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RESEARCH ARTICLE

Biochemical, physiological and molecular evaluation of rice cultivars differing in salt tolerance at the seedling stage

Mojtaba Kordrostami[1] · Babak Rabiei[2] · Hassan Hassani Kumleh[1]

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Abstract Changes in the antioxidant enzymes, lipid peroxidation, sodium and potassium, chlorophyll, H_2O_2 and proline content were monitored in the leaves of 42 rice varieties which were not yet well-documented for the salinity tolerance under different salinity levels. The tolerant varieties (FL478, Hassani, Shahpasand, Gharib and Nemat) showed signs of tolerance (lower Na^+/K^+ ratio, high proline accumulation, less membrane damage, lower H_2O_2 production, and higher superoxide dismutase and catalase activity) very well. The positive relationship between the level of salt tolerance and the amount of proline accumulation in the rice varieties support the important role of proline under the salt stress. The varieties were genotyped for 12 microsatellite markers that were closely linked to *SalTol* QTL. The results of association analysis indicated that RM1287, RM8094, RM3412 and AP3206 markers had the high value of R^2 for the regression models of the studied traits. It shows the important role of *SalTol* in controlling physio-biochemical traits. The results can be used in the future marker assisted selection (MAS) directly, if the results are confirmed.

Keywords Antioxidant enzymes · Hydrogen peroxide · Lipid peroxidation · Rice · Salt

Introduction

Salinity is the most important abiotic stress, which reduces the plant growth and productivity (Nazar et al. 2011; Kordrostami et al. 2016). The researchers express the salt stress as the accumulation of the ions such as sodium, sulphate and chloride in the rhizosphere in a way that disrupts the natural growth of the plant (Munns 2002; Ashraf and McNeilly 2004). Distribution and dispersion of saline lands is not uniform throughout the world, so that Australia and Asia have the highest surface of the saline lands. In Asia, Iran is in the fifth place for the area of saline soils after the former Soviet Union, China, India and Pakistan (ICARDA 2002). According to the various references, Iran saline lands are estimated between 23 and 34 million hectares. Furthermore, the statistics of FAO (2010) suggests that 25.5 million hectares of agricultural lands of Iran are saline and 8.5 million hectares are extremely saline. Most crops are sensitive to the salt stress and cannot survive in these circumstances. Plant response to salinity stress is complex and depends on various factors such as type and concentration of the solutes, plant growth stage, the genetic potential of the plant and environmental factors. Salinity reduces the growth in different ways (Dieriga et al. 2003). Reduced cell membrane stability, reduced photosynthesis and activity of the photosynthetic enzymes, reduced cells inflammation and thus reduced leaf

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✉ Babak Rabiei
rabiei@guilan.ac.ir

Mojtaba Kordrostami
kordrostami009@gmail.com

Hassan Hassani Kumleh
kumleh@guilan.ac.ir

[1] Department of Plant Biotechnology, Faculty of Agricultural Sciences, University of Guilan, P.O. Box 41635-1314, Rasht, Iran

[2] Department of Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, P.O. Box 41635-1314, Rasht, Iran

development, disorder in ion absorption; especially the accumulation of sodium and chlorine ions in the leaves, and ultimately reduced growth and economic performance are the effects of salinity on the crops (Munns 2002).

Rice is the world's most important food plant and the first food source for more than half of the world's population. It is also considered as a model for the cereals (Eckardt 2000). The reaction to the salt stress is very diverse among rice varieties. Therefore, understanding the mechanisms of the salt tolerance and the mechanisms for establishing the tolerance is very important in the agronomic and physiological studies. Rice is a salt sensitive crop (Grover and Pental 2003). Excessive salt always affects the underlying metabolic activities in rice including, the cell wall damage, plasmolysis, cytoplasmic degradation and the endoplasmic reticulum damage, accumulation of citrate, malate and inositol in the leaf blade, increase in the amount of proline (6–63 times), reduced the maximum quantum efficiency of photosystem II (Fv/Fm), overall reduction in germination and seedling growth, which ultimately leads to a lower growth and grain yield (Sahi et al. 2006). So, salinity is considered as one of the main constraints to produce of this crop, especially in the irrigated areas all over the world (Octávio et al. 1999).

Since rice is the main crop in the world, several studies have been conducted on salt tolerance of different varieties. The studies have shown that rice plants are relatively tolerant to salinity in the germination stage, very sensitive in the early seedling (3 leaves) and tolerant in the vegetative stage (Moradi and Ismail 2007). Researches showed that salinity strongly affects the photosynthesis. Photosynthesis in rice plants is controlled by various environmental factors. The factors depend on the variety, growth stage and environmental conditions. Availability of the nutrients in the environment is essential for rice and any environmental stress will reduce the growth (Postini and Bieker 1994). An increase in salinity can reduce nutrient uptake by the rice plants. Salinity is defined as the presence of the excessive amounts of soluble salts in the soil or irrigation water. The critical level of salinity for rice has been reported between 3 and 4 $dS.m^{-1}$. But the critical concentration of salt in the rice leaf tissue, causing damage to plants is different among the various varieties (Zeng et al. 2003). One of the effects of salinity on the rice is the leaf area reduction, a key factor in the photosynthesis decline. The researchers showed that in rice, the fully expanded leaves are affected by salinity before the young ones. In fact, due to salinity the leaf area will be reduced, as the first reaction of the plant (Alam et al. 2004). The soil water potential will be reduced by the increase in salinity and stomatal closure is an initial and rapid response of the plants to the salt stress. Quick stomatal closure may be due to low water potential and Na^+ harmful effects on the root signals and the stomatal guard cells of the leaves (Moradi and Abdelbagi 2007). The balance of K^+ and Na^+ ions within the cell are very important for the activity of many cytosolic enzymes, protection of membrane potential and as an osmotic regulator for regulating the cell volume (Koji et al. 2008). One of the secondary effects of high Na^+ concentrations in the root zone is preventing the absorption of essential nutrients like potassium and calcium. Salinity has direct effects on the levels of enzymatic activity, cell membrane function and metabolic processes (Moradi and Abdelbagi 2007). Salinity causes the water shortages which its result affects a wide range of metabolic activities of the plants. Reactive oxygen species arise due to the hyperosmotic effects and ionic stresses, causing decline in the membrane and cell death (Parida and Das 2005). Plants have mechanisms to prevent the toxic effects of the ROS, which divided to enzymatic (superoxide dismutase, catalase, glutathione reductase, ascorbate peroxidase and peroxidase) and non-enzymatic (tocopherol, ascorbic acid, glutathione, etc.) mechanisms (Reddy et al. 2004; Demiral and Türkan 2005; Sekmen et al. 2007). The relationship between antioxidant capacity and the salinity tolerance have been observed and discussed in some plant species (Jithesh et al. 2006; Cicek and Cakirlar 2008; Ashraf 2009).

