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Anju Mariam Joseph
Department of Plant Breeding and Genetics, CoA, Vellayani, Thiruvananthapuram, Kerala, India

Arya K
Department of Plant Breeding and Genetics, CoA, Vellayani, Thiruvananthapuram, Kerala, India

Veena Vighneswaran
Plant Breeding and Genetics, Rice Research Station, Vyttila, Ernakulam, Kerala, India

Bindhya Mathew
Department of Plant Breeding and Genetics, CoA, Vellayani, Thiruvananthapuram, Kerala, India

Corresponding Author:
Anju Mariam Joseph
Department of Plant Breeding and Genetics, CoA, Vellayani, Thiruvananthapuram, Kerala, India

Salinity screening of FL478 in Pokkali tracts of Kerala

Anju Mariam Joseph, Arya K, Veena Vighneswaran and Bindhya Mathew

Abstract
Saltol, the major QTL for seedling stage salinity tolerance was mapped on chromosome 1 in an F_8 Recombinant Inbred Line (RIL) population obtained by a cross between a saline-tolerant landrace, Pokkali and salt-sensitive IR 29. The resultant genotype FL478 is used as a salinity tolerance donor. The present study screens various aspects of salinity stress response at various stress levels of two major saline-tolerant genotypes. The *in vitro* salinity screening revealed that FL478 is a better performer in comparison to Pokkali regarding salinity responses. The field salinity screening had a different result. At 6 dS m^{-1}, FL478 and Pokkali showed responses on par. As the stress levels increased, FL478 tends to suffer more stress injury and displays adaptation mechanisms deficit in comparison to Pokkali, especially in the reproductive stage. Saltol based tolerance may not be the only salinity response mechanism in Pokkali. The flowering stage response of the landrace may be indicative of other genes or QTLs in the Pokkali genome associated with reproductive stage salinity tolerance.

Keywords: Saltol, salinity tolerance, rice, Pokkali, FL478

Introduction
Rice (*Oryza sativa* L.), one of the most important cereal crops, is categorized as a typical glycophyte, sensitive to salinity, particularly during seedling and reproductive stages. The response of rice plants to high salinity results from interactions involving various stress-responsive genes in a regulatory network. Saltol, QTL for seedling stage salinity tolerance had been mapped on chromosome 1 in an F_8 Recombinant Inbred Line (RIL) population obtained by a cross between Pokkali and IR 29 (Waziri *et al*, 2016) [14]. In the present study, differential manifestation in plants with respect to their morphological, physiological, biochemical or molecular parameters in response to salt stress between the landrace, Pokkali and the salinity donor FL478 derived from Pokkali were compared in the native environment of the former.

Materials and Methods
The experiment was conducted at Rice Research Station, Vyttila, Kerala in Rabi, 2021. The RIL population, FL-478 derived from IR-29 x Pokkali pertinent to Saltol QTL mapping was screened in the Pokkali tract of Kerala. The performance of the same was compared against Pokkali, the salinity tolerance donor native to the locale. The seedlings were phenotyped *in vitro* at electrical conductivity (EC) 6, 9 and 12 dS m^{-1} according to the standard protocol for salinity screening of IRRI (Gregorio *et al.* 1997) [7] for seedling stage salinity screening. Yoshida solution (Yoshida *et al*. 1976) [15] was the nutrient medium used for *in vitro* screening. The pH is maintained between 5.0 – 5.5 to ensure balanced availability of nutrients. The salinity was maintained at 6, 9 and 12 dS m^{-1} by adding NaCl to the nutrient solution. pH and EC were monitored and maintained daily. Test entries were scored 16 days after salinization using Standard Evaluation System (SES), 2002. With regard to field screening, seedlings were raised in pots filled with native soil of the Pokkali tract for physiological and biochemical characterization. Salinity was maintained at 6, 9 and 12 dS m^{-1} in pots into which 10 days old seedlings were transplanted. Sampling was done at 4 stages viz. 21st day, 40th day (active tillering), 60th day (panicle initiation) and flowering for assessment of various physiological and biochemical parameters. The molecular screening was done by extracting DNA from young leaves of 21 day old rice plants (Doyle and Doyle, 1987) [6]. Genome-wide molecular assay of both the genotypes was carried out using SSR markers comprising the foreground markers associated with the Saltol QTL located on the short arm of chromosome 1 and an array of genome-wide background markers.

