



# Genome wide association study of MAGIC population reveals a novel QTL for salinity and sodicity tolerance in rice

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**Abstract** The present study was conducted to identify the novel QTLs controlling salinity and sodicity tolerance using *indica* MAGIC rice population. Phenotyping was carried out in salinity (EC ~ 10 dS/m) and sodicity (pH ~ 9.8) at the seedling stage. Among 391 lines, 43 and 98 lines were found tolerant and moderately tolerant to salinity. For sodicity condition, 2 and 45 lines were showed tolerance and moderately tolerance at seedling stage. MAGIC population was genotyped with the help of genotyping by sequencing (GBS) and filtered 27041SNPs were used for genome wide marker trait association studies. With respect to salinity tolerance, 25 SNPs were distributed on chromosomes 1, 5, 11 and 12, whereas 18 SNPs were mapped on chromosomes 6, 4 and 11 with LOD value of > 3.25 to sodicity tolerance in rice. The candidate gene analysis detected twelve causal genes including *SKC1* gene at *Saltol* region for salinity and six associated genes for sodic stress tolerance. The significant haplotypes responsible for core histone protein coding gene (*LOC\_Os12g25120*) and three uncharacterized protein coding genes (*LOC\_Os01g20710*, *LOC\_Os01g20870* and

*LOC\_Os12g22020*) were identified under saline stress. Likewise, five significant haplotypes coding for ribose 5-phosphate isomerase (*LOC\_Os04g24140*), aspartyl protease (*LOC\_Os06g15760*), aluminum-activated malate transporter (*LOC\_Os06g15779*), OsFBX421-Fbox domain containing protein (*LOC\_Os11g32940*) and one uncharacterized protein (*LOC\_Os11g32930*) were detected for sodic stress tolerance. The identified novel SNPs could be the potential candidates for functional characterization. These candidate genes aid to further understanding of genetic mechanism on salinity and sodicity stress tolerance in rice. The tolerant line could be used in future breeding programme to enhance the salinity and sodicity tolerance in rice.

**Keywords** GWAS · MAGIC · GBS · Salinity · Sodicity · Rice

## Introduction

Rice is one of the important staple food crops, about 50 per cent of the global population consumes it as main source of energy. The global population is increasing day by day and it is expected to reach around 9.5 billion in 2050. Therefore, world food production needs to be increased by 70% (FAO 2013). Hence, there is a need to increase the productivity of rice by combating its low productivity caused by various biotic and abiotic stresses. Among the abiotic stresses, salinity and sodicity are the most important environmental factors hampering the crop productivity by ionic disequilibrium and disrupting the metabolic activities of the plants (Munns and Tester 2008). Salinity and sodicity stresses are highly influenced by environmental fluctuations like rise in temperature and relative humidity

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(Tack et al. 2015). Salt stress affects all the stages of plants but early seedling and reproductive stage are most sensitive in rice (Singh et al. 2010; Singh and Flowers 2010). Salinity stress reduces root length and shoot length at seedling stage, while tiller number, number of spikes, panicle length and spikelet fertility at reproductive stage (Ali et al. 2013; Krishnamurthy et al. 2016a). Sodicity also affects crop productivity. Many researchers have reported the effect of sodic soils on growth and development in rice (Singh et al. 2013; Krishnamurthy et al. 2017). However, less information is available on molecular basis for sodicity tolerance. Thus, it is necessary to study the molecular mechanisms underlying sodic stress.

It is very important to know the effect of salt stress on several traits in rice to breed the well adoptable salt tolerant varieties (Arshadullah et al. 2011). Various studies have been done on molecular breeding for abiotic stresses in rice. Several QTLs have been identified for salinity tolerance in rice (Lang et al. 2000; Tuan et al. 2000; Warraich et al. 2020; Mazumder et al. 2020). *Saltol* QTL was mapped on chromosome 1 using RIL population of Pokkali/IR29 by AFLP markers (Gregorio 1997). *Saltol* QTLs facilitates marker-assisted backcrossing breeding (MABB) to introgress it to popular rice varieties (Thomson et al. 2010) and has been introgressed into mega rice varieties (Singh et al. 2016; Babu et al. 2017; Geetha et al. 2017; Bhandari et al. 2019; Jaiswal et al. 2019; Krishnamurthy et al. 2021; Yadav et al. 2020). Majority of the salt tolerant rice varieties were developed based on the single crosses between two diverse parents. However, attempts were made to develop varieties employing multiple crosses involving three parents (Jansen et al. 2003). Due to technical complications like large population size and precision these multiple crosses were not frequently used by breeders. Only few reports were available on development of varieties by multi-parent advanced generation inter cross (MAGIC) population and also on identification of the QTLs for salt tolerance in rice. GWAS utilizes a wide range of diversity based on the groups of non-related individuals and take the advantage of the past crossing over and recombination events that have accrued over the generations (Korte and Farlow, 2013). Complex agronomic traits such as flowering time and grain related traits in rice have been detected by this approach (Huang et al. 2011). Linkage disequilibrium (Visscher et al. 2012) and non-identification of phenotypes caused by rare alleles (Kover and Mott, 2012) limits the application of GWAS by breeders. Earlier investigators have proposed the development of advanced intercross lines (AILs) to increase the number of recombination (Darvasi and Soller, 1995). But epistasis and the effect of genetic background were not considered in these populations. Recently, this AIL methodology has been extended into multiple parent

populations. Multi-parent advanced generation intercross (MAGIC) populations has been used in model plant species *Arabidopsis* for the first time to make accessible of distended recombination and high mapping resolution (Cavanagh et al. 2008). MAGIC has a better control over population structure along with kinship and highly effective in identifying major genes through GWAS strategy (Bandillo et al. 2013; Ponce et al. 2020). This MAGIC population was employed in different cereal crops by many workers. With this background, the purpose of the present study was to (i) know the effect of salinity and sodicity on *indica* MAGIC population (ii) identify the novel QTLs for salinity and sodicity tolerance at seedling stage in rice.

## Materials and methods

### Plant materials

The *indica* MAGIC population was developed using eight originator lines that include both elite and modern varieties. These founder lines known to exhibit tolerance to both biotic and abiotic stresses along with good grain quality (Table 1).

### Phenotyping of MAGIC population in saline stress

A set of 391 *indica* MAGIC lines procured from IRRI, Philippines were evaluated for salinity tolerance along with 8 parents, FL478 (tolerant check) and IR29 (sensitive check) using Yoshida nutrient solution (Yoshida et al. 1976). The experiment was piloted in a controlled glasshouse at Central Soil Salinity Research Institute (CSSRI), Karnal under two different stress conditions, namely salinity stress (EC ~ 10 dS/m) and normal (EC ~ 1.2 dS/m) by hydroponic technique at seedling stage (IRRI, 1996). The nutrient solution used in hydroponics was salinized by adding NaCl to acquire the desired levels of salinity. Saline stress of EC ~ 10 dS/m was induced at 14 days after sowing (DAS) and the anticipated level of salinity was maintained for the next 14 days. The revised standard evaluation system (SES) was used in screening the visual symptoms of salt injury (1 = highly tolerant, 3 = tolerant, 5 = moderately tolerant, 7 = susceptible and 9 = highly susceptible) and genotypes were scored after 14 days of salinization for salinity tolerance (IRRI, 1996). In this experiment, the data was recorded on salt injuries score (1–9), root length (cm) and shoot length (cm).