The research has shown that rice in seedling stage is more sensitive to salt than the reproductive stage (Sahi et al. 2006). The mechanisms of salinity resistance in higher plants include osmotic adjustment, ion adjustment and hormonal regulation (Chinnusamy et al. 2005). However, to improve the salt tolerance in crop plants via breeding programs, further exploration of the defense mechanisms against salinity in the susceptible and resistant varieties is essential (Cha-um et al. 2009). Therefore, understanding the mechanisms of salinity resistance in existing rice varieties and finding a way to transfer it to the high yielding varieties is one way of increasing the rice production in the world. On the other hand, one of the most important approaches for increasing the efficiency of the breeding for salt tolerance is to discover the genetic markers that are tightly linked to the tolerance related traits. The molecular breeding can facilitate the development of crop plants with improved salt tolerance, compared to traditional phenotypic selection methods (Foolad 2004; Collins et al. 2008; Witcombe et al. 2008). *SalTol* is a major quantitative trait locus (QTL) which is located on chromosome one. This QTL confers salinity tolerance to the rice varieties at the seedling stage and explains from 64 to 80% of the phenotypic variation (Bonilla et al. 2002). There are several reports about this QTL in some other rice varieties (Ren et al. 2005; Takehisa et al. 2004).

Based on the facts above, the present study was conducted to evaluate the best rice varieties which tolerate the

salt stress using physiological and biochemical characteristics, assess the genetic variation of rice varieties based on the SSR markers tightly linked to the *SalTol* QTL and identify salt tolerant rice varieties with putatively novel salinity tolerance sources.

Materials and methods

Plant materials

A total of 42 rice varieties (Supplementary Material 1) obtained from the Rice Research Institute of Rasht, Iran (RRII), together with salt-tolerant and susceptible varieties (FL478 and IR29) as the check varieties were screened for salinity tolerance. FL478 and IR29 are often used as check varieties to assess the salt tolerance (Gregorio et al. 1997). The other 42 varieties are not yet well-documented for salinity tolerance. This research was carried out in the form of three separate experiments based on the completely randomized design (CRD) with two factors, 44 rice varieties and salinity in three levels (control, 6 and 12 $dS.m^{-1}$ NaCl). The research was conducted at the seedling stage of the studied varieties at the Biotechnology laboratory of Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran, in 2015.

Screening for salinity tolerance

The plants grown in the greenhouse under natural light conditions. A preliminary test for salt tolerance was carried out using Gregorio et al. (1997) method. Seeds were surfaced sterilized with 5% sodium hypochlorite, imbibed for 48 h and sown on the plastic grids placed above 4 L black plastic pots containing distilled water. Three replications were used (each replication had 15 seedlings). When seedlings were 5 days old, distilled water was replaced with nutrient solution and after 14 days, nutrient solution was replaced with the nutrient solution containing the salinity with EC $\sim$6 and $\sim$12 $dS.m^{-1}$ corresponded to the studied salinity levels. Also, for the induction of severe stress at 12 $dS.m^{-1}$, nutrient solution was initially replaced with salinized nutrient solution at EC $\sim$6 $dS.m^{-1}$ for 3 days and finally at EC $\sim$12 $dS.m^{-1}$ for 14 days. The pH of the nutrient solution was maintained between 5.0 and 5.5 throughout the growth period. Salt stress symptoms were recorded according to the International Rice Research Institute standards (IRRI 1996) from all 15 samples in each replications. Visual rating of salinity tolerance was done according to Supplementary Material 2. After 14 days in the salinized solutions, plants were harvested (all 45 samples from three replications) for determination of physiological traits and enzyme assays.

H_2O_2 content

The H_2O_2 content from the leaves of rice seedlings was measured as described by Vellikova et al. (2000). The H_2O_2 content was determined using an extinction coefficient (ε) of 0.28 μM^{le} cm^{-1}.

Chlorophyll content

Leaf chlorophyll content was measured on the fully expanded leaves of all the plants per pot with a chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co. Ltd., Japan) at the harvest time.

Measurement of chlorophyll a fluorescence

The first intact, fully expanded leaf from the top was used for the measurement of chlorophyll *a* fluorescence using a plant efficiency analyzer (Handy PEA, Hansatech Instruments Ltd, Norfolk, UK). The data were recorded from 10 ms up to 1 s with data acquisition every 10 ms for the first 300 ms, then every 100 ms up to 3 ms and thereafter every 1 ms. The signal resolution was 12 bits (0–4000). The maximal quantum yield of PSII photochemistry (*Fv*/*Fm*) was calculated using the software supplied by the manufacturer.

Enzyme assay

Rice leaves (0.25 g) were homogenized in 1 mL of 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM of EDTA in the presence of PVP. The homogenate was centrifuged at 15,000 g for 15 min at 4 °C. The supernatant was used to measure the activities of SOD, POD and CAT and to determine total protein content. All assays were done at 25 °C using a spectrophotometer (T80, "PG Instrument", UK).

SOD (EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photo-reduction of nitro blue tetrazolium (NBT) according to the methods of Beauchamp and Fridovich (1971). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 200 mM methionine, 1.125 mM NBT, 1.5 mM EDTA, 75 µM riboflavin, and 0–50 µL of the enzyme extract. Riboflavin was added as the last component. The reaction was carried out in test-tubes at 25 °C under illumination supplied by two fluorescent lamps (20 W). The reaction was initiated by switching on the light for 15 min, and light switching off stopped the reaction. The tubes were then immediately covered with aluminum foil in order to stop the reaction, and absorbance of the mixture was then read at 560 nm. SOD activity of the extract was expressed as activity unit/g fr wt.

POD (EC 1.11.1.7) activity in leaves was assayed by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm (Chance and Maehly 1955). The reaction mixture contained 100 µL of crude enzyme extract, 500 µL of 5 mM H_2O_2, 500 µL of 28 mM guaiacol, and 1900 µL of 50 mM potassium phosphate buffer (pH 7.0). The POD activity of the extract was expressed as activity unit/(g fr wt min).

CAT (EC 1.11.1.6) activity was assayed according the method of Beers and Sizer (1952). The decomposition of H_2O_2 was monitored by the decrease in absorbance at 240 nm. The assay mixture contained 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0), 400 µL of 15 mM H_2O_2, and 40 µL of enzyme extract. The CAT activity of the extract was expressed as activity unit/(g fr wt min).

Malondialdehyde (lipid peroxidation) and proline content

For the measurement of lipid peroxidation, the thiobarbituric acid (TBA) test was used to measure MDA level as an end product of lipid peroxidation. The amount of MDA-TBA complex present was calculated using an extinction coefficient (ε) of 155 mM^{-1} cm^{-1}. Proline content was determined spectrophotometrically by adopting the ninhydrin method of Bates et al. (1973). Three hundred mg of fresh leaf samples were homogenized in sulfosalicylic acid; then 2 mL of each acid ninhydrin and glacial acetic acid were added. The samples were heated at 100 °C for 60 min. The mixture was extracted with toluene, free toluene was quantified at 520 NM using L-proline as a standard, and its content was expressed as µmol/g fr wt.