Results and Discussion

The study was divided into *in vitro* salinity screening, field salinity screening and genome wide molecular screening.

In vitro salinity screening

In vitro screening was used to register the germination percentage, survival percentage, percentage of leaf drying and SES scoring under salinity stress. The environmental effect was maintained at a minimum by conducting this screening in a polyhouse maintained at 29°/21 °C day/night temperature and minimum relative humidity of 50% during the day.

Table 1: *In vitro* salinity screening

Germination percentage				Survival percentage			
	6 dS m^{-1}	**9 dS m^{-1}**	**12 dS m^{-1}**		**6 dS m^{-1}**	**9 dS m^{-1}**	**12 dS m^{-1}**
FL 478	96.67	93.33	93.33	FL 478	96.55	92.86	78.57
Pokkali	93.33	90.00	86.67	Pokkali	100.00	77.78	73.08
Leaf drying percentage				**SES Score**			
	6 dS m^{-1}	**9 dS m^{-1}**	**12 dS m^{-1}**		**6 dS m^{-1}**	**9 dS m^{-1}**	**12 dS m^{-1}**
FL 478	32.14	71.79	65.15	FL 478	2.93	5.62	5.27
Pokkali	61.90	74.07	80.77	Pokkali	4.86	5.89	6.38

The results (Table. 1) revealed that the tolerance to salinity in controlled conditions is greater for FL478 surpassing its salinity tolerance donor paternal parent. FL478 showed better salinity tolerance in comparison to Pokkali at the seedling stage. Germination percentage and survival percentage were higher in FL478 except at 6 dS m^{-1}. Percentage leaf drying and SES Score showed a higher injury level in Pokkali (Fig.1).

Salinity screening at Field level

Screening at the field level included both biometric and biochemical characterization. Chlorophyll content was obtained using leaf greenness (SPAD-502 DL Plus Chlorophyll Meter) and acetone extraction method (Arnon, 1949) [2]. Sodium and potassium content was obtained using acid digestion and flame photometry (Jackson, 1973) [10]. Proline in plant samples was estimated by the method proposed by Bates et al. (1973) [3]. Estimation of catalase was done according to the method followed by Aebi (1974) [1]. Peroxidase assay was done using the method elucidated by Kar and Mishra (1976) [11]. Superoxide Dismutase assay was done following the method of Beauchamp and Fridovich (1971) [4]. The results of biometric observations at 21 days were recorded (Table 2). Grain yield per plant was recorded at various stress levels (Table 3).

