Received: 25 Jul 2025 | Revised: 08 Oct 2025 | Accepted: 09 Oct 2025

Electronic Journal of Plant Breeding

Research Article

Divergence studies in elite salt tolerant lines under direct seeded condition in rice (*Oryza sativa* L.)

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Abstract
Salinity is a major constraint in coastal agriculture due to seawater intrusion, tidal flooding and poor drainage. Hence it is imperative and viable option to grow salt tolerant lines with high yield potential and amenable for direct seeded conditions in flood prone coastal belts. In the present study 24 elite salt tolerant lines developed at ARS Machilipatnam (EC 9-10 dSm^{-1}, pH 7.9) under transplanted conditions were evaluated under direct seeded conditions along with two checks during *kharif*, 2023 at Agricultural College Farm, Bapatla, Andhra Pradesh in a randomized complete block design (RCBD) with two replications, in a normal soil condition (EC 2.5 dSm^{-1}, pH 7.2). Observations on yield and yield components; early vigour and lodging related traits were studied under direct seeded conditions. The results revealed that first six principal components accounted for 89.59 per cent of total divergence. PC_1 contributed maximum towards divergence (30.64 %) with eigen value of 6.435. Based on the analysis and loading values PC_5 contributed to most of direct seeded and yield traits *viz.*, root volume at 15 DAS, days to 50 per cent flowering, plant height, grain yield and harvest index in PC_5 followed by shoot length at 30 DAS, field emergence at 15 DAS, root volume at 30 DAS, spikelet fertility in PC_3 and root length at 15 DAS, panicle length, basal internodal length and culm thickness in PC_2. The 2D and 3D plots indicated that genotypes like MCM 148-2-1-1-1, MCM 141, MCM 153-2-1-2-1, MCM 103, MCM 153-1-1-1, MCM 305-32-2-1 and MCM 151-3-21-1 were divergent for most of the direct-seeded traits like root length, shoot length, root volume, basal internodal length and culm volume. Among the 14 SSR markers studied, RM5, RM10793, RM3412 and RM1287 recorded higher PIC values. Hence, these markers can be considered more informative and are capable of discriminating the genotypes more effectively.

Keywords: Direct seeding, salinity, Genetic divergence, PCA, Rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most essential cereal crops, meeting the nutritional needs of nearly half the global population. As a primary source of carbohydrates, it plays a critical role in the diet of much of the world's population. Indian rice systems are undergoing various types of changes in response to economic factors and technological opportunities in farming. One such change is to adopt direct seeding methods for rice establishment. Direct seeding helps to address problems of rising labour costs, declining water table and worsening soil health. Further, the availability of high-yielding, short-duration rice varieties, along with effective chemical weed control methods, has made this shift economically viable. Additionally, direct seeding can enhance water use efficiency. Direct-seeded rice with reduced or no tillage is a promising resource-conserving technology (RCT) for the future. Early seedling vigour is a key trait for success in direct-seeded rice (DSR) systems. Rice varieties suited for direct seeding in irrigated areas should have early seedling vigour, enhanced leaf

growth to suppress weeds during the vegetative phase, consistently high foliar nitrogen concentration during the reproductive stage, sturdy culms to resist lodging with improved reproductive sink capacity. (Gupta *et al.,* 2006, Cairns *et al.,* 2009).

Among various environmental stress factors, salinity is considered as one of the primary detrimental factors after drought as it restricts crop productivity. Paddy is categorized as salinity susceptible cereal. In India alone, 6.73 million hectares of land are degraded by salt on irrigated land which accounts to greater than 10 per cent of the agricultural crop land presenting a serious threat to global food security (Rani *et al.*, 2024).

All breeding efforts consider the genetic variability in yield-related traits and examine how yield interacts with these factors to develop new lines. Therefore, this study was conducted to evaluate and assess the performance and variability of elite salt tolerant lines developed at ARS Machilipatnam (EC 9-10 dSm^{-1}, pH 7.9) under transplanted conditions in normal soils under direct sown condition at Bapatla (EC 2.5 dSm^{-1}, pH 7.2) which is about 8 km away from Bay of Bengal. Further there is not much study on suitability of salt tolerant lines developed for natural coastal saline soils under direct seeded method of cultivation. Rice crop is also most sensitive to salinity at seedling and reproductive stage causing huge yield losses. Ullah *et al.,* 2007, conducted a field experiment in a salt-affected field (EC = 6.8 dSm^{-1} pH = 8.7, SAR = 45 silty clay) at Soil Salinity Research Institute, Pakistan to determine the best rice planting technique under salt-affected soils. Direct seeding and transplanting techniques were applied using two, three and four seedlings $hill^{-1}$. They reported that direct seeding gave the maximum returns under salt-affected soils reducing transplanting cost and concluded that direct sowing was the best planting technique for salt-affected soils related to coarse rice varieties in that region.

Keeping aforesaid aspects in view, the present work was carried out to identify the best performing genotypes among the ones developed at ARS, Machilipatnam, with high yield and good early vigour traits. The study was carried out at Bapatla, in normal field (EC 2.5 dSm^{-1}, pH 7.2), under direct sown conditions.