DNA extraction and SSR analysis

The fresh leaves were used for DNA extraction according to the CTAB method (Saghai-Maroof et al. 1984) with the slight modifications. The quality and quantity of the extracted DNA were evaluated by electrophoresis on a 0.8% agarose gel and spectrophotometer (LAMBDA 1050UV). A total of 12 SSR DNA markers, all of them near to the *SalTol* QTL region on the rice chromosome one, which were previously introduced by Bonilla et al. (2002), Gregorio et al. (2002), Islam (2004) and Niones (2004), were selected (Supplementary Material 3). A total of 3 µl PCR products were denatured and run on 6% polyacrylamide denaturing gels and electrophoretic bands were revealed using the silver staining described by Panaud et al. (1996). The observed alleles were scored in all studied varieties as (1) for existence and (0) for absence of each allele according to molecular weight using DNA size marker 100 bp Fermantas.

Data analysis

The combined analysis of variance was performed for combined data from three experimental conditions (non-stress and salinity stress of 6 and 12 $dS.m^{-1}$) to assess the effect of genotype, salinity and genotype × environment interaction using the SAS software ver. 9.2 (SAS Institute 2010). The Pearson's correlation coefficients among the studied traits were also calculated using the SPSS software ver. 19.0 (IBM Corp 2010). All the marker indices were previously reported by Kordrostami et al. (2016) for these varieties. Association analysis was performed to identify informative markers associated with each of the 12 studied traits using stepwise regression analysis between molecular data (as the independent variables) and morphological data (as the dependent variables) by SPSS software ver. 19 (SPSS 2010). To select the informative markers as the independent variables in the regression equation, 0.05 and 0.10 probability levels as the type I errors were used to enter and remove, respectively (Roy and Bargmann 1958; Affifi et al. 2004).

Results

The effect of salt stress on morphological and physiological traits

The results of combined analysis of variance for all the studied traits are shown in Table 1. The results showed that three mentioned salt stress conditions (0, 6 and 12 $dS.m^{-1}$ NaCl) were significantly different for all the evaluated traits. As shown in Table 1, the influences of genotype, salinity and genotype × salinity interaction were significant ($p < 0.01$) on all studied traits, indicating the significant differences of seedling characteristics among the 44 rice varieties and among the salinity levels and different physiological and biochemical responses of the varieties from one salinity condition to another (Table 1).

All the rice varieties had normal growth in the non-salinized condition. Under the salt stress conditions, the rice cultivars showed wide range in visual rankings ranging from score 1 (tolerant) to score 9 (susceptible) (Table 2). Totally, the mean comparisons of the genotype × salinity interactions showed that the most tolerant varieties were Ahlami-Tarom, Binam, Domzard, FL478, Gharib, Hassani, Nemat and Shahpasand according to visual scoring (under 12 $dS.m^{-1}$). Thirteen moderate salt tolerant rice varieties were identified as Ali Kazemi, Amol, Bahar, Domsiah, Domsefid, Dular, Gohar, Hasanjoo, Kadus, Sadri, Sahel and Salari. The most susceptible rice varieties were Anbarboo, IR29, Khazar and Sepidrood (Table 2).

Table 1 Combined analysis of variances for the studied traits of rice seedlings under three environmental conditions (non-stress and salinity stress of 6 and 12 dS.m^{-1})

S.O.V	df	MS											
		H_2O_2	T.Chl	Fv/Fm	POX	CAT	MDA	Proline	SOD	%Na	%K	Na/K	Tol.S
S	2	20183.76**	1575.69**	0.63**	14.16**	4574.05**	11702.51**	10488.95**	4574.05**	253.70**	117.45**	39.87**	582.66**
G	43	174.57**	8409.36**	0.91**	4.72**	322.90**	484.17**	78.24**	322.90**	1.17**	0.19**	0.23**	13.85**
G × S	86	52.95**	75.32**	0.03**	1.99**	51.68**	118.55**	56.52**	51.68**	0.22**	0.09**	0.16**	4.23**
Error	264	4.40	8.41	0.07	2.95	12.13	0.09	2.32	12.13	0.018	0.038	0.093	0.001
CV %	–	9.74	12.35	2.21	13.94	5.83	0.97	11.70	3.97	6.78	7.45	26.61	0.36

SOV source of variations, *S* salinity, *G* genotype, H_2O_2 H_2O_2 content, *T.Chl* total chlorphyll, *Fv/Fm* chlorophyll fluorescence, *POX* Peroxidase, *CAT* catalase, *MDA* malondialdehyde, *SOD* superoxide dismutase, *%Na* sodium percentage in shoot, *%K* potassium percentage in shoot, *Na/K* sodium/potassium ratio in shoot, *Tol.S* tolerance score

The results showed that sodium content increased significantly in all the varieties in the both salinity levels (Table 2). Maximum accumulation of sodium was observed in the susceptible varieties including IR29, Sepidrood, Khazar, Anbarboo, Neda and Bejar followed by moderately sensitive cultivars Gohar, Tarom Amiri and Tarom Molaee. The shoots of the tolerant varieties had the lowest sodium but a higher amount of potassium than the sensitive ones in the both salt stress conditions (Table 2).

The results also showed that the salt stress decreased the total chlorophyll content significantly in all the varieties, especially in the susceptible ones. The highest value for of total chlorophyll content was observed in Ghasroddashti, Chmpabudar, Gil 3, Sahel, Sadri, Hassani (non-stress conditions), Chmpabudar (EC ~6 dS), Dorfak, Nemat and Dasht (non-stress conditions), Sahel (EC ~6 dS), Domsiah, Tarmamiri and Sangetarom (non-stress conditions), Hassani, Gil- 3 and Sadri (EC ~6 dS). In addition, the lowest chlorophyll content was observed in Ghasroddashti (EC ~6 dS), Anbarboo, Bejar, Binam, IR29, and Bahar (EC ~12 dS), Binam, Bejar and Anbarboo (EC ~6 dS) and Hassansaraee (EC ~12 dS), respectively. The values of the *Fv*/*F*m changed dramatically in all the rice varieties, especially in the sensitive ones (Table 2). Under normal conditions, *Fv/Fm* were recorded in the range of 0.75–0.80 for all the rice varieties. Under the salt stress conditions, this ratio was reduced about 7–19.69% in all the varieties. The decrease in this ratio was dependent on the rice varieties. The lowest rate of reduction was observed in the tolerant genotypes such as Hassani, Nemat, Shahpasand and Ghasroldashti and the highest reduction was observed in Anbarbo, Khazar, Speedroud, Neda, Sangtaroom and Hashemi (Table 2).