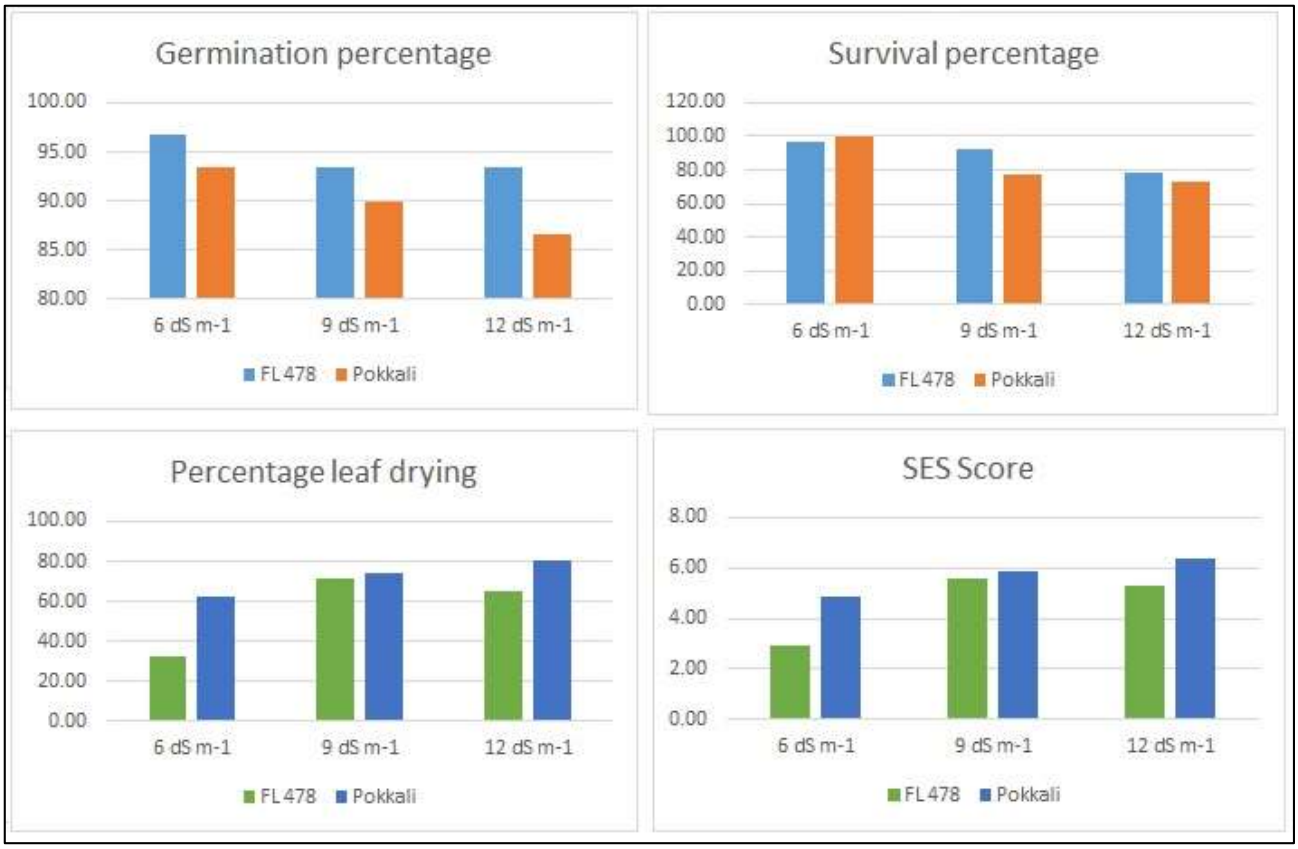

Fig 1: *In vitro* screening graphical representation

Table 2: Biometric observations from field screening

Sl. No.	Characteristics (@ 21 days)	EC (dSm^{-1})	Pokkali	FL 478
1	Plant height (cm)	6	30.5	23.93
		9	26.2	20.43
		12	17.2	14
2	Root length (cm)	6	15.5	13.9
		9	11.37	11.03
		12	10	8.43
3	Fresh weight of shoot (mg)	6	153.23	147.5
		9	107.3	103.6
		12	63.1	60.97
4	Dry weight of shoot (mg)	6	34.1	32.8
		9	20.3	19.7
		12	16.6	16.2
5	Fresh weight of root (mg)	6	77.7	74.9
		9	54	52.3
		12	19.1	18.6
6	Dry weight of root (mg)	6	26	25.13
		9	17.43	16.83
		12	11.77	11.3

Table 3: Grain yield at various stress levels

Grain yield per plant (g)	Pokkali	FL 478
6 dSm^{-1}	4.66	3.39
9 dSm^{-1}	2.11	0.75
12 dSm^{-1}	0.69	0.21