### Phenotyping of MAGIC population in alkaline stress

The same set of 391 *indica* MAGIC lines was evaluated for sodic stress in a controlled condition at CSSRI, Karnal. The

**Table 1** Agronomic relevance of the 8 founder lines used in developing the *indica* MAGIC population

Germplasm/variety	Varietal type	Origin	Agronomic relevance
Fedearroz 50	<i>Indica</i>	Colombia	Popular variety in several countries, with stay green/delayed senescence & quality traits, disease tolerance, progenitor of many breeding lines
Shan-Huang Zhan-2 (SHZ-2)	<i>Indica</i>	China	Blast resistant, high yielding; in the pedigrees of many varieties in south China
IR64633-87-2-2-3-3 (PSBRc82)	<i>Indica</i>	IRRI	High yielding and most popular variety of the Philippines
IR77186-122-2-2-3 (PSBRc 158)	<i>Indica / tropical japonica background</i>	IRRI	High yielding variety in New Plant Type II background
IR77298-14-1-2-10	<i>Indica</i>	IRRI	Drought tolerant in lowlands with IR64 background and tungro resistance
IR4630-22-2-5-1-3	<i>Indica</i>	IRRI	Good plant type, salt tolerant at seedling and reproductive stages
IR45427-2B-2-2B-1-1	<i>Indica</i>	IRRI	Fe toxicity tolerant
Sambha Mahsuri + Sub1	<i>Indica</i>	IRRI	Mega variety with wide compatibility, good grain quality and submergence tolerance

experiment was carried out using a sodic soil in the tray. To obtain the desired level of sodicity (pH ~ 9.7 to 9.8), required amount of sodium bicarbonate ( $\text{NaHCO}_3$ ) and sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) were added to the soil that simulating the field conditions. Visual indications of the salt injury (1 = highly tolerant, 3 = tolerant, 5 = moderately tolerant, 7 = susceptible and 9 = highly susceptible) was recorded by the revised standard evaluation system (SES) (and scoring of genotypes was done after 14 days of stress (IRRI, 1996). The observation was taken on salt injuries score (1–9) and shoot length (cm) for this investigation.

### Genotyping by sequencing (GBS) for genome-wide SNP analysis in *indica* MAGIC population

Genotyping by sequencing (GBS) is a novel technique for genotyping single nucleotide polymorphism (SNP) which has been utilized in implementation of GWAS (genome-wide association study) in different populations for crop improvement (Elshire et al. 2011; Huang et al. 2010). The raw GBS data of 391 *indica* MAGIC population was processed and 109,610 SNP markers were obtained. The processed data contains a large proportion of missing calls along with many heterozygous SNPs in the population. Further, filtering of data was done with missing rates > 30% and minor allele frequencies < 5%. Finally, we obtained 27,041 SNP sites and that was used for Marker Trait Association studies (MTA) (Raghavan et al. 2017).

### Statistical analysis

The filtered GBS data provided a total of 27,041 SNP marker sites across all the 12 chromosomes and that were used to create kinship matrix with the help of Trait Analysis by Association, Evolution and Linkage (TASSELV5.2) program (Bradbury et al. 2007). Genotypic, kinship matrix and phenotypic data of SES score for salinity and sodicity were combined for Marker Trait Association (MTA) analysis by mixed linear model (MLM) in 391 MAGIC lines. The model statistics gives the p-value along with  $R^2$  value for the marker and we have used a cut-off *P* value < 0.001 for detection of trait association (Zhang et al. 2010). The Manhattan and Q-Q plots were visualized through the qqman package in R programme (Turner, 2014). The significant SNPs (LOD of > 3.25) associated with traits were taken into consideration for candidate gene analysis by using the Rice Annotation Project (RAP) database genome browser (<http://rapdb.dna.affrc.go.jp/viewer/gbrowse/irgsp1>). The SNP positions of genes associated to trait of interest were recognized as candidate genes. The haplotype analysis for detected candidate genes was conducted by CandiHap V2. (<https://github.com/xukaili/CandiHap>).

### Results

In order to identify QTLs for salinity and sodic stress, we screened the 391 *indica* MAGIC populations in glasshouse condition and then 27,041 SNP sites were used for association studies. The results of Marker Trait Association (MTA) are presented below.

### Phenotyping of parents and MAGIC lines for non-stress and salinity stress at seedling stage

Based on the results of vigor score (SES-score), shoot and root length among eight *indica* parents, IR4630-22-2-5-1-3 and IR77186-122-2-2-3 (PSBRc 158) genotypes exhibited moderately tolerant response (SES-5); Fedearroz 50 and IR45427-2B-2-2B-1-1 genotypes were found susceptible (SES-7); remaining parents like Shan-Huang Zhan-2 (SHZ-2), IR64633-87-2-2-3-3 (PSBRc82), IR77298-14-1-2-10 and Sambha Mahsuri + Sub1 showed highly susceptible reaction (SES-9) with respect to salinity stress (supplementary table 1). The results of mean, range and per cent reduction of different traits for 391 MAGIC lines are represented in supplementary table 2. In general, all the MAGIC lines were showing reduction in their root and shoot length and these reductions varied among the lines. The frequency distribution of 391 MAGIC rice lines were estimated in all the stresses and furnished in supplementary Fig. 1. The findings indicate that tolerant and sensitive groups of MAGIC lines differed significantly in all the parameters, including reduction in shoot length, root length and vigor score (SES). Per cent root length reduction was 23% and shoot length reduction accounts for 30.56% by salinity stress in all the MAGIC lines. The root length (cm) ranged from 2.33 (IR 93,347:21-B-7-7-5-1RGA-2RGA-1-B-B) to 21.3 (IR 93,337:28-B-9-3-20-1RGA-2RGA-1-B-B) in non-stress and 2.27 (IR 93,327:27-B-8-19-6-1RGA-2RGA-1-B-B) to 14.50 (IR 93,353:15-B-3-22-7-1RGA-2RGA-1-B-B) in saline stress condition. The range of shoot length (cm) was 19.20 (IR 93,336:31-B-16-23-6-1RGA-2RGA-1-B-B) to 61.80 (IR 93,350:45-B-23-16-15-1RGA-2RGA-1-B-B) in non-stress and 14.40 (IR 93,343:14-B-12-5-20-1RGA-2RGA-1-B-B) to 35.73 (IR 93,347:2-B-10-14-4-1RGA-2RGA-1-B-B) in salt stress environment (supplementary table 2). With respect to SES, maximum number of lines scored 7 (sensitive) under salinity stress at seedling stage. Out of 391 lines, 43 genotypes were salinity tolerant and 98 genotypes were moderately tolerant (supplementary table 3). The lines expressing the tolerance to salinity are listed in supplementary table 4.