The results of our study showed that the amount of hydrogen peroxide varied among different cultivars in all three salinity conditions. The greatest amount of the hydrogen peroxide was observed in Anbarboo, IR29, Mehr, Bijar, Sangetarom, Khazar, Speedroud, Neda, Shiroodi and Hashemi (12 dS.m^{-1} NaCl). The minimum value for H_2O_2 was observed in Gohar, Taromamiri, Kadous, Saleh, Shafagh, Sadri, Sahel, Domsiah, Domsefid, Hassanjo, Sangjo, Dular, AliKazemi, Gil 1, Shiroodi, Salari, Neda, Hashemi, Bahar, Tarommolaee, Chmapaboodar, Mohammadi and Sepidrood (Nonstress conditions). Totally, the amount of H_2O_2 in the salt-tolerant varieties was lower than in the sensitive ones. Since the H_2O_2 content varied significantly among the varieties, the activity of major H_2O_2 scavenging enzymes, superoxide dismutase (SOD), guaiacol peroxidase (POD) and catalase (CAT), was variable in these varieties under the salt stress conditions (Table 2). Interestingly, CAT activity in control and stressed seedlings of FL478, Hassani, Shahpasand, Binam and Domzard was significantly higher than those of IR29, Seidrood, Khazar,

Table 2 Comparison of growth and physiological parameters in 44 rice cultivars exposed to 6 and 12 dS NaCl

	H_2O_2			T.Chl			FV/Fm			POX (unit/g fr wt min)			CAT (unit/g fr wt min)			MDA (nmol/gr fr wt)		
	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds
FL478	14.40	18.60	27.97	20.70	18.60	16.93	0.82	0.76	0.73	0.34	0.75	0.67	57.70	65.60	79.43	20.38	21.59	22.35
Sangejo	9.10	19.00	32.47	21.60	19.00	17.00	0.82	0.77	0.73	0.63	0.95	0.98	59.67	68.10	66.77	20.73	33.50	37.80
Shafagh	8.30	26.50	31.77	29.10	26.50	24.53	0.85	0.80	0.76	0.54	0.94	0.89	57.70	66.10	64.80	21.43	34.2	38.50
Hassansaraee	12.30	17.10	25.80	19.20	17.10	15.45	0.77	0.71	0.68	0.28	0.68	0.61	49.20	57.60	74.10	19.58	20.84	21.55
Hassani	13.10	29.60	26.67	31.70	29.60	27.90	072	0.66	0.63	0.30	0.68	0.63	48.20	56.60	74.80	20.20	21.40	22.20
Shiroodi	9.80	23.50	47.37	27.70	23.50	21.10	0.77	0.68	0.65	0.45	0.81	1.14	54.20	62.60	64.60	21.80	44.60	60.30
Anbarboo	12.90	15.00	50.40	19.20	15.00	12.50	0.76	0.67	0.64	0.41	0.85	1.10	62.20	70.50	70.30	22.20	44.50	61.30
Gharib	14.30	26.10	22.80	28.20	26.10	24.40	0.87	0.81	0.78	0.33	0.78	0.66	52.20	60.60	75.80	19.90	21.10	21.90
Kadous	7.10	26.50	30.50	29.10	26.50	24.50	074	0.69	0.65	0.62	1.00	0.95	58.70	67.10	62.10	21.70	34.40	38.70
Gohar	6.70	23.50	30.20	26.10	23.50	21.50	0.75	0.65	0.60	0.70	1.00	1.10	45.70	54.10	56.30	20.10	32.90	37.20
IR29	12.70	15.80	50.20	20.00	15.80	13.30	0.75	0.70	0.65	0.45	085	1.15	68.20	76.60	73.90	21.40	45.90	59.50
Bejar	11.90	15.00	49.40	19.20	15.00	12.60	0.85	0.75	0.70	0.55	0.85	1.20	62.20	70.60	69.30	22.10	46.10	60.20
Domzard	11.70	18.60	25.20	20.70	18.60	16.90	0.80	075	0.70	0.35	0.70	0.65	52.20	60.60	73.80	20.80	22.00	22.80
Shahpasand	14.70	26.60	28.20	28.70	26.60	24.90	0.85	0.80	0.75	0.30	0.75	0.60	41.20	49.60	70.10	19.10	20.30	21.00
Domsiah	8.70	27.50	32.10	30.10	27.50	25.50	0.85	0.80	0.75	0.60	0.95	0.95	53.20	61.60	60.60	20.20	32.90	37.30
Mohammadi	10.30	19.00	33.70	21.60	19.00	17.00	0.80	0.75	0.70	0.50	0.80	0.85	51.20	59.60	66.30	20.95	34.10	38.10
Ahlamitarom	13.70	21.60	27.20	23.70	21.60	19.95	0.75	0.70	0.65	0.41	0.75	0.75	48.20	56.60	74.80	20.60	21.80	22.60
Binam	12.90	14.60	26.50	16.70	14.60	12.95	0.75	0.65	0.60	0.35	0.70	0.70	52.20	60.60	76.50	19.80	21.00	21.75
Champaboodar	10.30	31.50	33.75	34.10	31.50	29.50	0.75	0.70	065	0.45	0.85	0.80	45.70	54.00	54.80	18.90	31.70	36.00
Hassanjo	8.85	20.50	32.30	23.10	20.50	18.55	0.80	0.75	0.70	0.45	0.90	0.80	49.70	58.00	53.80	19.50	32.30	36.60
Dorfak	11.80	20.50	35.30	31.10	28.50	26.60	0.85	080	0.75	0.45	0.95	0.85	38.70	47.10	50.10	20.10	32.90	37.20
Ghasroddashti	11.70	24.60	25.20	26.70	2.60	22.90	0.85	0.75	0.75	0.45	0.85	0.80	47.20	55.60	66.95	20.10	21.30	22.10
Nemat	11.35	28.60	24.90	30.70	28.60	26.95	0.75	0.75	0.65	0.50	0.85	1.15	48.20	56.60	62.60	20.10	21.20	22.00
Gil1	9.65	26.00	33.10	28.60	26.00	24.00	0.85	0.80	0.75	0.65	0.95	1.00	54.20	65.6	61.50	20.55	33.30	27.60
Gil3	7.10	29.60	30.50	32.20	29.60	27.65	0.85	0.80	070	0.55	0.95	0.90	52.70	61.10	60.60	20.40	33.20	37.50
Khazar	11.20	22.50	48.67	26.70	22.50	20.10	0.85	0.80	0.80	0.50	0.80	1.20	60.20	68.60	67.30	21.20	45.30	60.10
Sepidrood	10.40	22.00	47.95	26.20	22.00	19.55	0.80	0.75	0.69	0.40	0.80	1.10	58.20	66.60	65.30	22.30	45.10	60.70
Tarommolaee	10.10	19.00	33.45	21.60	19.00	17.10	0.75	0.70	0.65	0.60	1.10	0.95	59.65	68.10	67.80	21.10	33.90	38.20
Alikazemi	9.65	26.50	33.10	29.10	26.50	24.55	0.75	0.65	0.60	0.70	1.10	1.10	60.70	69.10	68.80	21.55	34.30	38.60
Dasht	11.45	28.00	34.90	30.60	28.00	26.10	0.85	0.80	0.75	0.60	1.00	0.95	49.70	58.10	55.80	18.50	31.20	35.50
Neda	9.90	22.50	47.40	26.70	2250	20.10	0.75	0.65	0.65	0.55	0.90	1.24	61.20	69.60	64.60	20.80	45.55	58.90
Taromamiri	7.10	27.50	30.50	30.10	27.50	25.60	0.75	0.70	0.70	0.55	0.99	0.90	51.70	60.10	62.10	21.15	33.90	38.20
Sahel	8.55	30.50	31.90	33.10	30.50	28.50	0.75	0.70	0.65	0.60	1.10	0.95	45.70	54.10	60.10	19.45	32.20	36.50
Salari	9.85	19.80	33.30	22.40	19.80	17.85	0.80	0.75	0.70	0.70	1.00	1.10	65.70	74.10	71.40	19.50	32.20	37.20