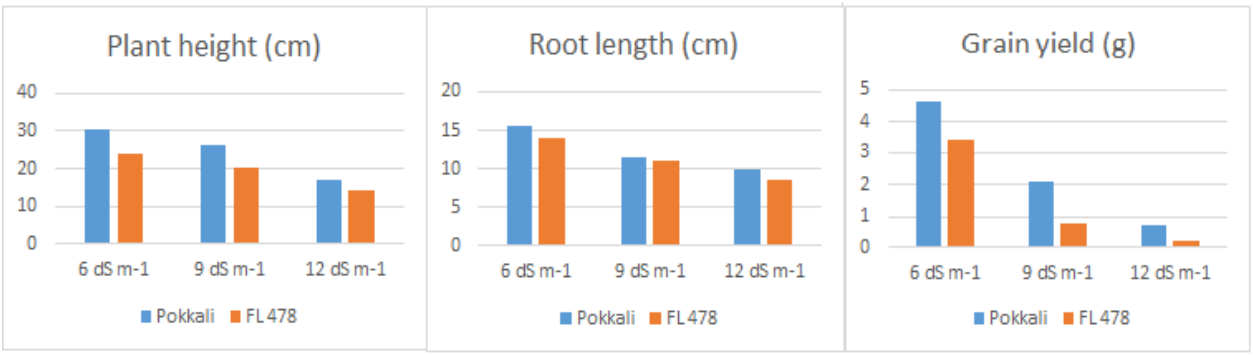

Fig 2: Biometric observations and grain yield from field screening

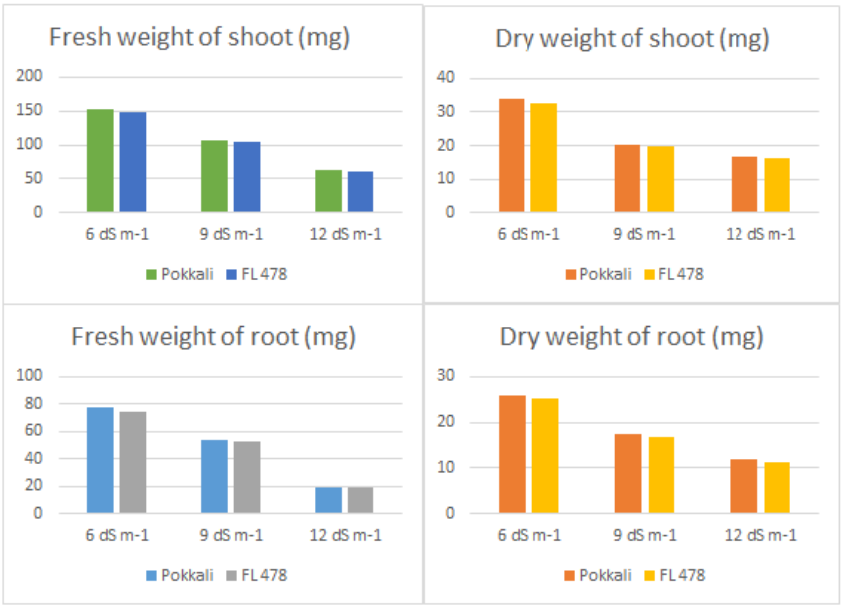

Fig 3: Observations on biomass at 21 days

Field screening revealed a better performance of Pokkali for the various character as against FL478 with regard to plant height, root growth, biomass accumulation and grain yield. The biochemical data were recorded at various stress levels at critical stages of growth and development.

Table 4: Chlorophyll content at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

EC (dS m-1)	Chlorophyll content (mg/g)	21 days	40 days	60 days	Flowering
6 dS m^{-1}	Pokkali	2.24	2.77	2.17	2.38
	FL 478	2.61	3.12	2.59	2.38
9 dS m^{-1}	Pokkali	1.89	1.92	1.77	1.82
	FL 478	2.22	2.23	2.04	1.97
12 dS m^{-1}	Pokkali	1.56	1.51	1.59	1.37
	FL 478	1.54	1.41	1.71	1.52

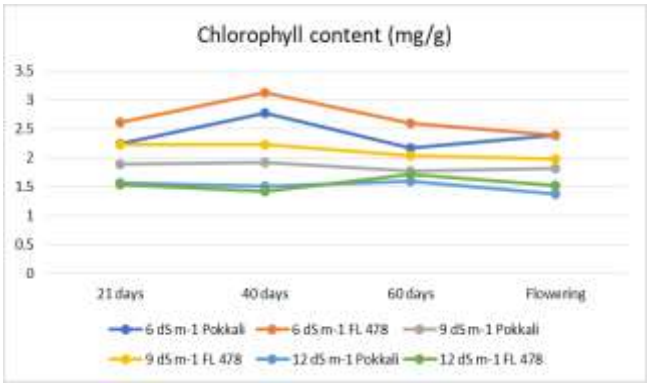

Fig 4: Chlorophyll content at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

The chlorophyll content at 6 dS m^{-1} was found to be lesser in Pokkali but at flowering both the genotypes had equivalent quantity. At 9 dS m^{-1}, the chlorophyll content is consistently lower in Pokkali but the difference minimizes by the flowering stage. At 12 dS m^{-1}, the chlorophyll content in Pokkali shows gradual increase and then decreasing at flowering while in FL478 the value had reduced at tillering stage but increased during panicle initiation and decreases at flowering. The impact of salinity on chlorophyll content seems inconclusive but FL478 maintains a better profile. The Na^+ : K^+ ratio was lower in FL478 during vegetative phase while higher in reproductive phase as against the values of Pokkali. Hence maintenance of ionic homeostasis is better in Pokkali across various growth stage when compared to FL478.