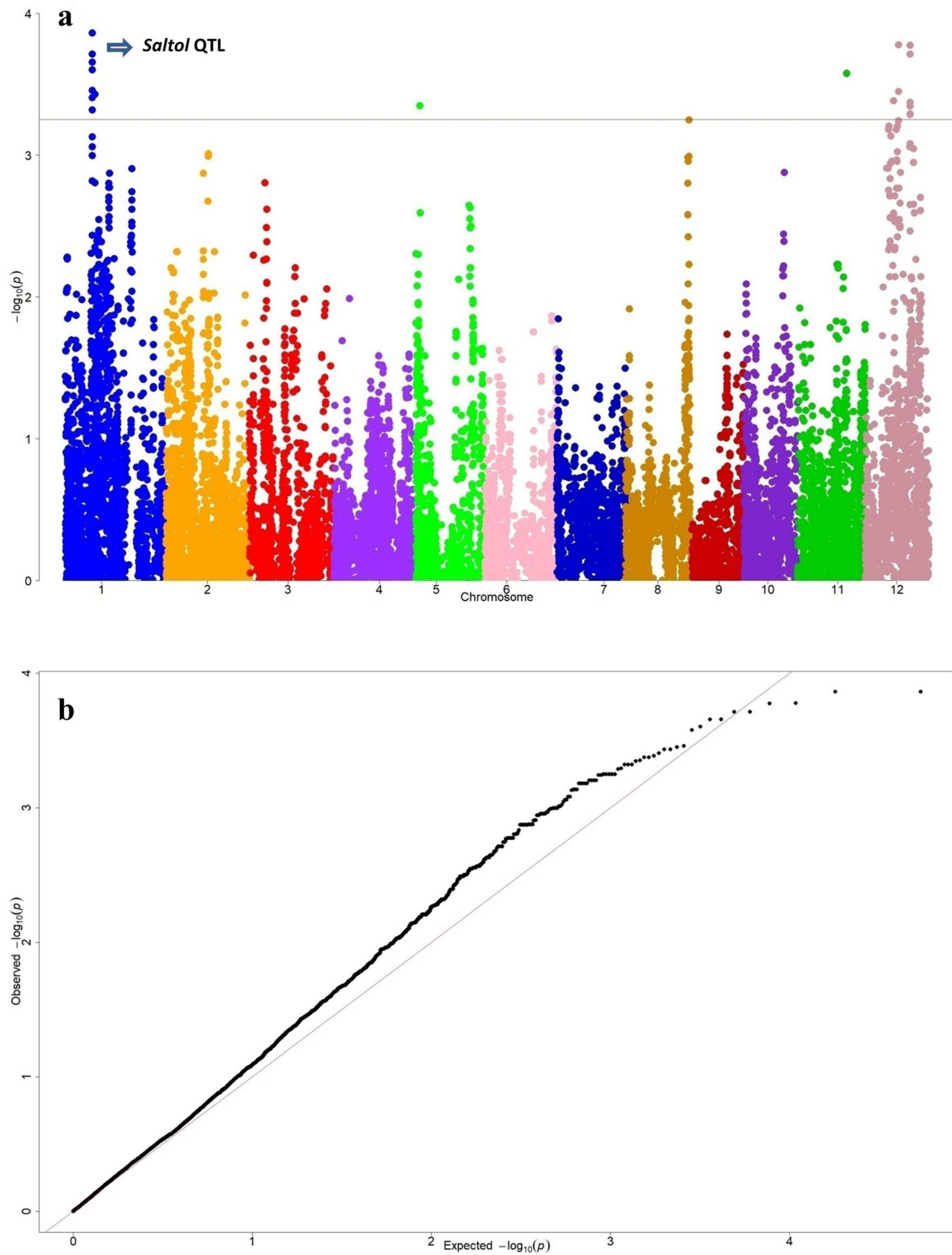
### Phenotyping of MAGIC lines for sodicity stress at seedling stage

Shoot length (cm) was ranged from 6.44 (IR 93,346:35-B-23-2-7-1RGA-2RGA-1-B-B) to 48.34 (IR 93,341:59-B-3-13-10-1RGA-2RGA-1-B-B) in sodic conditions (supplementary table 2). Maximum number of genotypes scored 9 (highly sensitive) under sodicity. Among the 391 lines, two genotypes were tolerant and 45 genotypes were found moderately tolerant to sodicity at seedling stage (supplementary table 3). The genotypes, IR 93,326:18-B-

12-7-15-1RGA-2RGA-1-B-B and IR 93,328:13-B-8-6-23-1RGA-2RGA-1-B-B were found to be tolerant in terms of sodicity stress (supplementary table 5).

### Association mapping and candidate genes haplotype analysis for salinity tolerance at seedling stage

The GWAS was conducted for salt injury score (1–9 vigour score) at salinity stress to identify causal genes. The genome wide Manhattan plot for salinity vigor score and Q-Q plot are shown in Fig. 1. In the present study, we detected a total of 25 SNPs significantly associated with (LOD value of > 3.25) the trait vigor score under salt stress at seedling stage. The associated markers were distributed across the chromosomes, among them 13 SNPs found on chromosome 1, 10 SNPs on chromosome 12 and single peaks were observed on chromosome 5 and 11. The chromosome 1 and 12 consists of QTL regions for salinity tolerance at seedling stage. The origin of GWAS peaks pertaining to salinity tolerance at chromosome 1 was tracked between the 128 kb region from 11.523 to 11.647 Mb and located eleven SNP markers. These markers situated near to *Saltol* region, a known and highly considered region for salinity tolerance at seedling stage in rice. Similarly, seven peak SNP markers on chromosome 12 were positioned in between 36 kb region from 19.369 to 19.405 Mb. At the chromosome 1, we found a peak at 11.523233 which is 60 kb apart from *SKC1*, the well known gene responsible for salinity tolerance at seedling stage. The identified SNPs responsible for salt tolerance were used for candidate gene analysis and detected eleven putative candidate genes lying in trait associated regions are presented in Table 2. Out of eleven genes, five genes *LOC\_Os01g20710*, *LOC\_Os01g20870*, *LOC\_Os12g22020*, *LOC\_Os12g32150* and *LOC\_Os12g32160* codes for uncharacterized hypothetical proteins. Remaining six genes were annotated for stress related proteins like C-NBS-LRR domain (*LOC\_Os01g20720*), heavy metal and copper transport associated protein (*LOC\_Os01g20830*), retrotransposon protein containing pectin lyase fold domain (*LOC\_Os01g22590*), WRKY-109 comprising of DNA binding domain (*LOC\_Os05g03900*) and core histone related (H2A/H2B/H3/H4) protein (*LOC\_Os12g25120*) for salt tolerance at seedling stage. Candidate gene haplotype analysis was carried out for these genes along with *SKC1* (*LOC\_Os01g20160*) which confers seedling stage salinity tolerance in rice is presented in Table 3. We observed a range of two to eight haplotypes for each putative locus. The significant haplotypes were found for *LOC\_Os01g20710*, *LOC\_Os01g20870*, *LOC\_Os12g22020* and *LOC\_Os12g25120* (Fig. 2 and supplementary Fig. 2) genes. The haplotype analysis indicates three haplotypes for *SKC1* (*LOC\_Os01g20160*) gene which was present on