Table 2 continued

	H_2O_2			T.Chl			FV/Fm			POX (unit/g fr wt min)			CAT (unit/g fr wt min)			MDA (nmol/gr fr wt)		
	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds
Sangetarom	11.35	25.60	48.90	29.80	25.60	23.10	0.85	0.75	0.75	0.40	0.80	1.15	56.20	64.60	63.30	21.80	45.70	59.90
Hashemi	9.95	19.50	47.10	23.70	19.50	17.10	0.75	0.65	0.60	0.50	0.85	0.85	48.20	56.60	58.10	21.20	45.55	59.30
Abjiboji	11.65	20.50	35.10	23.10	25.00	18.55	0.85	0.75	0.70	0.55	0.90	0.90	54.70	63.10	59.45	19.80	32.60	36.90
Saleh	7.65	26.00	31.10	28.60	26.00	24.10	0.80	0.75	0.70	0.55	0.95	0.90	55.70	64.10	62.30	21.30	34.00	38.30
Sadri	8.55	29.60	32.00	32.20	29.60	27.65	0.85	0.80	0.75	0.60	0.95	1.20	53.70	62.10	60.80	21.95	34.70	39.00
Bahar	10.10	16.50	33.60	19.10	16.50	14.55	0.75	0.65	0.65	0.40	0.85	0.75	49.70	58.10	56.50	19.95	32.70	37.00
Dular	9.45	19.00	32.90	21.60	19.00	17.10	0.75	0.70	0.70	0.45	0.80	0.80	46.70	55.10	54.10	19.20	31.90	36.20
Mehr	12.45	22.50	49.95	26.70	22.50	20.10	0.75	0.65	0.60	0.45	0.90	1.15	63.20	71.60	70.30	22.30	44.40	60.35
Amol	10.85	23.50	34.30	26.10	23.50	21.55	0.75	0.70	0.65	0.50	0.85	0.90	45.70	54.10	54.80	19.75	32.50	36.80
Domsefid	8.85	26.50	32.50	29.10	26.50	24.55	0.85	0.80	0.75	0.60	1.00	0.95	44.70	53.10	52.30	9.25	32.00	36.30
HSD (5%)	3.94			5.44			0.02			0.19			6.54			0.56		
HSD (1%)	4.37			6.05			0.03			0.22			7.26			0.63		

	Proline (μmol/g fr wt)			SOD (unit/g fr wt)			Tolerance score			Na%			K%			Na/K		
	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds
FL478	3.00	12.10	33.75	85.20	93.60	107.45	1	1	1	0.28	1.15	2.26	0.39	2.02	2.22	0.98	0.57	1.03
Sangejo	7.50	10.53	16.98	87.67	96.10	94.77	1	5	7	0.67	2.02	3.44	0.78	2.62	1.97	0.91	0.77	1.75
Shafagh	7.25	10.28	18.50	85.70	94.10	92.80	1	5	5	0.72	2.07	3.49	0.83	2.67	2.02	0.92	0.78	1.74
Hassansaraee	4.75	13.48	35.38	77.20	85.60	102.10	1	1	3	0.45	1.32	2.43	0.56	2.15	2.03	0.92	0.62	1.20
Hassani	5.25	13.98	35.90	76.20	84.60	102.80	1	1	1	0.48	1.35	2.45	0.58	2.17	2.05	0.91	0.62	1.22
Shiroodi	6.00	11.43	24.00	82.20	90.57	92.60	1	5	7	0.68	2.34	4.25	0.79	2.90	1.84	0.95	0.82	2.33
Anbarboo	6.45	10.45	23.10	90.20	98.60	98.30	1	5	7	0.65	2.31	4.22	0.75	2.87	1.81	0.91	0.81	2.35
Gharib	4.94	13.95	35.83	80.20	88.55	103.77	1	1	1	0.29	1.16	2.27	0.40	1.99	1.87	0.97	0.59	1.23
Kadous	5.75	8.80	18.90	86.70	95.10	90.10	1	5	5	0.80	2.16	3.58	0.92	2.76	2.11	0.92	0.79	1.71
Gohar	6.25	9.28	16.73	73.70	82.10	84.27	1	5	5	0.89	2.24	3.66	1.00	2.84	2.19	0.95	0.85	1.68
IR29	4.75	12.50	34.40	96.20	104.60	101.90	1	7	9	0.44	2.10	4.10	0.55	2.66	1.60	0.92	0.80	2.53
Bejar	6.25	9.20	21.75	90.20	98.60	97.30	1	5	7	0.48	2.14	4.05	0.59	2.70	1.64	0.91	0.80	2.49
Domzard	4.25	14.50	36.40	80.20	88.60	101.80	1	1	3	0.52	1.39	2.50	0.63	2.22	2.10	0.90	0.63	1.20
Shahpasand	4.70	13.50	35.40	69.20	77.60	98.10	1	1	3	0.49	1.36	2.47	0.60	2.19	2.10	0.90	0.65	1.22
Domsiah	6.00	9.10	15.50	81.20	89.60	88.60	1	5	5	0.75	2.10	3.52	0.85	2.70	2.10	0.92	0.80	1.73
Mohammadi	7.50	10.53	17.10	79.20	87.60	94.30	1	5	5	0.83	2.18	3.60	0.94	2.78	2.13	0.92	0.79	1.69
Ahlamitarom	4.50	12.20	34.10	76.20	84.60	102.80	1	1	1	0.32	1.19	2.30	0.43	2.12	1.90	0.95	0.59	1.22
Binam	4.25	13.73	35.63	80.20	88.57	104.45	1	1	1	0.37	1.24	2.35	0.48	2.10	1.95	0.93	0.60	1.21
Champaboodar	6.50	9.55	16.95	73.70	82.10	82.77	1	3	5	0.66	2.10	3.45	0.77	2.61	1.96	0.91	0.77	1.76