Table 5: Na^+ : K^+ ratio at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

EC (dS m-1)	Na^+ : K^+ ratio	21 days	40 days	60 days	Flowering
6 dS m^{-1}	Pokkali	0.95	1.25	1.59	1.20
	FL 478	0.92	1.18	1.69	1.46
9 dS m^{-1}	Pokkali	1.12	1.48	1.64	1.55
	FL 478	1.08	1.40	2.02	2.04
12 dS m^{-1}	Pokkali	1.42	2.07	1.71	1.13
	FL 478	1.42	1.95	2.20	1.61

The highest overall proline content was observed in FL478 during 12 dS m^{-1} while the lowest was in Pokkali at 6 dS m^{-1}. Proline content remains on par for both the genotypes in the vegetative stages but in the reproductive stages, Pokkali accumulates lesser proline in comparison with FL478 though there is a steady increase in proline content across various stress levels.

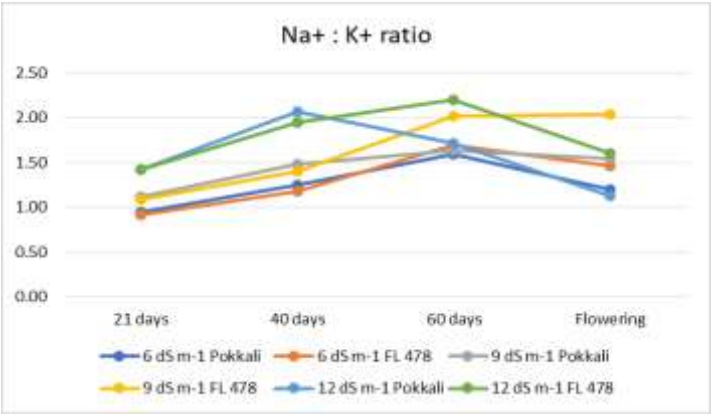

Fig 5: Na^+ : K^+ ratio at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

Table 6: Proline content at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

EC (dS m-1)	Proline content (µg/g)	21 days	40 days	60 days	Flowering
6 dS m^{-1}	Pokkali	713.66	745.49	778.66	840.25
	FL 478	691.35	726.6	926.1	1048.34
9 dS m^{-1}	Pokkali	1121.06	1167.03	1185.06	1275.1
	FL 478	1100.06	1147.22	1430.68	1488.59
12 dS m^{-1}	Pokkali	1708.41	1825.88	2053.59	2136.71
	FL 478	1592.36	1830.48	2112.55	2364.27

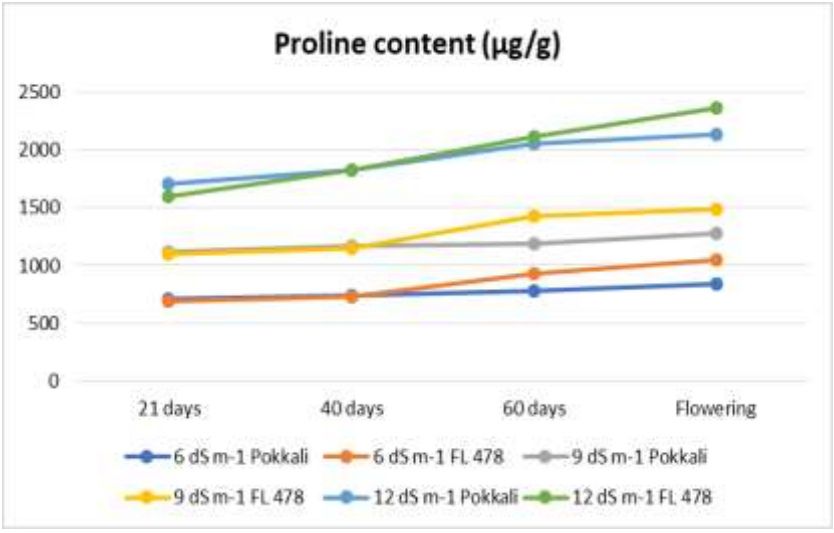

Fig 6: Proline content at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

Chunthaburee et al. (2016) [5] observed a 7.95 fold increase in proline in salt susceptible IR29, while tolerant cultivars Pokkali and FL496 had only a 1.83 and 2.57 fold increase compared to the control. In this context, the accumulation of proline implies the stress experienced by the plant rather than the countermeasure adopted.