MATERIALS AND METHODS

The present investigation was carried out using 24 elite salt tolerant advanced cultures with two checks FL478 (resistant salt tolerant check) and BPT5204 (yield check). These salt tolerant lines developed at ARS, Machilipatnam were evaluated in randomized complete block design (RCBD) for yield and yield related traits under direct seeded condition at Agricultural College Farm, Bapatla, Andhra Pradesh under normal soil conditions during *kharif,* 2023. Each genotype was grown in five rows of 3.0 m length with a spacing of 20 cm between rows and 15 cm between plants, within the row. The data was recorded on five competitive plants taken from each replication. The early vigour traits *viz.,* root length (cm), shoot length (cm), root volume (cm^3/plant) and field emergence (%) were taken at 15 and 30 DAS as these measurements helps to determine the effectiveness of establishment method and potential issues related to root development that could affect nutrient and water uptake which ultimately influence yield. Data was also collected on days to 50 per cent flowering, plant height (cm), number of productive tillers per plant, panicle length (cm), number of filled grains per panicle, spikelet fertility (%), culm strength, per cent lodging (%), basal internodal length (cm), culm diameter (mm), culm thickness (mm), biological yield (kg/ha), grain yield (kg/ha) and harvest index (%). Culm diameter was measured at 4^{th} internode from the top at 20 days after heading using vernier calipers. For culm thickness, the culm internodes were cut transversely at 4^{th} inter node from the top of the panicle at 20 days after heading with a scalpel to measure the inner and outer diameters of the inter node with a vernier calipers. The averaged culm wall thickness was then calculated by the equation; culm wall thickness = (outer diameter – inner diameter)/2. Principal component analysis was carried according to procedure described by Banfield (1978) using WINDOSTAT *version 9.3* The molecular characterization of 24 rice genotypes conducted using trait-specific simple sequence repeats (SSR) markers, following a modified CTAB method and agarose gel electrophoresis were utilized to analyse the SSR markers. The genetic dissimilarity between the genotypes and dendrogram for 24 rice genotypes was assessed by UPGMA (Unweighted Pair Group Method with Arithmetic Averages) using DARwin software ver. 6.0.12 (Perrier and Jacquemoud, 2006) (**Table 1**).

RESULTS AND DISCUSSION

The results revealed that first six canonical roots accounted for 89.59 per cent of total divergence. PC_1 contributed maximum towards divergence (30.64 %) with eigen value of 6.4354. The second, third, fourth, fifth and sixth canonical vectors contributed 25.81 %, 12.77 %, 9.40 %, 7.11 % and 3.86 % respectively to total divergence (**Table 2**). In first principal component (PC_1) the traits that contributed positively to total divergence were root length at 15 DAS (0.2118), root volume at 15 DAS (0.0512), root volume at 30 DAS (0.2168), number of productive tillers per plant (0.3509), culm diameter (0.1744), biological yield (0.2424), grain yield (0.2924) and harvest index (0.2492). In second principal component (PC_2) the traits that contributed positively to total divergence were root length at 15 DAS (0.2781), shoot length at 30 DAS (0.0301), root volume at 30 DAS (0.1620), panicle length (0.3196), number of filled grains per panicle (0.1286), basal internodal length (0.3467), culm thickness (0.3437) and biological yield (0.0712). In third principal component (PC_3) the traits that contributed positively to total divergence were root length at 15 DAS (0.1715), shoot length at 15 DAS (0.0187), shoot length at 30 DAS (0.3030), field emergence at 15 DAS (0.1924), root volume at 15 DAS (0.1863), root volume at

Table 1. Details of SSR markers used in the present investigation to study molecular diversity

S. No.	SSR marker	Forward and Reverse sequence	Trait linked	Reference
1	RM472	F: CCATGGCCTGAGAGAGAGAG R: AGCTAAATGGCCATACGGTG	Grain yield	Sweeney *et al*. (2006), Bernier *et al*. (2007)
2	RM3412	F: TGATGGATCTCTGAGGTGTAAAGAGC R: TGCACTAATCTTTCTGCCACAGC	Salinity tolerance	Krishnamurthy *et al.* (2016)
3	RM6329	F: CAGCAGAGACTATAGACACTCAAGC R: TGCCTAGCTACTCTAGGTGAAACC	Chlorophyll content	Sweeney *et al*. (2006)
4	RM10694	F: TTTCCCTGGTTTCAAGCTTACG R: AGTACGGTACCTTGATGGTAGAAAGG	Salinity tolerance	Krishnamurthy *et al.* (2016)
5	RM10793	F: GACTTGCCAACTCCTTCAATTCG R: TCGTCGAGTAGCTTCCCTCTCTACC	Salinity tolerance	Ravikiran *et al*. (2017)
6	RM3231	F: CACCGGCGTCAAGCTCATCG R: GTCAGTCCAGGTAGGAGCATGAGAGC	Chl a, catalase	Nayak *et al*. (2022)
7	RM493	F: GTACGTAAACGCGGAAGGTGACG R: CGACGTACGAGATGCCGATCC	Salinity tolerance	Krishnamurthy *et al.* (2016)
8	RM9	F: GGCCCTCATCACCTTCGTAGC R: CGTCCTCCCTCTCCCTATCTCC	Plant yield, harvest index	Moniruzzaman *et al*. (2012), Chattopadhyay *et al*. (2021)
9	RM336	F: CTTACAGAGAAACGGCATCG R: GCTGGTTTGTTTCAGGTTCG	Shoot dry weight	Uyoh *et al*. (2019), Sanghamitra *et al*. (2021)
10	RM24	F: GAAGTGTGATCACTGTAACC R: TACAGTGGACGGCGAAGTCG	Na/K	Kumari *et al*. (2016)
11	RM1287	F: CCATTTGCAGTATGAACCATGC R: ATCATGCAATAGCCGGTAGAGG	85% flowering Na/K	Ghomi *et al*. (2013)
12	RM5	F: TGCAACTTCTAGCTGCTCGA R: GCATCCGATCTTGATGGG	MDA	Anyomi *et al*. (2018)
13	RM8094	F: GATCTGTAAATGCTTCATGG R: ACTCAATTTCAACAATGGTG	Salinity tolerance	Chowdhury *et al*. (2016)
14	RM140	F: CTTGCACAAGAGATGATGATGAGC R: CATGCTGAGAAATAGTACGCTTGG	K%	Ghomi *et al*. (2013)
15	RM10852	F: GAATTTCTAGGCCATGAGAGC R: AACGGAGGGAGTATATGTTAGCC	Salinity tolerance	Krishnamurthy *et al*. (2016)

