle number, sterility, plant height, and panicle length. The Gramene database (https://www.gramene.org/) was searched for the thirteen MTA (36). Because only three MTA were rediscovered within the range of functional genes, these three SNP will need to be studied further to determine their role in regulating salinity stress in rice. There are many reports regarding the effect of salinity on the biology, physiology and biochemical of different plants (37-40) that the effect of genome and genes on these mechanisms can be investigated.

5. Conclusion

Screening by SES score identified the study's target salt donor genotypes, which will be the primary source for developing salt-tolerant rice in the future. A diversity panel of the germplasm tested revealed four major clusters within the population. The rice samples were divided into four major groups based on the molecular diversity analysis (groups I, II, III, and IV). By combining better alleles from different sources, crossing genetically diverse and distant germplasm will help to broaden the genetic base and increase the degree of tolerance. This population's GWAS study suggested that multiple mechanisms for higher salt tolerance in rice can be combined. In the tested genotypes, GWAS revealed thirteen significant associations (MTA) between 1 K RiCA SNP markers and the studied traits. Salinity screening revealed seven tolerant genotypes: Koijuri, Asha, Kajal, Kaliboro, Hanumanjata, Akundi and Dular. They also have some common (minor) alleles for specific MTAs. As a result, the presence of minor alleles may be responsible for their salt tolerance mechanism. Further research into the functional genes that localize the identified MTAs is possible. Tolerant germplasm can be used to improve salt tolerance and provide new sources of salt tolerance for future breeding.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions

Nusrat Jahan, Mohammad Sharif Raihan, M. Moshiul Islam, Farzana Mustafa Era, A. K. M. Aminul Islam , designed, conducted the research, analysis the data as well as prepared the manuscript. Adel I. Alalawy, Awatif M. E. Omran, Yasmene F. Alanazi, Mohamed Sakran, Abdulrahman Alasmari, Fahad M. Alzuaibr, Danial Kahrizi, Ayman El Sabagh , also contributed during writing the manuscript and advised scientific suggestion as well as revised/edited the manuscript.

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