Enzymatic antioxidant defense machinery was analyzed based on the activity of catalase, peroxidase and superoxide dismutase. Superoxide dismutase enzyme functions by dismutating superoxide radicals into hydrogen peroxide. The scavenging activity at this point is taken over by catalase, ascorbate peroxidase, glutathione reductase, etc. which decomposes hydrogen peroxide into water and oxygen (Rossatto et al., 2017) [12]. The activity of catalase in the present study remained on par at the vegetative phase for both genotypes. FL478 showed reduced activity in the reproductive phase at 9 and 12 dS m^{-1} contrary to Pokkali where catalase activity kept increasing with increasing stress levels.

Table 7: Catalase activity at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

EC (dS m-1)	Catalase activity (Units/min/ g fresh wt)	21 days	40 days	60 days	Flowering
6 dS m^{-1}	Pokkali	123.83	131.12	134.82	174.13
	FL 478	129.1	135.67	105.75	137.28
9 dS m^{-1}	Pokkali	193.47	198.89	208.33	211.99
	FL 478	205.49	209.67	219.1	179.56
12 dS m^{-1}	Pokkali	217.39	223.76	230.95	243.46
	FL 478	220.62	227.43	234.62	209.36

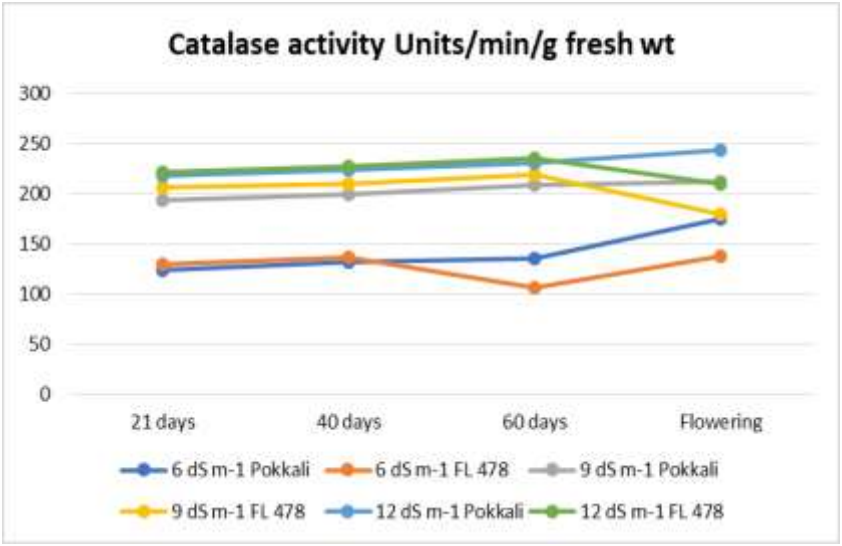

Fig 7: Catalase activity at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

Peroxidase activity was found to increase with an increase in stress levels in both genotypes at the vegetative stage. Towards panicle initiation and flowering, there is a decreasing trend in peroxidase activity in both genotypes with a greater reduction in FL478. In Pokkali though the trend is decreasing the activity remains greater than at 21 days.

Table 8: Peroxidase activity at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

EC (dS m-1)	Peroxidase activity (Units/min/g fresh wt)	21 days	40 days	60 days	Flowering
6 dS m^{-1}	Pokkali	1.006	1.918	1.706	1.143
	FL 478	1.034	1.994	1.723	0.81
9 dS m^{-1}	Pokkali	1.089	2.184	1.842	1.44
	FL 478	1.185	2.2284	1.757	1.071
12 dS m^{-1}	Pokkali	1.275	2.334	1.869	1.794
	FL 478	1.361	2.393	2.03	1.357

