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IDENTIFICATION OF FAVOURABLE ALLELES FOR MULTIPLE TRAITS AND NUTRIENT MANAGEMENT IN SALT TOLERANT RICE VARIETIES TO ENHANCE PRODUCTIVITY IN COASTAL SALINE ECOSYSTEM

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Abstract– Rice crop is most sensitive to salinity at seedling and reproductive stages causing huge yield loss. Cultivation of salt tolerant rice varieties along with management practices will minimize the yield loss in saline soils. Four salt tolerant rice varieties MCM 100, MCM 103, MCM 109 and MCM 125 developed by pedigree breeding in typical coastal saline soils of Andhra Pradesh at Agricultural Research Station, Machilipatnam with moderate tolerance to BLB, BPH. The present study aimed to identify favourable alleles and suitable best nutrient management practices for four salt tolerant rice varieties developed at Agricultural Research Station(ARS), Machilipatnam of Acharya NG Ranaga Agricultural University (ANGRAU). Variation in haplotype of saltol region was found among the four varieties using 11 SSR markers and none of the variety showed complete positive alleles of Saltol region. Maximum number of favourable alleles of 7 was noticed in MCM 109 followed by MCM 103, MCM 125 with 5 alleles and minimum number of 3 alleles was observed in MCM 100. Diagnostic markers for saltol i.e., RM 3412 and RM 8094 expressed in all varieties except MCM 100 which conferred positive allele for RM 8094. This indicates that there is variation in salt tolerance at genetic level and there might be other minor genes contributing for salt tolerance are to be traced out by using metabolomic studies. Molecular profiling using 1K RICA (Rice Custom Amplicon) genotyping with 217 SNPs revealed that all the four rice varieties possess favorable alleles for anaerobic germination (*qAG3*), Heading date (DTH8, ehd1), reproductive stage drought tolerance (DTY 12.2), BLB resistant alleles for Xa21, eating quality (*SBElib*), Grain Size (GS3). MCM 103 and MCM 109 have nitrogen use efficient alleles (*NGR5*), MCM 109 has additional resistant allele for BLB (*Xa4, qXa26*), MCM125 has resistant alleles for BPH (*Bph32*), blast (*pi54, pita, pitr*). More number of favourable alleles for heading date genes was identified in MCM 109 followed by MCM 100. The results of best management practices under coastal saline soils implied that MCM 103 and MCM 109 can respond to RDF (recommended dose of fertilizer) (90:60:60 kg/ha) with foliar application of Zinc sulphate. Salt tolerant rice varieties MCM 100 and MCM 125 can give potential yield under 25 % extra over RDF and application of N,K in 3 splits+ Application of 0.2% Zinc sulphate at 15DAT, 20DAT.

INTRODUCTION

Agricultural land of 833 million ha of soils are salt-affected (FAO, 2021) globally in more than100 countries. Soil salinity is the major constraints for rice productivity in coastal areas. Salt affected soils of 2501369 ha in coastal India consists of 1828361 ha saline and 673008 ha sodic (Mandal *et al*., 2018). The rice crop is salt sensitive crop particularly at seedling and reproductive stages and threshold electrical

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conductivity of 3 dS m-1 considered as the salinity tolerance (Mohammadi *et al.,* 2013). Poor correlation exists between sensitivity at seedling stage and reproductive stage suggests that the effects of salt are determined by different mechanisms and sets of genes (QTLs) in seedlings and during flowering stage (Singh *et al.,* 2021). Molecular profiling would help in assessing genetic purity, varietal identification, detecting presence of useful genes (Girija rani *et al*., 2021; Shivani *et al*., 2021; Kumari *et al*., 2020) and high throughput genotyping helps in identification of favorable alleles (Maniruzzaman *et al.,* 2022).

The deployment of salt-tolerant rice varieties combined with improved nutrient management found to be a strategy for improving farmers' income and livelihoods in coastal saline areas of Indonesia (Subekti *et al*., 2019). Best agricultural management practices for salt-affected soils in rice cropping systems of the South Asian region considering net GHG (Green house gas) emissions were identified besides cultivation of salt tolerant trice varieties (Lokupitiya *et al*., 2020). Salt stress jeopardizes plant growth by ionic imbalance, triggering reactive oxygen species (ROS), osmotic and oxidative stress (Pan *et al.,* 2019). In saline and alkali soils, nutrient management strategies are important to tap potential yield of the cultivar (Choudhary and Yaduvanshi, 2019). Saline soils have poor fertility, generally with low availability of nitrogen, calcium, zinc, iron and manganese (Sandeep and Vinay, 2022). Balanced Nitrogen management increased N, P, and K mobilization to the leaves (from FH to maturity) by 49, 43, and 67%, respectively in saline alkaline soils (Xiaohong *et al*., 2020).