Table 2 continued

	Proline (μmol/g fr wt)			SOD (unit/g fr wt)			Tolerance score			Na%			K%			Na/K		
	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds	C	6ds	12ds
Hassanjo	5.50	8.53	17.51	77.71	86.10	81.77	1	5	5	0.71	2.10	3.48	0.82	2.66	2.10	0.92	0.78	1.74
Dorfak	5.95	8.98	18.48	66.71	75.10	78.10	1	5	5	0.68	2.10	3.45	0.79	2.60	1.90	0.91	0.77	1.76
Ghasroddashti	2.75	14.20	36.10	75.20	83.60	94.90	1	1	3	0.45	1.33	2.44	0.57	2.20	2.10	0.95	0.62	1.20
Nemat	3.25	11.90	33.85	76.20	84.60	90.60	1	1	3	0.54	1.40	2.50	0.65	2.20	2.10	0.90	0.63	1.20
Gil1	4.00	7.10	17.23	82.20	90.60	89.40	1	5	7	0.66	2.10	3.45	0.77	2.61	1.96	0.91	0.77	1.75
Gil3	4.50	7.50	14.95	80.70	89.10	88.60	1	5	5	0.70	2.10	3.48	0.81	2.65	2.00	0.92	0.78	1.74
Khazar	6.00	10.70	23.30	88.17	96.60	95.30	1	5	7	0.55	2.20	4.10	0.65	2.75	1.70	0.91	0.80	2.45
Sepidrood	6.50	10.45	23.00	86.20	94.60	93.30	1	7	9	0.61	2.30	4.20	0.72	2.85	1.77	0.90	0.80	2.38
Tarommolaee	7.70	10.73	18.23	87.70	96.10	95.80	1	5	5	0.85	2.20	3.60	0.95	2.80	2.10	0.95	0.80	1.70
Alikazemi	7.95	10.95	18.70	88.70	97.10	95.80	1	5	5	0.64	1.99	3.41	0.75	2.60	1.95	0.90	0.77	1.77
Dasht	6.20	9.25	19.95	77.70	86.10	83.75	1	5	7	0.48	1.85	3.25	0.60	2.45	1.75	0.90	0.75	1.84
Neda	4.50	11.10	23.70	89.20	97.60	92.60	1	5	7	0.60	2.30	4.20	0.75	2.85	1.80	0.91	0.80	2.45
Taromamiri	7.25	10.30	19.25	79.70	88.10	90.10	1	5	7	0.90	2.22	3.60	0.95	2.80	2.20	0.92	0.80	1.69
Sahel	4.50	7.55	15.00	73.70	82.10	88.10	1	5	5	0.70	2.10	3.50	0.85	2.70	2.10	0.92	0.80	1.73
Salari	6.00	9.00	15.50	93.67	102.1	99.40	1	3	5	0.65	1.95	3.40	0.75	2.60	1.90	0.91	0.75	1.77
Sangetarom	7.00	10.93	23.51	84.20	92.60	91.30	1	5	7	0.60	2.30	4.20	0.75	2.85	1.80	0.90	0.80	2.36
Hashemi	5.00	8.95	21.50	76.20	84.60	86.10	1	5	7	0.70	2.40	4.30	0.80	2.90	1.90	0.92	0.80	2.32
Abjiboji	4.25	7.30	22.00	82.70	91.10	87.40	1	3	5	0.50	1.80	3.25	0.60	2.40	1.80	0.90	0.80	1.84
Saleh	7.75	10.80	18.20	83.70	92.10	90.80	1	5	5	0.80	2.20	3.60	0.90	2.75	2.10	0.92	0.80	1.71
Sadri	8.25	11.30	18.70	81.70	90.1	88.80	1	3	5	0.80	2.20	3.60	0.90	2.80	2.10	0.92	0.80	1.70
Bahar	5.50	8.50	16.70	77.70	86.10	84.40	1	5	5	0.60	1.90	3.30	0.70	2.50	1.90	0.90	0.80	1.80
Dular	6.00	9.00	16.50	74.50	83.10	82.10	1	5	5	0.65	1.99	3.40	0.75	2.60	1.95	0.90	0.77	1.77
Mehr	6.70	10.90	23.50	91.20	99.60	98.30	1	5	7	0.45	2.10	4.10	0.60	2.70	1.60	0.92	0.80	2.52
Amol	5.80	8.80	15.20	73.70	82.10	82.80	1	5	5	0.50	1.90	3.30	0.60	2.50	1.80	0.91	0.80	1.83
Domsefid	4.00	7.00	17.20	72.70	81.10	80.10	1	5	5	0.70	2.00	3.40	0.80	2.60	1.95	0.91	0.77	1.75
HSD (5%)	2.86			6.54			0.002			0.25			0.37			0.57		
HSD (1%)	3.17			7.26			0.005			0.28			0.41			0.64		

H_2O_2 H_2O_2 content, *T.Chl* total chlorphyll, *Fv/Fm* chlorophyll fluoroscence, *POX* Peroxidase, *CAT* catalase, *MDA* malondialdehyde, *SOD* superoxide dismutase, *Tol.S* tolerance score, *%Na* sodium percentage in shoot, *%K* potassium percentage in shoot, *Na/K* sodium/potassium ratio in shoot

Anbarboo in both salinity levels. Thus, it seems that the varieties had an efficient enzymatic detoxification system for H_2O_2 scavenging.

Mean comparisons of the genotype × salinity interactions showed an increase in proline in all cultivars, especially in the tolerant varieties (Table 2). The salt-tolerant varieties FL478, Hassani, Shahpasand, Binam and Domzard accumulated the highest amount of proline. The shoot proline content in FL478, Hassani, Shahpasand, Binam and Domzard treated with 14 days m^{-1} NaCl showed a high increase, respectively compared with the control plants in both salinity levels. Under both 6 and 12 $dS.m^{-1}$ NaCl, MDA content was increased in all the varieties (Table 2). Under control condition, the MDA level in Sepidrood and Khazar was higher than it in the other varieties. At 12 $dS.m^{-1}$ NaCl, the increase in Malondialdehyde (MDA) content in FL478, Hassani, Shahpasand, Binam and Domzard were 9.65, 9.75, 10.56 and 10.85%, respectively compared with the control plants, which means less increase.