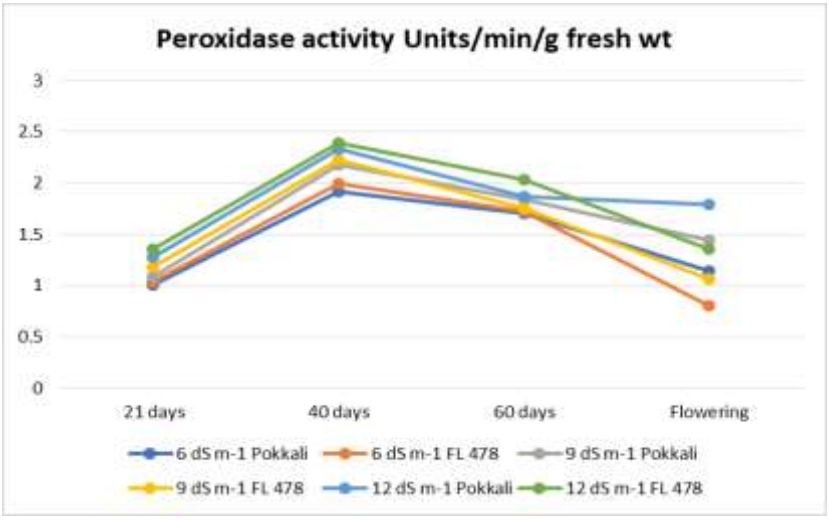

Fig 8: Peroxidase activity at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

Superoxide dismutase activity appeared to be increasing till the active tillering phase and gradually decline during the reproductive phase in both genotypes at different levels of stress. Pokkali shows higher activity in comparison to FL478 regardless of the stress levels.

Table 9: Superoxide dismutase activity at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

EC (dS m-1)	Superoxide dismutase activity (Units/min/g fresh wt)	21 days	40 days	60 days	Flowering
6 dS m-1	Pokkali	65.88	73.47	56.24	54.7
	FL 478	70.37	69.46	39.59	54.96
9 dS m-1	Pokkali	81.17	82.6	73.95	68.87
	FL 478	81.94	72.67	63.23	58.63
12 dS m-1	Pokkali	86.31	94.88	79.62	75.44
	FL 478	87.08	86.49	77.04	62.52

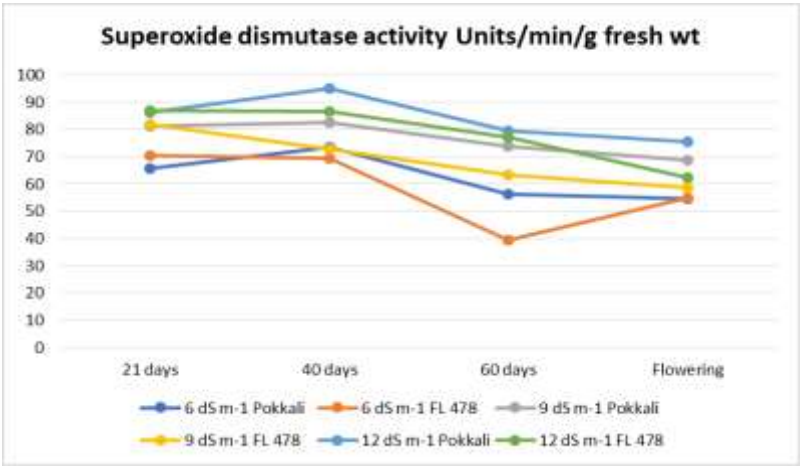

Fig 9: Superoxide dismutase activity at 4 stages at stress levels of 6, 9 and 12 dS m^{-1}

Molecular Screening

Genome-wide molecular screening using SSR markers was conducted. Foreground markers specific to Saltol QTL and a series of background markers were used. The assay can be summarized in Table 10. The Saltol locus as mapped on FL478 extends from 10.7 – 12.2 Mb. Three markers RM1287 (10.8 Mb), RM10711 (11.2 Mb) and RM10713 (11.2 Mb) falling within the QTL locus showed polymorphism between the genotypes. This observation might account for the better performance of FL478 in seedling stage.