The present study aimed to identify favourable alleles and suitable best nutrient management practices for four salt tolerant rice varieties developed at Agricultural Research Station(ARS), Machilipatnam of Acharya NG Ranaga Agricultural University (ANGRAU).

MATERIALS AND METHODS

Genotyping

The four salt tolerant rice varieties MCM 100, MCM 103, MCM 109 and MCM 125 were developed by Agricultural Research Station, Machilipatnam of ANGRAU by pedigree method of breeding in typical coastal saline soils with pH 7.5-8.5 and EC 4-10 ds m^{-1}. These four rice varieties were genotyped with 1K RiCA (Rice Custom Amplicon) using 217 SNPs (Single Nucleotide polymorphism) associated with about 80 genes of various biotic, abiotic and agronomic traits.The leaf sample was punched into small piece in a specific 96 well plates as per sample number and sample paltes were oven dried at 50 ^{0}C for 24 hours and the sample was sent to Intertek, Hyderabad for mid density 1K RiCA genome platform developed by IRRI (International Rice Research Institute), Philippines. Genotyping using 11 SSR markers linked to saltol region was performed as per the protocol described by Girija Rani *et al*. (2021).

Nutrient management studies

Field experiment was carried out by adopting split plot design with three salt tolerant rice varieties MCM 109, MCM 100, MCM 103 as main plots and four treatments (T1: RDF (Recommend dose of fertilizer) (90:60:60kg/ha)+ 0.2% Zinc sulphate at 15DAT, 20DAT, T2: 25% extra over RDF and application of N,K in 3 splits+ Application of 0.2% Zinc sulphate at 15DAT, 20DAT, T3: RDF, T4: Control) as sub plots. Twelve treatments were replicated 3 times and 30 days old seedlings were transplanted at 20X15 cm in 18m^2 plot in saline soil of soil pH 7.95 EC 9.7 ds m^{-1} during wet season 2020. The same treatments were repeated for four salt tolerant rice varieties MCM 109, MCM 100, MCM 103, MCM 125 and 16 treatments were replicated twice with 10m^2 plot in saline soil pH 7.52 EC 6.45 ds m^{-1} during wet season 2021. Data collected on plant survival %, days to 50% flowering, plant height, Ear bearing tillers m^{-2}, grain yield kg ha^{-1}, panicle length (cm), spikelet fertility % and Na and potassium ratio.

Representative soil samples from surface (0-15 cm) were collected from the experimental sites before puddling. The collected samples were then air dried and passed through a 2 mm sieve. The processed soil samples were analyzed for selected basic properties like pH and electrical conductivity (EC). Soil pH and electrical conductivity of the samples were measured on a 1:2.5, soil: water suspension separately using digital pH meter and digital conductivity meter at ARS, Machilipatnam. Sodium Na^+ and Potassium K^+ in the diacid (9.4 ratio of Nitric acid, per chloric acid) extract of plant samples was determined using flame photometer. The statical analysis was performed using excel.