**Fig. 1** **a** Genome wide distribution of SNPs presented in Manhattan plot for salt injury score (vigour score) for salinity stress through MLM model, **b** The expected and observed  $-\log_{10}$  probability distribution of SNPs presented in Q-Q plot for salt injury score

(vigour score) for salinity stress through MLM model. *Saltol* region, a known QTL responsible for salinity tolerance at seedling stage on chromosome 1 was highlighted

**Table 2** Associated QTLs with SNP position and probable candidate genes for salinity tolerance in MAGIC population

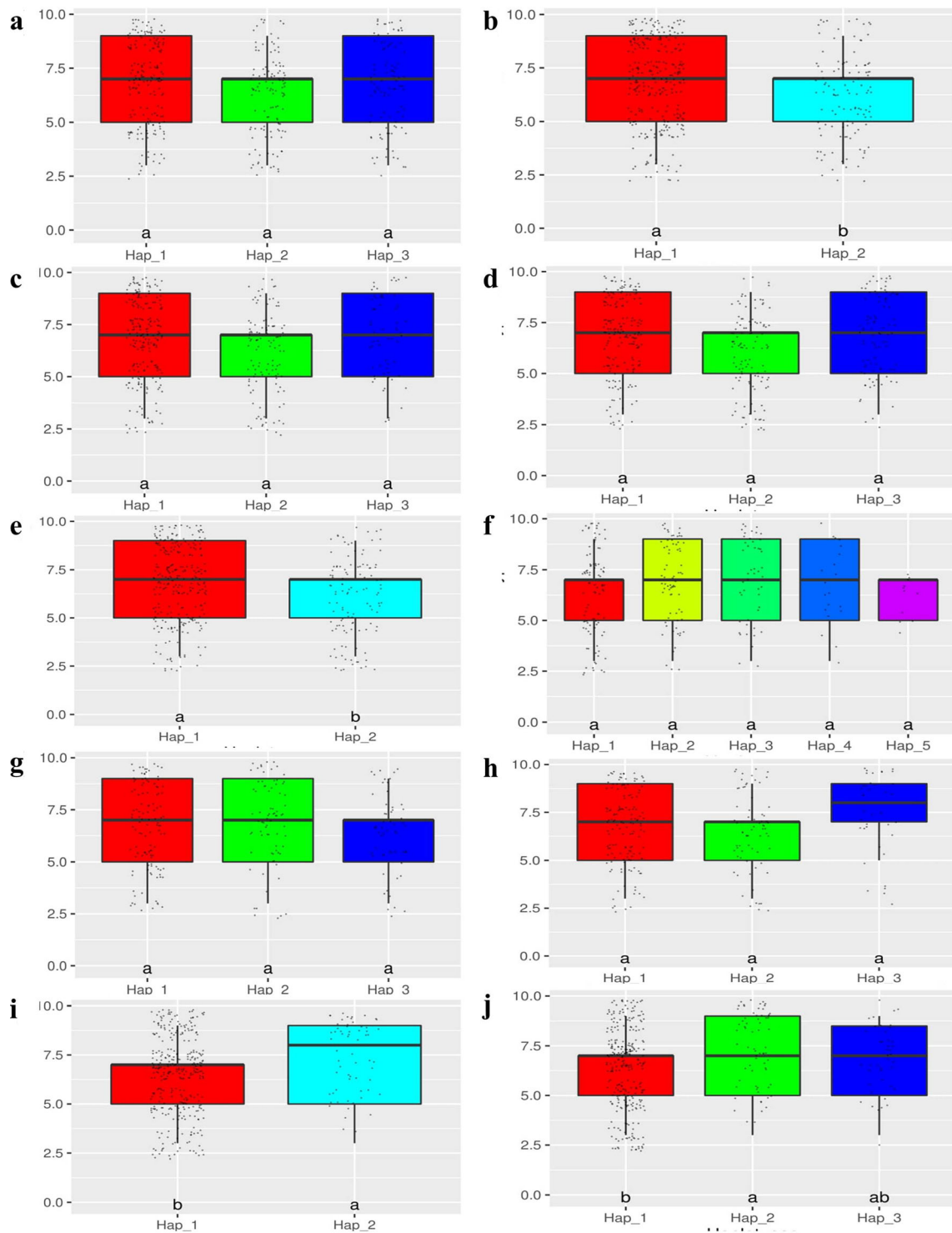
Sl.No	Chr	Peak SNP	<i>P</i> value	LOD	MarkerR <sup>2</sup>	FDR	Locus ID	Gene annotation
1	1	11,523,233	4.81E-04	3.32	0.031	0.416	LOC_Os01g20710	Uncharacterized expressed protein
2	1	11,529,325	4.81E-04	3.32	0.031	0.416	LOC_Os01g20720	CC-NBS-LRR,Putative expressed and response to stress
3	1	11,529,368	4.81E-04	3.32	0.031	0.416		
4	1	11,587,558	3.49E-04	3.46	0.032	0.416		
5	1	11,596,445	3.94E-04	3.40	0.032	0.416		
6	1	11,601,232	2.21E-04	3.66	0.035	0.416		
7	1	11,611,121	2.21E-04	3.66	0.035	0.416	LOC_Os01g20830	Heavy metal associated protein
8	1	11,623,953	1.38E-04	3.86	0.037	0.416		
9	1	11,624,067	1.38E-04	3.86	0.037	0.416		
10	1	11,624,734	1.94E-04	3.71	0.035	0.416		
11	1	11,647,832	2.51E-04	3.60	0.034	0.416	LOC_Os01g20870	Expressed hpothetical protein
12	1	12,702,701	3.71E-04	3.43	0.032	0.416	LOC_Os01g22590	Retrotransposon pectin lyase fold domain containing protein
13	1	12,702,721	3.71E-04	3.43	0.032	0.416		
14	5	1,743,516	4.47E-04	3.35	0.031	0.416	LOC_Os05g03900	WRKY 109- DNA-binding domain containing protein
15	11	21,055,879	2.66E-04	3.58	0.034	0.416	LOC_Os11g35870	RING finger family RWD domain containing protein
16	12	12,383,067	4.14E-04	3.38	0.032	0.416	LOC_Os12g22020	Expressed protein
17	12	14,424,756	1.67E-04	3.78	0.036	0.416	LOC_Os12g25120	Core histone H2A/H2B/H3/H4,
18	12	14,430,101	3.56E-04	3.45	0.032	0.416		
19	12	19,369,099	4.23E-04	3.37	0.031	0.416		
20	12	19,369,102	4.23E-04	3.37	0.031	0.416		
21	12	19,392,199	1.94E-04	3.71	0.035	0.416	LOC_Os12g32150	Expressed conserved hypothetical protein
22	12	19,392,202	1.68E-04	3.77	0.036	0.416		
23	12	19,392,459	5.18E-04	3.29	0.031	0.416		
24	12	19,400,490	5.11E-04	3.29	0.031	0.416		
25	12	19,405,473	4.52E-04	3.34	0.031	0.416	LOC_Os12g32160	Expressed hpothetical protein