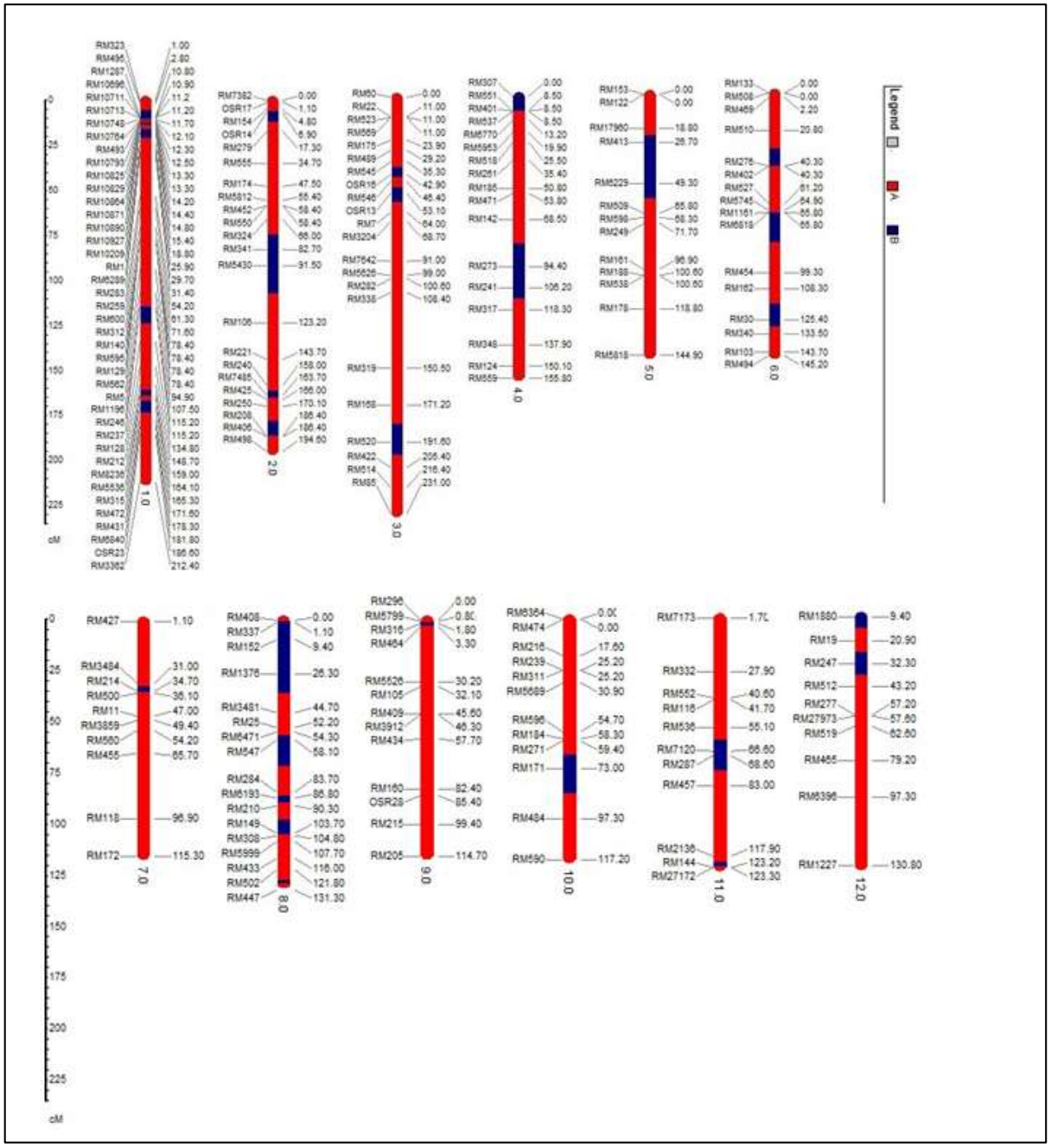

Fig 10: GGT of Genome-wide molecular screening of Pokkali and FL478

Table 10: Genome-wide molecular screening

Sl. No.	Characters	Observation
1	Total no. of markers	206
2	Monomorphic	163
3	Polymorphic	43
4	Monomrphism %	80.8
5	Polymorphism %	19.2

Conclusion

The present study has screened various aspects of salinity stress response at various stress levels of two major saline-tolerant genotypes. The *in vitro* salinity screening revealed that FL478 is a better performer in comparison to Pokkali regarding salinity responses. The field salinity screening had a variable response. At 6 dS m^{-1}, FL478 and Pokkali showed responses on par despite the stage of development. As the stress levels increased, FL478 tends to suffer more stress injury and displays poorer adaptation mechanisms in comparison to Pokkali, especially in the reproductive stage. Saltol based tolerance may not be the only salinity response mechanism in Pokkali. Pokkali indeed may be a source of reproductive stage salinity tolerance with an unknown QTL. Furthermore, the superiority of the landrace may be attributed to evolutionary consequences resulting in specific adaptation to the environment.

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