RESULTS AND DISCUSSION

Presence of saltol QTL region of 5 Mb between 10.4Mb – 15.6Mb conferring for seedling stage salinity tolerance alleles varied among the four salt tolerant rice varieties and no variety has expressed all the 11 positive alleles of saltol region of FL 478 derived from Pokkali (Table1). Maximum number of favourable alleles of 7 was noticed in MCM 109 followed by MCM 103, MCM 125 with 5 alleles and minimum number of 3 alleles was observed in MCM 100. Diagnostic marker RM 3412 and RM 8094 expressed in all the varieties except MCM 100 only RM 8094 expressed. The difference in allelic pattern of saltol region might be caused by several minor genes associated with salt tolerance in these genotypes which are to be traced out. Variation in expression of alleles in the saltol region was also found by earlier workers (Krishnamurthy *et al*., 2016; Krishnamurthy *et al*., 2020). Expression of tolerance to salinity is also cultivar specific (Amber and Birendra, 2020; Singha *et al*., 2021). The results of 1 K RiCA genotyping revealed that equal number of favourable alleles for anaerobic germination(*qAG3*), cold tolerance at seedling stage (*qSCT1*), grain size (*GS3*), eating quality (*SBElib*), amylase (*waxy*) was expressed in all the four genotypes (Table 2). More number of alleles of nitrogen use efficiency was observed in MCM 103 and MCM 109 reflecting on phenotyping results of fertilizer response with recommended dose of 90:60:60kg/ha in saline soils (Table3). More number of drought tolerances at reproductive stage (*DTY 12.1, DTY 12.2, DTY 3.2*) was observed in MCM 125. Presence of favorable alleles for other abiotic stress like anaerobic germination, drought and cold tolerance implies that alleles for multiple abiotic stresses were conserved in these genotypes. MCM 125 has Brown plant hopper (BPH) resistance alleles for *Bph 32* conferred with phenotypic score of 3, blast (*pi54, pita, pitr*) and Bacterial Leaf Blight (BLB) gene *Xa 21* were also detected. MCM 109 has more number of alleles for heading date as this variety has plasticity to come to maturity in 130 days during wet and 120 days during dry season. In all the genotypes BLB resistance alleles *Xa21* was conserved and MCM 109 has additional alleles of *qXa26, Xa4* alleles confirmed by moderate tolerance for BLB with phenotypic score of 3. Detection of positive alleles for multiple biotic and abiotic stresses helps in future gene deployment of desired traits for crop improvement (Girija Rani *et al*., 2021, Roy *et al*., 2023, Ali *et al*., 2021). Presence of multiple alleles for biotic stresses like BPH, BLB, blast, nutrient use efficient genes in the salt tolerant rice varieties reduces cost of cultivation on pesticides and fertilizers. Abiotic stress tolerance like anaerobic germination, drought tolerance in salt tolerant lines gives scope for adoption of direct seeding of salt tolerant rice varieties.

Low grain yield was recorded under high salinity of pH 7.95 and EC 9.7 ds m^{-1} during 2020 (Table 3) and higher yield was observed under moderate salinity level of pH 7.52 and EC 6.45 ds m^{-1} during 2021 (Table 4). This can be attributed as intensity of soil salinity plays a major role on grain yield in saline soils. There is significant difference for grain yield, days to 50% flowering, plant height, panicle

Table 1. Summary of haplotype variation of *saltol* positive alleles on chromosome 1

S. No.	SSR marker	Position of the marker (Mb)	Amplicon size bp for presence of alleles	MCM100	MCM103	MCM 109	MCM125	Check FL478
1	RM10694	*10.96*	200				+	+
2	AP3206	*11.2*	90		+	+		+
3	RM8094	*11.2*	70	+	+	+	+	+
4	RM10720	*11.39*	210			+		+
5	RM3412	*11.6*	270		+	+	+	+
6	RM10773	*12.16*	390	+				+
7	RM493	*12.3*	300			+		+
8	RM10800	*12.73*	110		+		+	+
9	RM10825	*13.3*	130					+
10	RM10852	*14.0*	200			+		+
11	RM10843	*13.9*	120	+	+	+	+	+
	Total positive alleles			3	5	7	5	11

+: positive alleles

Table 2. Summary results of 1K RICA genotyping in salt tolerant rice varieties for number of favourable alleles for multiple traits

Trait	Anaerobic germination	Drought tolerance at reproductive stage			Cold tolerance Seedling	Nitrogen use efficiency	BPH	BLB			blast			
Gene														
Variety	qAG3	*qDTY12.1*	*qDTY2.2*	*qDTY3.2*	qSCT1	NGR5	Bph 32	Xa21	qXa26	qXa4	pi54	pik	pita	ptr
MCM100	2	0	1	2	4	1	0	1	1	2	0	1	0	0
MCM103	2	0	2	0	4	2	0	1	0	0	0	0	0	0
MCM125	2	1	2	2	4	1	2	1	0	0	1	0	1	1
MCM109	2	0	2	0	4	2	0	1	1	2	0	1	0	0