**Table 3** Alternative haplotypes for candidate genes for salinity tolerance identified by GWAS in MAGIC population

Locus ID	Haplo group	Number of lines	SNP positions
LOC_Os01g20160	Hap_1	180	11,460,324 GG
	Hap_2	115	11,460,344 GG
	Hap_3	96	11,460,344 TT
LOC_Os01g20710	Hap_1	276	11,523,233 CC
	Hap_2	115	11,523,233 TT
	Hap_3	62	11,523,233 GG
LOC_Os01g20720	Hap_1	214	11,529,325 GG
	Hap_2	115	11,529,325 TT
	Hap_3	62	11,529,325 GG
LOC_Os01g20830	Hap_1	181	11,611,121 GG
	Hap_2	116	11,611,121 TT
	Hap_3	94	11,611,121 CC
LOC_Os01g20870	Hap_1	274	11,647,832 GG
	Hap_2	117	11,647,832 AA
	Hap_3	96	11,647,832 TT
LOC_Os01g22590	Hap_1	96	12,701,866 TT
	Hap_2	73	12,701,866 TT
	Hap_3	48	12,701,866 CC
LOC_Os05g03900	Hap_1	20	12,702,701 TT
	Hap_2	11	12,702,701 CC
	Hap_3	2	12,702,701 TT
LOC_Os11g35870	Hap_1	142	21,055,876 GG
	Hap_2	70	21,055,876 TT
	Hap_3	38	21,055,876 TT
LOC_Os12g22020	Hap_1	329	12,383,067 GG
	Hap_2	62	12,383,067 CC
	Hap_3	62	12,383,067 CC







**Fig. 2** Boxplots indicating phenotypic responses for salinity vigor score among *indica* MAGIC rice lines with alternative haplotypes for candidate genes determined from significantly associated SNPs by GWAS analysis (X-axis = Haplotypes; Y-axis = Vigor score).

**a** *LOC\_Os01g20160*; **b** *LOC\_Os01g20710*; **c** *LOC\_Os01g20720*; **d** *LOC\_Os01g20830*; **e** *LOC\_Os01g20870*; **f** *LOC\_Os01g22590*; **g** *LOC\_Os05g03900*; **h** *LOC\_Os11g35870*; **i** *LOC\_Os12g22020*; **j** *LOC\_Os12g25120*

chromosome 1 between 11.458 Mb to 11.466 Mb (Fig. 2a). The probable candidate genes haplotype contributing parents and haplotype specific MAGIC lines for salinity tolerance are presented in supplementary table 6.

### Association mapping and candidate genes haplotype analysis for sodicity tolerance at seedling stage

As similar to salinity, GBS data of 391 *indica* MAGIC rice lines was used for association analysis with respect to vigor score (SES) under sodic stress at seedling stage. The genome wide Manhattan plot for sodicity vigor score and Q-Q plot are presented in Fig. 3. GWAS peaks revealed that, QTLs mapped on chromosome 6, 4 and 11 plays an important role in controlling the sodicity tolerance at seedling stage. Significant GWAS loci peaks related to sodicity at chromosome 6 was formed between the coordinates of 8.91 and 8.96 Mb in 45 kb region, whereas at chromosome 11 it was traced between 19.4 to 19.5 Mb in 80 kb region. For sodicity tolerance, around 18 SNPs found distributed on three chromosomes i.e., one on chromosome 4, ten on chromosome 6 and seven on chromosome 11 with LOD value of > 3.25. The associated 18 SNPs were subjected to candidate gene analysis and identified six putative causal genes prevailing in genomic regions are presented in Table 4. Among six genes, *LOC\_Os11g32930* gene on chromosome 11 is uncharacterized and responsible for expression of hypothetical protein. There is a peak on chromosome 4 that mapped a candidate gene *LOC\_Os04g24140* (13.8–13.805 Mb) which is known for ribose-5-phosphate isomerase. The three candidate genes were detected on chromosome 6 between 8.91 and 8.96 Mb of 45 kb region. The gene *LOC\_Os06g15730* regulates NB-ARC domain containing protein and it is similar to disease resistance protein RGA3 (XM\_026025943.1). *LOC\_Os06g15760* locus encodes aspartyl protease and peptidase domain containing protein, while the locus *LOC\_Os06g15779* plays an crucial role for aluminium-activated malate transporter (ALMT1) which is one of the key genes that performs the multiple functions like osmotic adjustment, maintenance of electro neutrality and influencing tolerance at sodicity (pH ~ 9.8). We found a candidate gene *LOC\_Os11g32940* on chromosome 11 related to OsFBX421 an F-box domain containing protein. Candidate gene haplotype analysis was performed for the six candidate genes that were involved in the sodic stress tolerance in rice and are presented in Table 5. We noticed two significant haplotypes for the genes *LOC\_Os04g24140*, *LOC\_Os06g15760*, *LOC\_Os06g15779*, *LOC\_Os11g32930*, *LOC\_Os11g32940* and four non-significant haplotypes for the candidate gene *LOC\_Os06g15730* which are shown in Fig. 4. The probable candidate genes haplotype contributing parents and

haplotype specific MAGIC lines for sodicity tolerance are provided in supplementary table 6.