Table 2. Canonical vectors for 21 characters in 24 genotypes of rice (*Oryza sativa* L.)

S. No.	Parameter	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6
1.	Eigen Value (Root)	6.4354	5.4207	2.6820	1.9756	1.4933	0.8108
2.	% Var. Exp.	30.64	25.81	12.77	9.40	7.11	3.86
3.	Cum. Var. Exp.	30.64	56.45	69.22	78.63	85.74	89.60
S. No.	**Character**						
1.	Root length at 15 DAS	0.2118	0.2781	0.1715	0.2411	0.0232	0.0421
2.	Root length at 30 DAS	-0.0876	-0.0378	-0.4114	0.1002	0.3917	-0.2931
3.	Shoot length at 15 DAS	-0.2596	-0.0162	0.0187	-0.3046	0.1658	0.3862
4.	Shoot length at 30 DAS	-0.2334	0.0301	0.3030	-0.0768	0.0756	0.1586
5.	Field emergence (%) 15 DAS	-0.2412	-0.1996	0.1924	-0.2011	0.1462	-0.2916
6.	Root volume (cm^3) at 15 DAS	0.0512	-0.0495	0.1863	0.3853	0.4954	-0.3731
7.	Root volume (cm^3) at 30 DAS	0.2168	0.1620	0.3483	0.1809	0.1889	0.1734
8.	Days to 50 per cent flowering	-0.2319	-0.2188	0.0745	-0.2353	0.2953	0.2118
9.	Plant height (cm)	-0.2047	-0.1557	-0.4048	0.1434	0.1968	-0.0026
10.	Panicle length (cm)	-0.0642	0.3196	0.2070	0.1525	0.2199	0.1348
11.	No. of productive tillers per plant	0.3509	-0.1452	-0.0114	-0.1714	-0.0188	-0.0502
12.	No. of filled grains per panicle	-0.2383	0.1286	-0.1337	0.3339	0.2141	0.3356
13.	Spikelet fertility (%)	-0.3724	-0.0076	0.1090	0.0012	-0.0972	-0.0379
14.	Culm strength	-0.1392	-0.3141	0.1802	0.2599	-0.0051	0.2573
15.	Per cent lodging	-0.0916	-0.3493	-0.0203	0.2769	-0.2155	0.0520
16.	Basal internodal length	-0.0841	0.3467	-0.2336	-0.0758	-0.0443	0.0110
17.	Culm diameter (mm)	0.1744	-0.3772	0.0167	0.0657	0.0147	0.0766
18.	Culm thickness (mm)	-0.1654	0.3437	-0.0800	-0.2475	0.1050	-0.1510
19.	Biological yield (kg/ha)	0.2424	0.0712	-0.3685	0.0643	0.0765	0.4261
20.	Grain yield (kg/ha)	0.2924	-0.1717	-0.1321	-0.1581	0.2962	0.1512
21.	Harvest index (%)	0.2492	-0.0584	0.1622	-0.3556	0.3561	0.0410

F: Forward sequence R: Reverse sequence

30 DAS (0.3483), days to 50 per cent flowering (0.0745), panicle length (0.2070), spikelet fertility % (0.1090), culm strength (0.1802), culm diameter (0.0167) and harvest index (0.1622). In fourth principal component (PC_4) the traits that contributed positively to total divergence were root length at 15 DAS (0.2411), root length at 30 DAS (0.1002), root volume at 15 DAS (0.3853), root volume at 30 DAS (0.1809), plant height (0.1434), panicle length (0.1525), number of filled grains per panicle (0.3339), spikelet fertility % (0.0012), culm strength (0.2599), per cent lodging (0.2769), culm diameter (0.0657) and biological yield (0.0643). In fifth principal component (PC_5) the traits that contributed positively to total divergence were root length at 15 DAS (0.0232), root length at 30 DAS (0.3917), shoot length at 15 DAS (0.1658), shoot length at 30 DAS (0.0756), field emergence at 15 DAS (0.1462), root volume at 15 DAS (0.4954), root volume at 30 DAS (0.1889), days to 50 per cent flowering (0.2953), plant height (0.1968), panicle length (0.2199), number of filled grains per panicle (0.2141), culm diameter (0.0147), culm thickness (0.1050), biological yield (0.0765), grain yield (0.2962) and harvest index (0.3561). In sixth principal component (PC_6) the traits that contributed positively to total divergence were root length at 15 DAS (0.0421), shoot length at 15 DAS (0.3862), shoot length at 30 DAS (0.1586), root volume at 30 DAS (0.1734), days to 50 per cent flowering (0.2118), panicle length (0.1348), number of filled grains per panicle (0.3356), culm strength (0.2573), per cent lodging (0.0520), basal internodal length (0.0110), culm diameter (0.0766), biological yield (0.4261), grain yield (0.1512) and harvest index (0.0410).