The results of correlation analysis are presented in Table 3. The results showed that Na and Na/K ratio had a significant negative correlation with all the studied physiological and biochemical traits except H_2O_2, MDA and tolerance score. It showed that the sensitive genotypes with higher tolerance score had high levels of H_2O_2, MDA and sodium in their tissues. All the antioxidant enzymes had a negative correlation with H_2O_2 except CAT. It is because CAT had a low affinity to H_2O_2 and the amount of the enzyme increases only when Hydrogen peroxide is increased. Proline followed the same model and had a negative correlation with H_2O_2, MDA, Na and Na/K ratio.

The results of cluster analysis

Cluster analysis based on the physiological and biochemical data using Euclidean distance coefficient grouped the rice varieties into three main clusters (Supplementary Material 4). The first cluster included Ahlami-Tarom, Binam, Domzard, FL478, Gharib, Ghasroddashti, Hassan-Saraee, Hassani, Nemat and Shahpasand. Anbarboo, Bijar, Hashemi, IR29, Khazar, Mehr, Neda, SangeTarom, Sepidrood and Shiroodi were clustered in the second group. Finally, the third cluster included Abjibooji, AliKazemi, Amol, Bahar, ChampaBoodar, Dular, Dasht, Dorfak, DomSefid, DomSiah, Hassnjoo, Gil.1, Gil.3, Gohar, Kadus, Mohammadi, Sadri, Sahel, Salari, Saleh, Sangjoo, Shafagh, TaromAmiri and TaromMolaee respectively. UPGMA-dendrogram clearly separated the varieties according to their tolerance. In this regard, the first cluster included the varieties with the highest tolerance (Binam, FL478 and Hassani), while the second cluster had the sensitive varieties (IR29, Khazar, Anbarboo). Some moderate tolerant varieties were clustered together in the third group.

Association analysis

The association analysis of microsatellite markers with the salt tolerance related traits were investigated using the

Table 3 Correlation coefficients among physiological and biochemical parameters from 44 rice cultivars exposed to 12 dS NaCl

	H_2O_2	Total Chl	Fv/Fm	POX	CAT	MDA	Proline	SOD	Tol score	Na	K	Na/K
H_2O_2	1											
Total Chl	−0.340*	1										
Fv/Fm	−0.305*	0.305*	1									
POX	−0.723**	−0.060	−0.241	1								
CAT	0.550**	−0.386**	−0.107	−0.154	1							
MDA	0.966**	−0.260	−0.319*	0.791**	−0.173	1						
Proline	0.405**	−0.207	−0.011	0.368*	0.740**	−0.375*	1					
SOD	−0.450**	−0.386**	−0.107	−0.154	0.998**	−0.173	0.740**	1				
Tol. score	0.788**	−0.133	−0.130	0.753**	−0.379*	0.867**	−0.542**	−0.379*	1			
Na	0.842**	−0.121	−0.288	0.774**	−0.393**	0.943**	−0.618**	−0.393**	0.905**	1		
K	−0.775**	0.345*	0.161	−0.489**	−0.036	−0.664**	−0.001	−0.036	−0.526**	−0.462**	1	
Na/K	0.954**	−0.243	−0.291	0.792**	−0.247	0.984**	−0.432**	−0.247	0.893**	0.937**	−0.734**	1

* Significant at the $p < 0.05$

** Significant at the $p < 0.01$

H_2O_2 H_2O_2 content, *T.Chl* total chlorophyll, *Fv/Fm* chlorophyll fluoroscence, *POX* Peroxidase, *CAT* catalase, *MDA* malondialdehyde, *SOD* superoxide dismutase, *Tol.S* tolerance score, *%Na* sodium percentage in shoot, *%K* potassium percentage in shoot, *Na/K* sodium/potassium ratio in shoot

Table 4 The characteristics of informative markers related to salinity tolerance in seedling stage under 12 dsm^{-1}

Trait	Informative markers	Regression coefficient	Significant level	Coefficient of determination (R^2)
H_2O_2	RM493	0.51	0.0002	0.751
	RM1287	−0.48	0.0001	
T.Chl	RM3412	0.28	0.004	0.482
Fv/Fm	RM140	0.41	0.001	0.314
	RM5	−0.38	0.0001	
POX	RM10793	−0.43	0.0005	0.652
	RM490	0.55	0.0004	
CAT	RM3412	0.42	0.0005	0.812
MDA	RM5	0.49	0.0001	0.722
Proline	RM809	0.51	0.0001	0.654
SOD	RM341	−0.39	0.0001	0.554
Na%	RM8094	0.42	−0.0009	0.523
	RM341	0.35	0.004	
	AP3206	0.31	0.044	
K%	RM140	0.76	0.031	0.485
	RM490	−0.17	0.007	
Na/K	RM3412	0.61	0.005	0.781
	RM1287	−0.36	0.008	
	RM5	0.37	0.047	
	RM10793	0.39	0.007	
Tol.S	AP3206	−0.12	0.027	0.452
	RM10793	0.62	0.0035	
	RM490	0.65	0.038	
	RM3412	0.37	0.0072	
	RM493	0.39	0.0003	

H_2O_2 H_2O_2 content, *T.Chl* total chlorphyll, *Fv/Fm* chlorophyll fluoroscence, *POX* Peroxidase, *CAT* catalase, *MDA* malondialdehyde, *SOD* superoxide dismutase, *%Na* sodium percentage in shoot, *%K* potassium percentage in shoot, *Na/K* sodium/potassium ratio in shoot, *Tol.S* tolerance score

stepwise regression analysis. Twelve highly polymorphic SSR loci were used for association analysis. In this regard, at the significant threshold of $p \leq 0.01$, 10 out of 12 markers (AP3206, RM5, RM140, RM490, RM493, RM562, RM1287, RM3412, RM8094 and RM10793) were detected to be associated with physio-biochemical traits (Table 4). The high value of R^2 for the regression models of the studied traits demonstrated the important role of *SalTol* in controlling physio-biochemical traits associated with SSR markers linked to *SalTol* QTL. The most important markers in the study included AP3206, RM1287, RM3412 and RM8094 which showed a significant association even with more than two physio-biochemical traits (Table 4).

Discussion

The salinity can cause negative effects on the plant growth and development. The decrease in the activity of meristem cells and knock out the important physio-biochemical processes of the plant, prevents plant growth and subsequently grain yield. The results of this study showed that rice cultivars examined in this research responded variably to the salt stress in terms of sodium, potassium and Na^+: K^+ ratio in the leaves after 14 days salt stress. Under salinity, by increasing in the Na concentration, ion toxicity, imbalance and nutrient deficiency occurs. The lack of K and Ca absorption due to the high concentrations of Na, will reduce plant growth and development (Mousa et al. 2013). Like this study, the results show that salinity increases the sodium entry to the plants and its accumulation causes Na: K replacement and ion toxicity effects (Munns 2002). Maintaining a high ratio of K/Na in the plants tissues is necessary for the plant salt tolerance (Ashraf and McNeilly 2004; López-Aguilar et al. 2012) and normal cell activities (Munns 2002; Azuma et al. 2010). These results are in line with ours. Potassium/sodium ratio has a strong relationship with growth and yield in the plants and like this study, has been introduced as an effective indicator for salt tolerance by many researchers (Aktas et al. 2006; Maggio

et al. 2007; Rubio et al. 2009; Niu and Cabrera 2010; Niu et al. 2010; Babu et al. 2012; Zhani et al. 2012; Mousa et al. 2014).