### Discussion

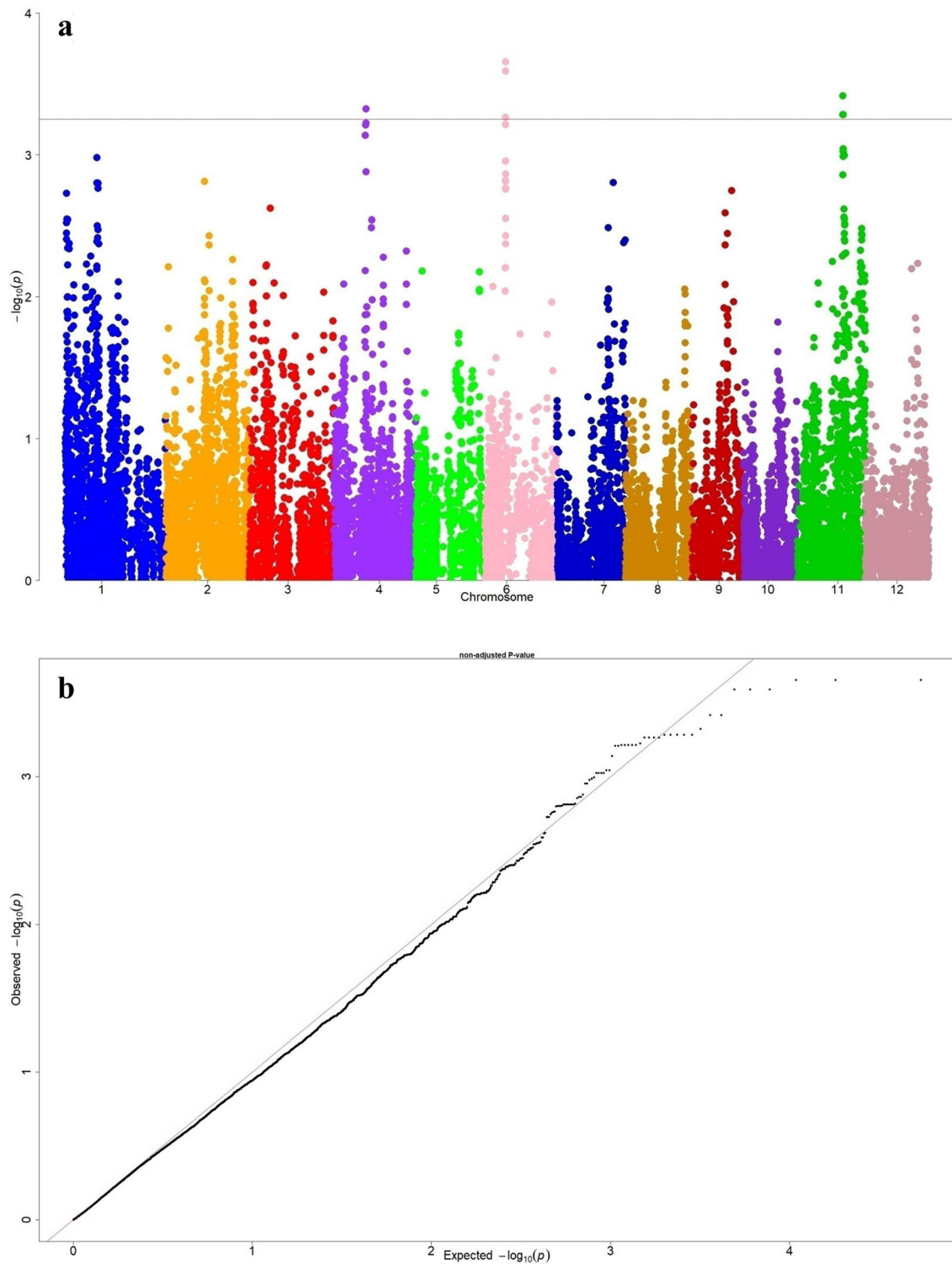
Agricultural production is adversely influenced by the presence of excess salts in the soil. According to statistical data, nearly four million hectare of rice crop is affected badly by salinity in India. Salt tolerance is governed by many genes with different genetic interactions and it is regulated by multigenic traits in rice. Identification of QTLs which contribute to the salt tolerance helps in improving this trait through marker assisted back cross breeding and producing new rice cultivars with desirable traits DNA markers linked to the salt tolerant trait can considerably shorten breeding time to develop varieties for salt tolerance.

### Effect of salinity and sodicity on phenotypic traits

Significant variation was observed in salt injury score (vigor) and other growth parameters in MAGIC lines. This was reflected due to strong interaction in the lines and could be from salt tolerant donor parent IR4630-22-2-5-1-3 or IR77186-122-2-2-3. Among the 391 genotypes, 43 were tolerant to salinity and 2 were tolerant to sodicity. Reduction in seedling height was the major trait that clearly showed variation between the tolerant and sensitive lines under salt stress. Decline in survival and growth of seedlings were the major causes of reduction in plant height, biomass, root length and crop stand failure which leads to yield loss in salt-affected areas (Singh et al. 2014; Heenan et al. 1988; Zeng and Shannon, 2000a, b). Root length and shoot length reduction was noticed in all the lines for salinity stress. Earlier researchers found that the degree of decrease in shoot height was due to intensity and duration of saline stress (Singh et al. 2013; Munns and Tester, 2008; Zeng and Shannon, 2000b; Pundir et al. 2016).

### Association studies for salinity and sodicity tolerance

GWAS in *indica* MAGIC population was conducted by using their corresponding genotype datasets. QTLs for salt stress have been mapped repeatedly on chromosome- 1, 4, 6 and 7, whereas very few on chromosome 2, 3, 5, 8, 9, 10, 11 and 12 (Tiwari et al. 2016; Negrao et al. 2011; Pandit et al. 2010). Salinity and sodicity tolerance are highly influenced by environment (Krishnamurthy et al. 2016b) as it is controlled by multiple complex genes. These environmental factors affect the early vigor and grain yield.



**Fig. 3 a** Genome wide distribution of SNPs presented in Manhattan plot for sodic injury score (vigour score) under sodic stress through MLM model **b** The expected and observed  $-\log_{10}$  probability

distribution of SNPs presented in Q-Q plot for sodic injury score (vigour score) under sodic stress through MLM model

The success of marker assisted back cross breeding (MABB) encouraged the rice breeders in further exploration of the novel QTLs that covers the complex traits.

Thus, mapping of QTLs for salinity tolerance is very helpful for rice breeders to enhance the salt tolerance. In this experiment, we carried out GWAS in MAGIC

**Table 4** Associated QTLs with SNP position and probable candidate genes for sodicity tolerance in MAGIC population

Sl.No	Chr	SNP position	P value	LOD	MarkerR <sup>2</sup>	FDR	Locus ID	Gene annotation
1	4	13,803,235	4.75E-04	3.32	0.032	0.641	LOC_Os04g24140	Ribose 5-phosphate isomerase family protein
2	6	8,917,185	5.44E-04	3.26	0.031	0.641	LOC_Os06g15730	NB-ARC domain containing protein part of the gene is similar to disease resistance protein RGA3 (XM_026025943.1)
3	6	8,917,933	5.44E-04	3.26	0.031	0.641		
4	6	8,917,936	5.44E-04	3.26	0.031	0.641		
5	6	8,917,962	5.44E-04	3.26	0.031	0.641		
6	6	8,921,200	2.57E-04	3.59	0.035	0.641		
7	6	8,921,201	2.57E-04	3.59	0.035	0.641		
8	6	8,932,488	2.57E-04	3.59	0.035	0.641		
9	6	8,956,202	2.22E-04	3.65	0.036	0.641	LOC_Os06g15760	Aspartyl protease/Peptidase A1 domain containing protein
10	6	8,962,742	2.22E-04	3.65	0.036	0.641	LOC_Os06g15779	Aluminum-activated malate transporter protein
11	6	8,962,796	2.22E-04	3.65	0.036	0.641		
12	11	19,448,111	5.19E-04	3.28	0.031	0.641	LOC_Os11g32930	Expressed protein
13	11	19,448,156	5.19E-04	3.28	0.031	0.641		
14	11	19,452,795	5.19E-04	3.28	0.031	0.641	LOC_Os11g32940	OsFBX421—F-box domain containing protein
15	11	19,468,613	3.83E-04	3.42	0.033	0.641		
16	11	19,468,625	3.83E-04	3.42	0.033	0.641		
17	11	19,528,488	5.19E-04	3.28	0.031	0.641		
18	11	19,528,524	5.19E-04	3.28	0.031	0.641		

population and it provides adequate power for association analysis because of its larger population size and wider genetic diversity. The combination of expression pattern and QTL mapping for salt tolerance offers significant information on expression of genes associated with a particular QTL region that are affected by a salt stress condition.