In principal component analysis by examining the variable loadings of the first six PCs, it was recorded that fifth principal component (PC_5) has high loadings for all yield contributing characters (including yield). Hence, the fifth principal component PC_5 can be termed as 'sexual reproduction axis'. Based on the trait loadings, the second and fourth principal components (PC_2 and PC_4) can be called as 'lodging axis', because these two principal components recorded high values for basal internodal length, culm thickness, culm strength and per cent lodging. High loading values were recorded for some early vigour traits and vegetative characters in third and fifth principal components (PC_3 and PC_5) like root volume, shoot length, field emergence, plant height and days to 50% flowering. Therefore, PC_3 and PC_5 can put together be called as 'vegetative axis'. Therefore, it can be inferred that principal component analysis was able to discriminate and separate 24 rice genotypes (including checks) in terms of these three dimensions represented by sexual reproduction, lodging and vegetative (early vigour traits) indicating the use of principal component analysis for in depth analysis of genetic divergence (**Table 2**). The 2D and 3D plots indicated that genotypes like MCM 148-2-1-1-1, MCM 141, MCM 153-2-1-2-1, MCM 103, MCM 153-1-1-1, MCM 305-32-2-1 and MCM 151-3-21-1 were divergent (away from the axis) for all

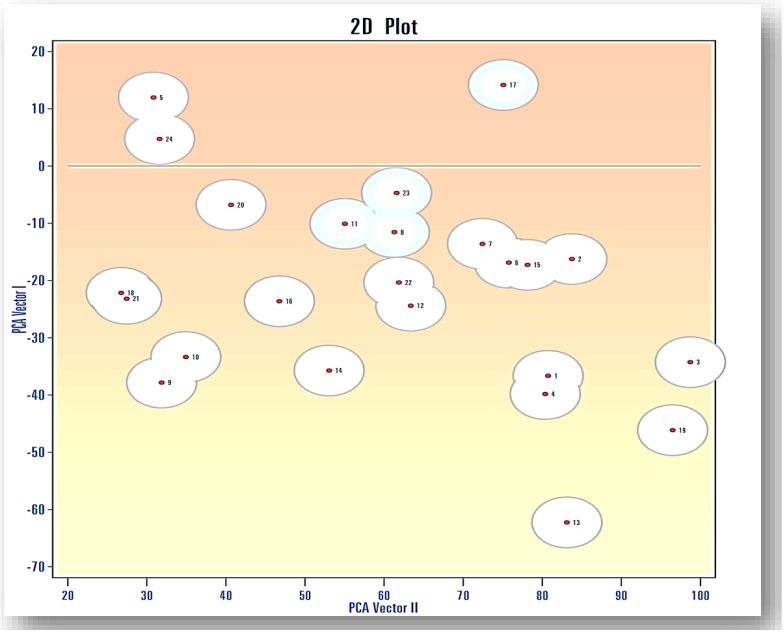


Fig. 1. Two-dimensional (2D) graph based on PCA scores showing relative positions of 24 genotypes of rice

1. MCM 142	2. MCM 208-14-1-1	3. MCM 147-1-1-2-1	4. MCM 258-8-2-7	5. MCM 148-21-1
6. MCM 149-3-1-1-1-1	7. MCM 151-3-21-1	8. MCM 153-1-1-1	9. MCM 253-6-2-2	10. MCM 100
11. MCM 103	12. MCM 125	13. MCM 109	14. IR17F1107	15. MCM 253-5-3-1
16. MCM 143	17. MCM 141	18. BPT 5204 (check)	19. FL 478 (check)	20. MCM 153-2-1-2-1
21. MCM 253-8-2-2	22. MCM 106-2-10-2-2	23. MCM 305-32-2-1	24. MCM 305-14-1-1-1	

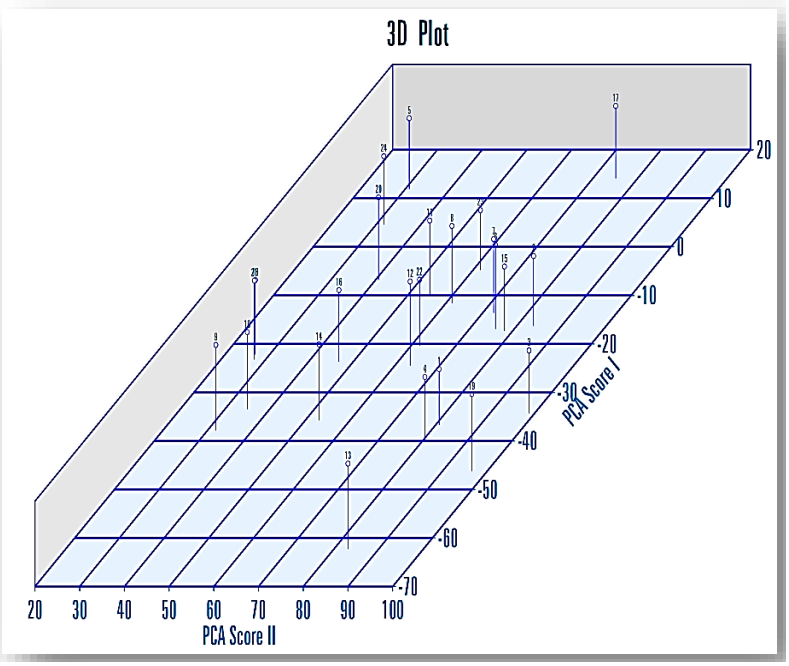


Fig. 2. Three-dimensional (3D) graph showing relative positions based on PCA scores in 24 genotypes of rice