Trait	Grain size	Eating quality	Heading date						GT	Amylose	Chalkiness	Grain filling
Gene Variety	GS3	SBE Lib	ehd1	DTH8	Hd1	RFT1	Hd3a	Hd2	Alk	Waxy	Chalk5	GFR1
Varieties MCM100	1	4	1	1	1	0	0	7	0	3	2	0
MCM103	1	4	1	1	1	1	1	2	0	3	2	2
MCM125	1	4	1	1	1	0	0	2	1	3	3	2
MCM109	1	4	1	1	1	1	2	7	0	3	1	2

length for MCM 109, MCM 100, MCM 103 varieties but significant difference was observed for flowering duration and grain yield with treatments during 2020. Interaction of salt tolerant varieties with treatments for grain yield revealed that MCM 109, MCM 103 can give yield under saline soils with recommended dose of fertilizers 90:60:60kg/ha with the foliar application of 0.2% Zinc sulphatic at 15 DAT and 20 DAT. Higher yields of MCM 100 can be realized at 25% extra over RDF and application of N, K in 3 splits+ Application of 0.2% Zinc sulphate at 15DAT, 20 DAT under high saline soils. Lower yields might be due to stunted plant growth, shorter panicle length under high salinity (Pan *et al.,* 2019).

All the traits showed significant treatments for both varieties and treatments except days to 50% flowering do not have impact with treatments under moderate level of salinity. Short stature of plant at initial stages at seedling establishment resulted in poor survival as the crop was suffered with flash floods for a week caused by heavy rains during 2021. All the three MCM 100, MCM 103, MCM 125 varieties with 140 days duration gave higher yield than MCM 109 with 130 days duration in both years. Among the treatments, RDF+ Application of 0.2% Zinc sulphate at 15 DAT, 20DAT and without Zinc foliar application gave higher yield than control under high saline soils and 25% extra over RDF and application of N,K in 3 splits+ Application of 0.2% Zinc sulphate at 15 DAT, 20 DAT found to be better than control under moderate saline soils. The results of two years data revealed that MCM 103 and MCM 109 can respond to recommended dose of fertilizer with foliar application of Zinc sulphate and MCM 100, MCM 125 salt tolerant rice varieties can give potential yield under25 % extra over RDF and application of N, K in 3 splits+ Application of 0.2% Zinc sulphate at 15DAT, 20DAT. Balanced application of fertilizer dose for cultivating salt tolerant rice varieties improved crop yield under saline alkaline soils (Subekti *et al*., 2019; Lokupitiya *et al*., 2020; Xiaohong *et al*.,2020).

CONCLUSION

Results of 1K RICA genotyping with 217 SNPs revealed that all the four genotypes possess favorable alleles for anaerobic germination (*qAG3*), Heading date (*DTH8, ehd1*), reproductive stage drought (*DTY 12.2*), BLB resistant alleles for *Xa21*, eating quality (*SBElib*), Grain Size (*GS3*). MCM 103 and MCM 109 has nitrogen use efficient alleles

Table 3. Summary results of evaluation of salt tolerant rice varieties with best management practices to enhance productivity under salinity during 2020

Treatments	Plant survival %	Days to 50% flowering	Plant height (cm)	EBT/m²	Grain yield (Kg ha⁻¹)	Panicle length (cm)	Spikelet Fertility %	Na/K ratio
Entries (V)								
V_1 : MCM 109	97	93	108	457	2391	21	92	0.31
V_2 : MCM 100	95	115	97	498	3703	23	94	0.27
V_3 : MCM 103	95	115	84	491	3222	21	93	0.23
SEm±	1.4	0.8	1.4	12	80.0	0.2	1.8	
CD (P=0.05)	NS	3.0	6.0	NS	314	1.0	NS	
Fertilizer levels (F)								
T_1 : RDF + Application of 0.2% Zinc sulphate at 15 DAT, 20 DAT	96	104	95	469	3520*	21	94	0.29
T_2: 25% extra over RDF and application of N, K in 3 splits + Application of 0.2% Zinc sulphate at 15 DAT, 20 DAT	95	109	97	472	2939	22	95	0.25
T_3 : RDF	96	110	97	525	3253*	21	92	0.27
T_4 : control	96	107	97	462	2711	21	91	0.27
SEm±	0.9	0.8	1.4	36	115.6	0.4	2.1	0.02
CD (P=0.05)	NS	3	NS	NS	343	NS	NS	NS
Interaction (V × F) CD (P=0.05)	NS	NS	NS	NS	595	NS	NS	NS
CV % varieties	4.9	2.5	5.1	8.6	8.9	8.6	6.6	5.0
CV % Inter action	2.5	2.4	4.4	7.5	11.2	7.5	6.6	5.0