Earlier workers have reported the presence of salinity tolerance genes using bi-parental population on chromosome 1 (Ganie et al. 2014; Thomson et al. 2010). Previous studies focused on the *Saltol* QTL regions marks the presence of *SKC1*, *Salt* and *pectin esterase* genes as the key

factors for the salinity tolerance (Bonilla et al. 2002; Claes et al. 1990; Ren et al. 2005). The *SKC1* gene was found to be overexpressed under salinity stress in *Arabidopsis* (Ren et al. 2005). This gene could be one of the potential targets that are being affected during salinity stress. Our findings showed the presence of a peak (60 kb) near to *SKC1* gene at chromosome 1 of rice. But, non-significant haplotype results was observed for *SKC1* (*LOC\_Os01g20160*) locus, this may be due to its association with K<sup>+</sup>/Na<sup>+</sup> phenotypic value under salt stress and not because of SES-vigor score. Similarly, the stress responsive CC-NBS-LRR-encoding gene (*LOC\_Os01g20720*) belongs to a class of resistance gene

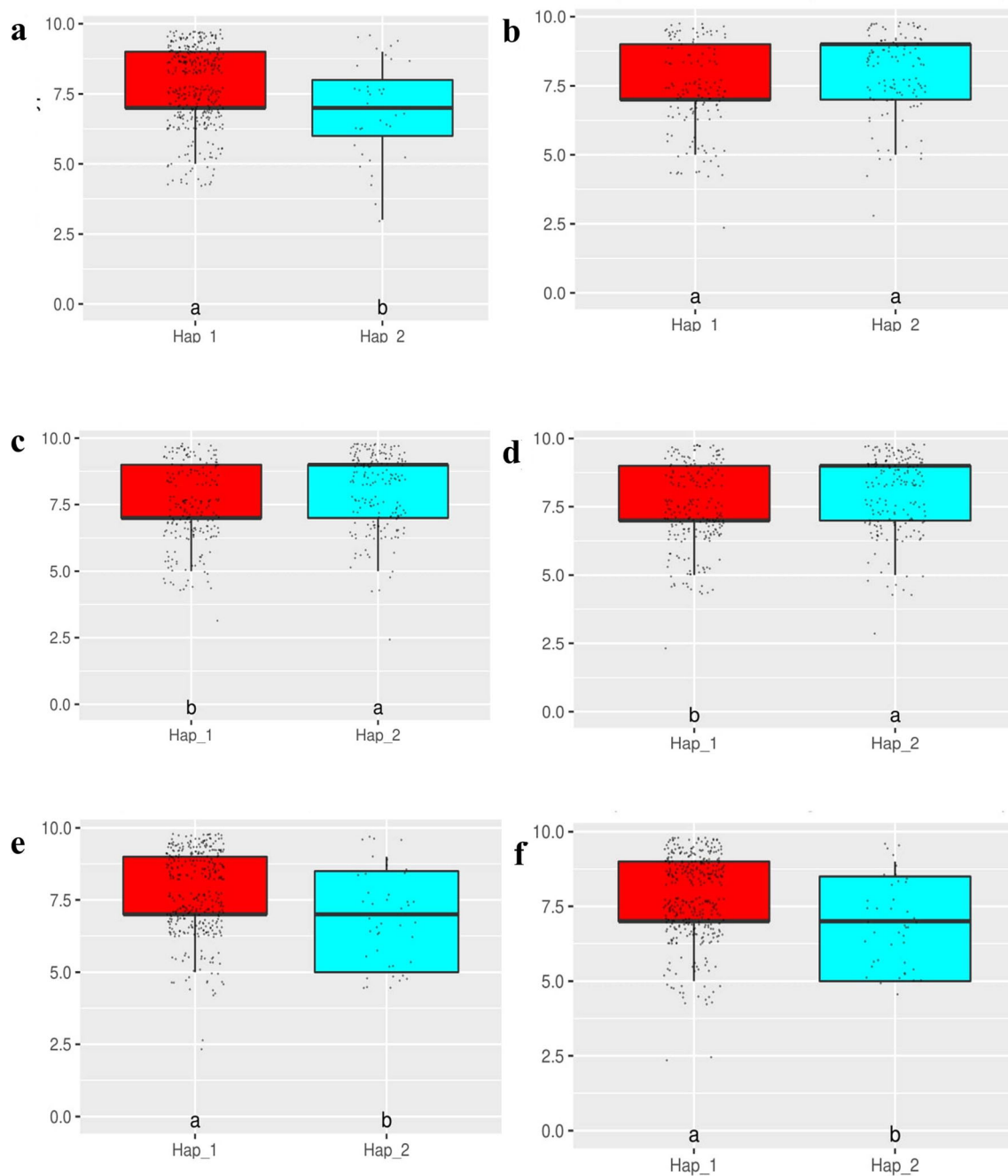
**Table 5** Alternative haplotypes for candidate genes for sodicity tolerance identified by GWAS in MAGIC population

Locus ID	Haplo group	Number of lines	SNP positions							
LOC_Os04g24140			13,803,235							
	Hap_1	356	CC							
	Hap_2	35	GG							
LOC_Os06g15730			8,914,643	8,914,650	8,914,651	8,914,671	8,914,769	8,917,185	8,917,933	8,917,936
	Hap_1	136	CC	GG	CC	TT	TT	CC	TT	GG
	Hap_2	112	TT	AA	TT	CC	CC	TT	CC	CC
	Hap_3	1	TT	AA	TT	CC	CC	CC	TT	GG
	Hap_4	1	CC	GG	CC	TT	TT	TT	CC	CC
LOC_Os06g15760			8,956,202							
	Hap_1	210	CC							
	Hap_2	181	GG							
LOC_Os06g15779			8,962,742	8,962,796						
	Hap_1	210	TT	TT						
	Hap_2	181	CC	CC						
LOC_Os11g32930			19,448,111	19,448,156						
	Hap_1	349	CC	GG						
	Hap_2	42	TT	CC						
LOC_Os11g32940			19,452,795							
	Hap_1	349	CC							
	Hap_2	42	TT							