1. MCM 142	2. MCM 208-14-1-1	3. MCM 147-1-1-2-1	4. MCM 258-8-2-7	5. MCM 148-21-1
6. MCM 149-3-1-1-1-1	7. MCM 151-3-21-1	8. MCM 153-1-1-1	9. MCM 253-6-2-2	10. MCM 100
11. MCM 103	12. MCM 125	13. MCM 109	14. IR17F1107	15. MCM 253-5-3-1
16. MCM 143	17. MCM 141	18. BPT 5204 (check)	19. FL 478 (check)	20. MCM 153-2-1-2-1
21. MCM 253-8-2-2	22. MCM 106-2-10-2-2	23. MCM 305-32-2-1	24. MCM 305-14-1-1-1	

the traits included in the study (**Fig. 1&2**). Kashyap and Yadav (2020) conducted work on high and normal salt conditions to study genetic variation existing among the 78 accessions of rice, including seven checks and concluded that characters with largest absolute value closer to unity within the principal component influence the clustering more than those with lower absolute value closer to zero. Further genotypes NDRK 11-24, KR15006, NDRK 11-21, CSR36, Usar Dhan 3 recorded high variation in factor 2 in their study which represented important yield contributing character, productive tillers plant^{-1} and test weight.

Among the 15 SSR markers included in the study, 14 markers *viz.,* RM3412, RM6329, RM10694, RM10793, RM3231, RM493, RM9, RM336, RM24, RM1287, RM5, RM8094, RM140 and RM10852 exhibited polymorphism. Among these 14 markers, RM3412, RM10793, RM1287 and RM5 recorded higher PIC values (**Table 3**). These markers can be considered more informative and with better capability to discriminate genotypes more effectively. These markers can be utilised for marker assisted selection and for screening of rice germplasm.

Cluster analysis revealed that the *saltol* genotype FL 478 (tolerant to salinity at seedling stage) was in one cluster and the remaining genotypes were in another cluster. FL 478 showed strong seedling stage salinity tolerance with *saltol* locus on chromosome 1 spanning 10.6-11.5 Mb and contains several genes with a key functional gene *SKC 1.* In general clustering with SSR markers reflects genome wide relatedness. Hence, cosegregation with *saltol* does not force the genotypes to cluster with FL 478. In the present study, all the alleles present in *saltol* region may not be necessarily be present in salt tolerant lines included in the study which were confirmed for seedling and reproductive salinity tolerance under transplanted field condition at ARS, Machilipatnam (EC 9-10dSm^{-1}, pH 7.9). Further introgression and recombination can also place *saltol* alleles on to diverse backgrounds and matching with the size of SSR allele does not always reflects identical functional haplotype. This has to be traced out using advanced biotechnological tools. These results were supported by previous work of Manohara *et al.*, 2021 in their study on haplotype analysis of *saltol* QTL region in 71 diverse rice land races which were phenotypically tolerant for salinity. But when screened with 14 *saltol* linked SSR markers, some of the phenotypically tolerant genotypes had different allelic constitution at *saltol* region which is different from check FL 478. Hence, they concluded that some tolerant lines in their study did not share the same haplotype as FL 478 at *saltol* supporting the idea that sharing the *saltol* markers does not always equate to being clustered with check FL 478.

Table 3. Number of alleles and polymorphic information content (PIC) value of the 14 polymorphic SSR markers used in the present study

S. No.	SSR Marker	Chr. No.	Na*	Ne*	PIC	Amplification size (bp)
1.	RM3412	1	3	2.81	0.64	100
2.	RM10694	1	2	1.84	0.45	150
3.	RM10793	1	3	2.94	0.66	150
4.	RM493	1	2	1.54	0.35	150
5.	RM9	1	3	2.09	0.55	100
6.	RM24	1	3	2.44	0.62	150
7.	RM1287	1	3	2.65	0.62	150
8.	RM8094	1	3	2.12	0.52	100
9.	RM140	1	2	1.72	0.45	100
10.	RM10852	1	2	1.47	0.39	150
11.	RM6329	3	2	1.92	0.48	100
12.	RM336	7	3	2.16	0.53	150
13.	RM3231	8	2	1.30	0.33	100
14.	RM5	8	3	2.98	0.66	100
	Minimum		2	1.30	0.33	100
	Maximum		3	2.98	0.66	150
	Total		36	29.98	7.25	
	Mean		2.57	2.14	0.51	

Na* = Number of alleles; Ne* = Number of effective alleles; **PIC** = Polymorphic information content; **bp**=Base pairs

Table 4. Summary of positive alleles for salt tolerance in 24 rice genotypes.