Interaction table Grain yield kg/ha

Fertilizer levels (F)	Entries (V)			Mean
	V1(MCM 109)	V2(MCM 100)	V3(MCM 103)	
T_1: RDF+ Application of 0.2% Zinc sulphate at 15DAT, 20DAT	3097*	3765	3697*	3520
T_2: 25% extra over RDF and application of N,K in 3 splits+ Application of 0.2% Zinc sulphate at 15DAT, 20DAT	1274	4378*	3165	2939
T_3: RDF	2645	3591	3521	3253
T_4: control	2547	3079	2505	2711
Mean	2391	3703	3222	

Table 4. Summary results of evaluation of salt tolerant rice varieties with best management practices to enhance productivity under salinity during 2021

Treatments	Plant survival %	Days to 50% flowering	Plant height (cm)	EBT/m^2	Grain yield (Kg ha^{-1})	Panicle length (cm)	Spikelet Fertility %	Na/K ratio
Entries (V)								
V_1 : MCM 109	95	97	112	347	5558	23	87	0.93
V_2 : MCM 100	91	110	110	293	5959	22	92	0.51
V_3 : MCM 103	89	112	97	343	5954	23	88	0.84
V4: MCM 125	98	120	115	345	6368	24	90	0.57
SEm±	0.43	2.51	0.46	11.10	136	0.08	0.33	0.02
CD (P=0.05)	1.48	8.69	1.41	38.4	469	0.26	1.13	0.07
Fertilizer levels (F)								
T_1: RDF + Application of 0.2% Zinc sulphate at 15DAT, 20DAT	88	111	108	304	5996	24	89	0.77
T_2: 25% extra over RDF and application of N,K in 3 splits+ Application of 0.2% Zinc sulphate at 15DAT, 20DAT	97	110	107	355	6315*	23	90	0.67
T_3 : RDF	92	110	105	326	5874	21	90	0.73
T_4 : control	95	109	114	342	5654	23	87	0.69
SEm±	0.41	2.83	0.89	10.23	156	0.09	0.39	0.04
CD (P=0.05)	1.21	8.25	2.61	29.86	456	0.25	1.13	0.13
Interaction (V×F) CD (P=0.05)	2.42	NS	5.22	59.22	912	0.51	2.26	0.26
CV % varieties	0.83	5.65	1.79	14.20	9.65	1.36	1.55	11.64
CV % Inter action	2.42	16.50	5.22	13.08	11.12	1.57	1.84	26.76

Interaction table Grain yield kg/ha

Fertilizer levels (F)	Entries (V)				Mean
	V_1(MCM 109)	V_2(MCM 100)	V_3(MCM 103)	V_4(MCM 125)	
T_1 : RDF+ Application of 0.2% Zinc sulphate at 15 DAT, 20 DAT	5859*	6095	5712	6319	5996
T_2: 25% extra over RDF and application of N, K in 3 splits + Application of 0.2% Zinc sulphate at 15 DAT, 20 DAT	5650	6331	6455	6824	6315
T_3 : RDF	5891*	5899	5307	6398	5874
T_4 : control	4832	5509	6342	5932	5654
Mean	5558	5959	5954	6368	

*Significance at 0.05 probability, EBT: ear bearing tillers

(*NGR5*), MCM 109 has additional resistant allele for BLB (*Xa4, qXa26*), MCM125 has resistant alleles for BPH (Bph32), blast (*pi54, pita, pitr*). More number of favourable alleles for heading date genes was identified in MCM 109 followed by MCM 100. Salt tolerance in these varieties might be due to other minor alleles in addition to saltol which has to be traced out with advanced biotechnological approaches. MCM 103 and MCM 109 can be cultivated with RDF and potential yield of MCM 100 and MCM 125 can be realized under saline soils with 25% extra dose of fertilizers in saline soils.

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