which helps in the recognition of pathogen-derived avirulence protein. It provides a durable blast resistance in rice through the activation of signal transduction pathway by interacting with WRKY45 transcription factor (Inoue et al. 2013). It was also involved in the drought tolerance mechanism by the overexpression of *ADR1* gene encoding a CC-NBS-LRR in *A. thaliana* (Chini et al. 2004). Leon et al. (2016) recorded the QTL region between 11.53 to 11.58 Mb on chromosome 1 which confers high shoot  $K^+$  concentration (*qK1.11*) in the vicinity of *LOC\_Os01g20720* from pokkali derived RIL population. Another candidate gene *LOC\_Os01g20830* responsible for heavy metal transport/detoxification superfamily protein was overexpressed during biotic stress (*Magnaporthe oryzae*) in rice was identified in this study (Zhang et al. 2010). Retrotransposon protein containing pectin lyase fold domain was encoded by the *LOC\_Os01g22590* gene. The pectin lyase is an extracellular enzyme and induced by pectin. Divergent functions of the pectin lyase members for stress response was reported in *Arabidopsis* (Cao 2012). *LOC\_Os05g03900* gene regulates WRKY 109 DNA-binding domain containing protein. Usually, WRKY TF family plays a key role in controlling the tolerance to biotic and abiotic stresses response (Li et al. 2020). Likewise, *LOC\_Os11g35870* controlling Really Interesting Novel Gene (RING) finger family protein was involved in the ubiquitination pathway. The stress-induced RING E3 ligase

related to RING family genes inferred to play a role in regulation of different physiological responses like protein stabilization, maintenance of cell membrane integrity, stomatal opening, heavy metal levels and reactive oxygen species through ubiquitination in plants (Chapagain et al. 2017). However, haplotype analysis of these candidate genes showed non-significant results. It may be due to the use of broad ranged phenotype (vigor score) for identification of QTLs. Even though vigor score is an important trait for salt injury, which may be unable to capture the minute phenotypic differences caused from each of the candidate genes. The significant haplotypes were observed for the genes *LOC\_Os01g20710*, *LOC\_Os01g20870* and *LOC\_Os12g22020* related to uncharacterized proteins and the gene *LOC\_Os12g25120* regulating core histone H2A/H2B/H3/H4 protein. Histones play an important role in chromosomal stability, transcription regulation, DNA repair and replication. The modification of histone protein like acetylation associated with up-regulation of the cell wall related genes under salt stress condition (Li et al. 2014).

Sodicity is believed to be one of the major factors of abiotic stresses which limit the crop productivity. The effect of sodicity was investigated in the different crops like leaf and roots of a xerophilous grass (*Leymus chinensis*) (Jin et al. 2006), tomato (Biatczyk et al. 1994) and wheat (Millar et al. 2007) reveals that the plants have developed various tolerant mechanisms to adapt



**Fig. 4** Boxplots indicating phenotypic responses for sodic vigor score among *indica* MAGIC rice lines with alternative haplotypes for candidate genes determined from significantly associated SNPs by

GWAS analysis (X-axis = Haplotypes; Y-axis = Vigor score). **a** *LOC\_Os04g24140*; **b** *LOC\_Os06g15730*; **c** *LOC\_Os06g15760*; **d** *LOC\_Os06g15779*; **e** *LOC\_Os11g32930*; **f** *LOC\_Os11g32940*

themselves under sodic stress conditions during the course of evolution. No such studies have been reported in rice. Our investigation targeted the genes related to sodicity in rice using MAGIC population. Out of six candidate genes, all the genes showed significant haplotypes except the locus *LOC\_Os06g15730* which codes for NB-ARC domain containing disease resistance protein. These genes recognizes the pathogen-associated molecular patterns (PAMPs)

during the pathogen attack, while plants also sense the abiotic stress through the surface-localized pattern recognition receptors (PRRs) that were present in the NB-ARC domain containing proteins (Głowacki et al. 2011; Fujita et al. 2006; Kim et al. 2014). The gene, *LOC\_Os06g15760* encoding Aspartyl protease domain containing protein regulates biotic and abiotic stressors. Overexpression of grapevine aspartic protease (*API7*) gene in transgenic

*Arabidopsis* plants exhibited salt- and drought-tolerance (Guo et al. 2015). An aluminium activated malate transporter (which is an ortholog for *ALMT1* gene in *Arabidopsis*) confers sodicity stress on chromosome 6. The *AtALMT1* gene was found to be one of the several genes critical for aluminium toxicity tolerance in *Arabidopsis* (Hoekenga et al. 2006). Recently, an aluminium resistant gene *rall* has been isolated and characterized on chromosome 6 in rice (Liu et al. 2016), Barley (Delhaize et al. 2004) and Wheat (Sasaki et al. 2004). Sodicity tolerance seems to be controlled by the *LOC\_Os06g15779* gene. Aluminium is considered as toxic to plants at high pH, which considerably reduces the growth and development of plants more than that caused by sodicity (high pH) alone. The impact of both sodicity and aluminium became evident at pH ~ 9.0 and waning at pH > 9.2. Hence, it is important to know the pH where aluminium becomes dominant, that probably can cause phytotoxicity (Brautigam et al. 2012). Therefore, correlation of markers associated with aluminium toxicity and sodicity needs to be further studied. *LOC\_Os04g24140* (Ribose-5-phosphate isomerase) is one of the key genes influencing tolerance at sodicity (pH ~ 9.8). Deficiency in a cytosolic ribose-5-phosphate isomerase causes chloroplast dysfunction, late flowering and premature cell death in *Arabidopsis* (Xiong et al. 2009). Thus, this gene also plays an important role in expressing tolerance at sodic stress in rice. OsFBX421 F-box domain containing protein was controlled by the *LOC\_Os11g32940* gene. F-box proteins constitute large family encoding genes for different abiotic stress conditions, floral transition, panicle and seed development (Jain et al. 2007). The function of *LOC\_Os11g32940* gene in cold tolerance at germination stage in chromosome segment substitution lines (CSSL) developed from cold tolerant wild rice donor Y11 (*Oryza rufipogon* Griff.) was reported by Pan et al. (2020). Identified salinity and sodicity tolerant SNPs and respective candidate genes responsible for abiotic stress can be further reinvestigated to confirm their role in salt tolerance in rice. After testing in multiplication trail identified tolerant lines could be released as salt tolerant commercial variety and they can also be used as salt tolerant donor for future breeding programme.

## Conclusions

The results revealed that, salinity and sodicity plays a significant role in the seedling stage of rice growth and development. In this study, we identified 25 SNPs associated with salinity tolerance along with twelve candidate genes including *SKC1* gene on chromosome 1, 5, 11 and 12. The GWAS analysis detected a *Saltol* region, a major QTL with

11 SNPs related to salinity on chromosome 1. Trait linked 18 SNPs and six candidate genes were noticed on chromosome 4, 6 and 11 for sodicity tolerance. An aluminium activated malate gene transporter conferring sodic stress tolerance was located on chromosome 6. About 10 candidate genes showing significant haplotypes were recorded for salinity and sodicity tolerance in rice. The genotypes tolerant to salinity and sodicity stress could be used in the development of salt tolerant variety. This population could be further used in the QTL mapping at the reproductive stage.

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**Author contributions** SLK, PCS, RKS, HL design the experiment, edit the manuscript, SLK, ASW conducted experiments, draft the manuscript and analyze the data, DD, SR NMV, ASW, BML performed the analysis of data, wrote and revised the manuscript.

## Declaration

**Conflict of interest** The authors declare no conflict of interest.

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