S. No	SSR marker	PIC	Amplification size (bp)	Trait linked	QTL/Gene	Chromosome	No. of genotypes	No. of genotypes with positive alleles
1.	RM472	-	200	Grain yield	*qDTY1.1*	1	0	NIL
2.	RM3412	0.64	100	Salinity tolerance	*Saltol*	1	8	DS1,DS2, DS3,DS4, DS6,DS9, DS20,DS21
3.	RM6329	0.48	100	Chlorophyll content	*qSES3.1*	3	6	DS1, DS3, DS9, DS16, DS20, DS21
4.	RM10694	0.45	150	Salinity tolerance	*Saltol*	1	0	NIL
5.	RM10793	0.66	150	Salinity tolerance	*Saltol*	1	5	DS20,DS21, DS22,DS23, DS24
6.	RM3231	0.33	100	Chl a, catalase	*qChla8.1*	8	2	DS22, DS23
7.	RM493	0.35	150	Salinity tolerance	*Saltol*	1	0	NIL
8.	RM9	0.55	100	Plant yield, harvest index	*qS1S1.1*	1	4	DS1, DS2, DS3, DS21
9.	RM336	0.53	150	Shoot dry weight	*Saltol*	7	2	DS22, DS23
10.	RM24	0.62	150	Na/K	*Saltol*	1	2	DS20, DS21
11.	RM1287	0.62	150	85% flowering, Na/K	*Saltol*	1	3	DS8, DS22, DS23
12.	RM5	0.66	100	MDA	*Saltol*	8	4	DS6, DS15, DS20, DS21
13.	RM8094	0.52	100	Salinity tolerance	*Saltol*	1	4	DS1, DS3, DS22, DS23
14.	RM140	0.45	100	K%	*Saltol*	1	5	DS1, DS6, DS9, DS20, DS22
15.	RM10852	0.39	150	Salinity tolerance	*Saltol*	1	5	DS5, DS8, DS11, DS22, DS23

DS1	MCM 142	DS7	MCM 151-3-21-1	DS13	MCM 109	DS19	FL 478
DS2	MCM 208-14-1-1	DS8	MCM 153-1-1-1	DS14	IR17F1107	DS20	MCM 153-2-1-2-1
DS3	MCM 147-1-1-2-1	DS9	MCM 253-6-2-2	DS15	MCM 253-5-3-1	DS21	MCM 253-8-2-2
DS4	MCM 258-8-2-7	DS10	MCM 100	DS16	MCM 143	DS22	MCM 106-2-10-2-2
DS5	MCM 148-2-1-1-1	DS11	MCM 103	DS17	MCM 141	DS23	MCM 305-32-2-1
DS6	MCM 149-3-1-1-1-1	DS12	MCM 125	DS18	BPT 5204	DS24	MCM 305-14-1-1-1

Table 5. Grouping into different clusters based on Jaccard's similarity coefficient using UPGMA method in 24 genotypes of rice

S.No.	Cluster	Sub cluster	Number of genotypes	Name of the genotypes
1.	I	IA	20	MCM 208-14-1-1, MCM 142, MCM 258-8-2-7, MCM 147-1-1-2-1, MCM 151-3-21-1, MCM 149-3-1-1-1-1, MCM 253-8-2-2, MCM 153-2-1-2-1, BPT 5204 (check), MCM 148-2-1-1-1, MCM 141, IR17F1107, MCM 253-5-3-1, MCM 143, MCM 109, MCM 125, MCM 103, MCM 100, MCM 253-6-2-2, MCM 153-1-1-1
		IB	3	MCM 305-14-1-1-1, MCM 305-32-2-1, MCM 106-2-10-2-2
2.	II	-	1	FL 478 (check)

Table 6. Details of the promising genotypes identified based on *per se* performance in the present study

S. No.	Genotypes	Grain yield (kg/ ha)	Number of productive tillers per plant	Number of filled grains per panicle	Panicle length (cm)	Spikelet fertility (%)	Lodging (%)
1.	MCM 208-14-1-1	7800.0	13.0	266.0	25.40	90.90	1.75
2.	MCM 147-1-1-2-1	6816.0	15.5	243.0	23.10	93.00	0.00
3.	MCM 149-3-1-1-1-1	8533.0	16.0	189.0	23.55	85.88	6.15
4.	MCM 151-3-21-1	7166.5	19.0	131.0	24.00	90.58	10.40
5.	MCM 253-5-3-1	6433.0	14.0	189.5	24.70	81.47	0.00
6.	MCM 141	7500.0	15.5	190.0	23.75	92.08	1.75
7.	MCM 305-32-2-1	6433.0	16.5	168.5	22.70	85.76	4.35
Check varieties							
1.	FL 478	3449.5	8.0	132.5	22.95	90.28	3.05
2.	BPT 5204	5666.5	18.0	190.0	22.05	94.54	90.80

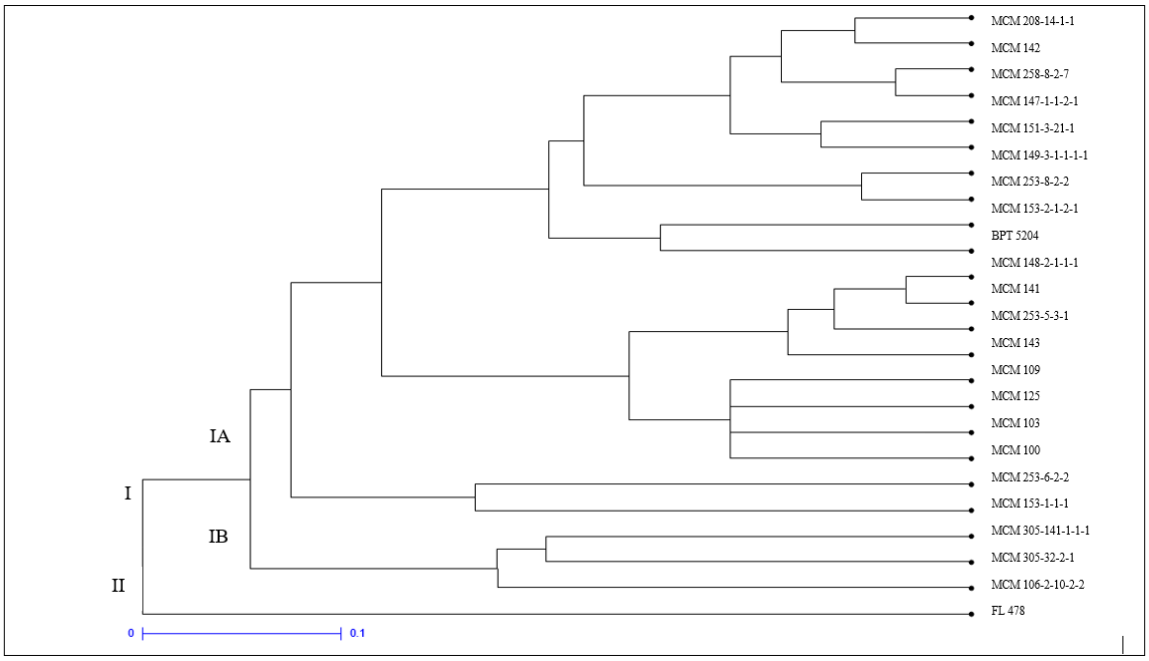
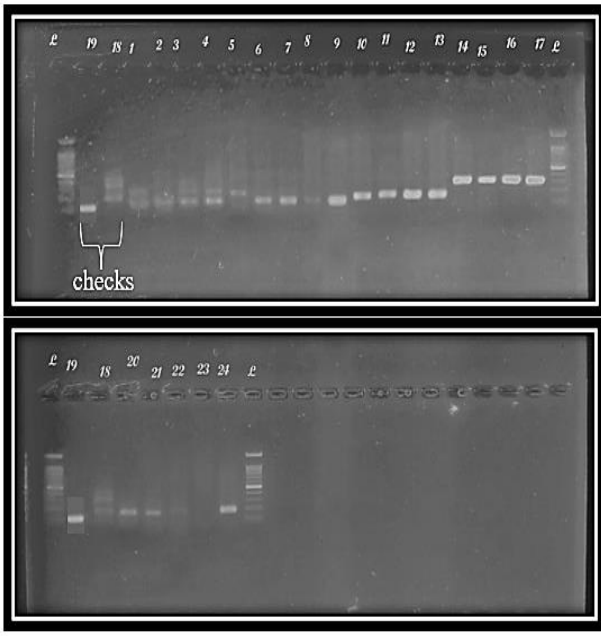
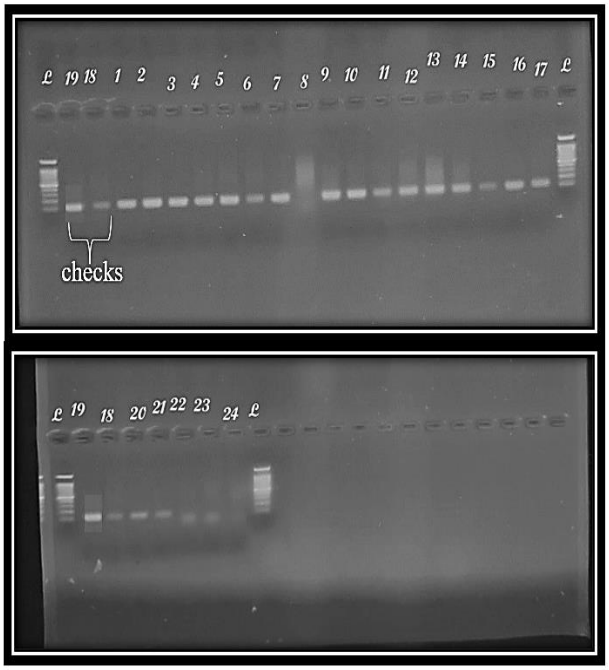


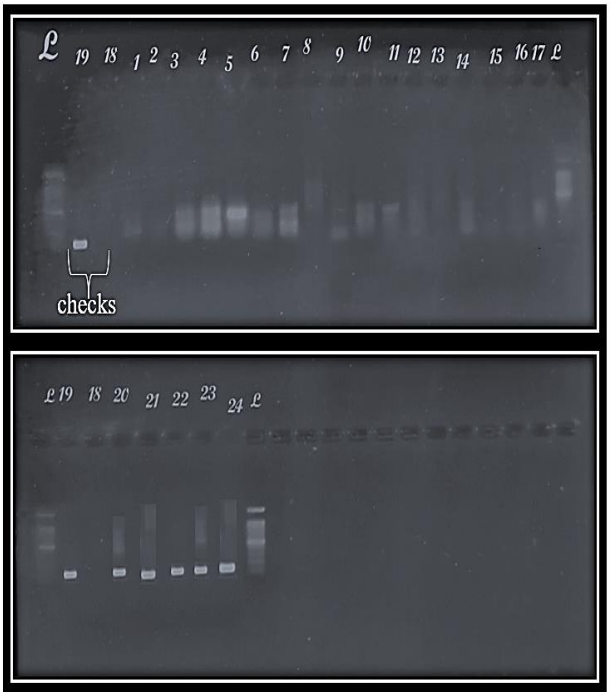
Fig. 3. UPGMA cluster dendrogram showing the genetic relationships among 24 genotypes based on the alleles detected by 15 SSR markers in rice



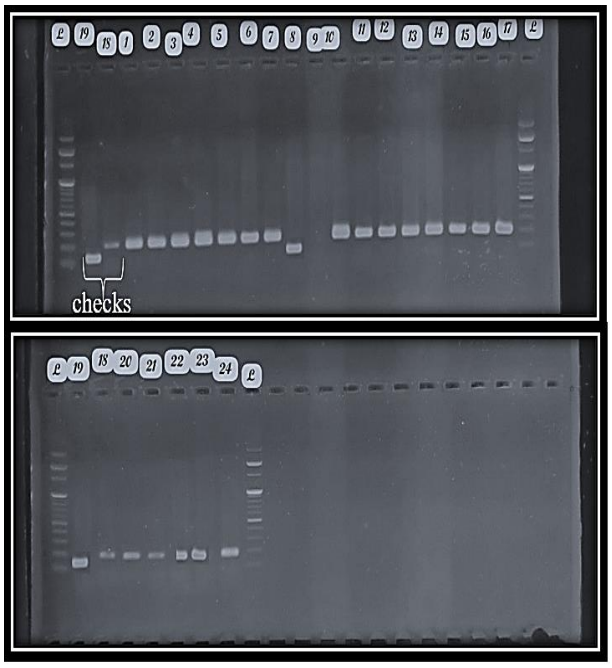
A. Primer RM3412.



B. Primer RM5.



C. Primer RM10793.



D. Primer RM1287

Plate 1. Amplification profile of the DNA of 24 rice genotypes

L: 100bp ladder

1. MCM 142; 2. MCM 208-14-1-1; 3. MCM 147-1-1-2-1; 4. MCM 258-8-2-7; 5. MCM 148-21-1; 6. MCM 149-3-1-1-1-1; 7. MCM 151-3-21-1; 8. MCM 153-1-1-1; 9. MCM 253-6-2-2; 10. MCM 100; 11. MCM 103; 12. MCM 125; 13. MCM 109; 14. IR17F1107; 15. MCM 253-5-3-1; 16. MCM 143; 17. MCM 141; 18. BPT 5204 (check); 19. FL 478 (check); 20. MCM 153-2-1-2-1; 21. MCM 253-8-2-2; 22. MCM 106-2-10-2-2; 23. MCM 305-32-2-1; 24. MCM 305-14-1-1-1

In the present study SSR markers with high PIC *viz;* RM1287 (0.62), RM3412 (0.64) and RM10793 (0.66) were previously reported across the *saltol* QTL on the chromosome 1 which is a major QTL contributing for salinity tolerance especially in the seedling stage (Aliyu *et al.,* 2011). The genotypes MCM 153-1-1-1, MCM 106-2-10-2-2 and MCM 305-32-2-1 showed marker trait co-segregation for *saltol* and positive alleles for the marker RM1287 at amplicon size of 100 bp, and genotypes MCM 142, MCM 208-14-1-1, MCM 147-1-1-2-1, MCM 258-8-2-7, MCM 149-3-1-1-1-1, MCM 253-6-2-2, MCM 153-2-1-2-1 and MCM 253-8-2-2 for the marker RM3412 at amplicon size 100 bp and MCM 153-2-1-2-1, MCM 253-8-2-2, MCM 106-2-10-2-2, MCM 305-32-2-1 and MCM 305-14-1-1-1 genotypes for the marker RM10793 at amplicon size 100 bp indicating that these elite salt tolerant lines are showing positive alleles with check FL 478. Further, it can also be concluded that salt tolerance in the remaining genotypes might be due to other alleles which could be traced out with advanced biotechnological approaches. These results were supported by previous works of Sogir *et al.,* (2023) and Rani *et al.* (2024).

In the present study, the salt tolerant lines developed after confirming field studies at ARS, Machilipatnam (EC 9-10 dSm^{-1}, pH 7.9) *viz.,* 208-14-1-1, MCM 147-1-1-2-1, MCM 149-3-1-1-1-1, MCM 253-5-3-1 and MCM 305-32-2-1 recorded some positive alleles for *saltol* besides good *per se* performance for most yield contributing characters under direct sown conditions in normal soils (EC 2.5 dSm^{-1}, pH 7.2) of Bapatla. Rice crop is most sensitive to salinity at seedling and reproductive stage causing huge yield losses. Hence it is viable option to grow salt tolerant lines with high yield potential and amenable for direct seeded conditions in flood prone coastal belts. Hence the best performing genotypes identified in the present study with high yield and early vigour can be further tested in saline soils under direct seeded conditions. Direct sowing is a climate-smart adaptation practice in coastal ecosystems. Further, it allows early establishment before salinity levels peak, ensuring better germination and survival, because coastal areas are increasingly affected by salt water intrusion and rising ground water salinity due to climate change.

ACKNOWLEDGMENTS

The authors are thankful to the Department of Genetics and Plant Breeding, Agricultural College, Bapatla, ANGRAU for providing resources for carrying out the research.

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