Perhaps the photosynthetic processes are the most important cellular reactions that are affected by salinity. The disruption of these processes directly reduces the carbon fixation and biomass production in the plants (Flowers 1999). In our study, the chlorophyll content was affected by the salinity which is in line with Wang et al. (1997). They reported that under salinity stress, sodium is accumulated in the shoots, while the concentration of potassium, calcium, magnesium and subsequently the photosynthesis are reduced. Salt stress reduces the amount of chlorophyll in the leaves by degradation or inhibiting the synthesis of chlorophyll (Ashraf and Harris 2013). High levels of salinity increases the chlorophyllase enzyme activity (enzymes degrading chlorophyll). This can be one of the most important factors in the reduction of photosynthesis under salt stress. Salt stress causes the oxidative stress which decreases the number and size of chloroplasts and destroys it (Santos 2004; Khafagy et al. 2009). Hence, variation in the chlorophyll content can be used as a stress indicator (Naumann et al. 2008), because chlorophyll content decreased in the sensitive crop plants under the salt stress conditions (Ashraf and Harris 2013). In our research, the sensitive varieties had lower chlorophyll content. The results of our research was in line with the other plants such as wheat (Raza et al. 2006), peas (Yildirim et al. 2008), safflower (Siddiqi et al. 2009) and rapeseed (Mukhtar et al. 2013).

Among the photosynthetic indices, Ashraf (1999) offered Fv/Fm as a valid indicator to determine the stress tolerance. Our results also confirmed this fact, so that the proportion of Fv/Fm in the salt sensitive varieties significantly decreased, while in the salt tolerant ones a significant decrease in the ratio was not observed. The results of the other researches show that increasing the sodium chloride in the chloroplasts of the plants, inhibit the activity of photosystem II and increase the sensitivity to photo inhibition (Sudhir and Murthy 2004). In different studies, the most photochemical efficiency (Fv/Fm) for leaves were recorded in the range of 0.75–0.85 under normal conditions and a decrease in this amount show the photo inhibition damage (Kaouther et al. 2013). In this study, Fv/Fm ratio decreased significantly by the salinity. Accordingly, it can be concluded that salinity can cause disruption in the electron transport of photosystem II (Megdiche et al. 2008). In the other words, salinity prevents the transfer of electrons from the first acceptor, quinine Qa to the second acceptor quinine Qb in the receptors of photosystem II which implies reduced Fv/Fm ratio (Shu et al. 2012). Effects of salinity on Fv/Fm ratio depend on the salinity tolerance and can be varied among species or genotypes (Lee et al. 2004; Jiang et al. 2006). Our results were consistent with the previous studies on pepper (Kaouther et al. 2013), eggplant (Wu et al. 2012), tomatoes (Al-aghabary et al. 2005), cucumbers (Shu et al. 2012), wheat (Kanwal et al. 2011) and Brassica species (Wani et al. 2013; Jamil et al. 2014). According to the results of the different studies it can be concluded that Fv/Fm ratio is widely used as a technique for rapid diagnosis of the stress (Baker and Rosenqvist 2004).

In the various sources, it is specified that salt stress increases ROS production and the activity of antioxidant enzymes in the plants. NaCl-induced H_2O_2 accumulation reduces plant growth, development and productivity (Uchida et al. 2002; Vaidyanathan et al. 2003). The results of this study showed that the H_2O_2 content was lower in the tolerant varieties (FL478, Shahpasand, Hassani, Gharib) than the sensitive ones (IR29, Sepidrood, Khazar and Anbarboo) in both salinity levels. On the other hand, removal of the free oxygen radicals is an important mechanism of salt tolerance in the plants (Motohashi et al. 2010). In this study, the salinity stress affected the activity of antioxidant system which is in line with Wi et al. (2006). They reported that the activity of antioxidant enzymes in the rice tolerant varieties was more than the susceptible ones under salt stress conditions. Catalase, peroxidase and superoxide dismutase are the most effective antioxidant enzymes. Increase in the activity of these enzymes increases the salinity tolerance. In the present study, it was found that salinity significantly increased the antioxidant enzyme activity in the tolerant varieties. For instance, the tolerant varieties such as FL478, Hassani, Shahpasand and Gharib poses higher amount of SOD activity in high salinity levels. SOD plays an important role in the immune system of the cells against oxidative stress, so that its activity directly modifies the amounts of O_2^- and H_2O_2 (two Haber–Weiss reaction precursors which generate OH radicals) (Sudhakar et al. 2001). Super oxide dismutase (SOD) catalyzes the conversion of superoxide anion to hydrogen peroxide and oxygen (Stepien and Klobus 2005). There were significant differences among the rice varieties in SOD enzyme activity, so that Hassani and FL478 (which are more tolerant to salinity) showed totally higher enzyme activity. The differences in SOD activity can be seen among and within species (Ashraf 2009). The higher activity of SOD was also observed in other salt tolerant plants (Sekmen et al. 2007; Sairam et al. 2002).

Guaiacol peroxidase activity rose sharply in all the varieties under the salt stress conditions. Previous studies have determined that peroxides plays a key role in the metabolism of reactive oxygen species, biosynthesis of plant cell walls by accelerating the last stage of the synthesis of lignin and suberin (Quiroga et al. 2000). Salinity induces peroxidase activity by the production of reactive

oxygen species. Like this study, many researchers stated the activity of this enzyme is a key factor to protect plants against environmental stresses. Ashraf and Ali (2008) observed that salinity increases peroxidase activity in the leaves of canola and reduces the adverse effects of salinity stress. Meloni et al. (2003) by studying the cotton plants under salinity condition, observed that an increase of peroxidase activity influenced by salinity leading to reduction of H_2O_2 and decrease in cell membranes damage. Given the role of this enzyme in the oxidative stress, it can be concluded that increasing the activity of peroxidase reduces the effects of oxidative stress and improving the plant stress tolerance (Vaidyanathan et al. 2003). Overall, our results are confirmed by other researchers which have suggested that salinity tolerance could be a result of increased activity of antioxidant enzyme defense system (Hernandez et al. 2003; Stepien and Klobus 2005). Sariam et al. (2002) reported that Na concentration influenced by salinity in wheat, leading to the increasement of cell membranes destruction, while research has shown that peroxidase activity plays an important role in decreasing the activity of free radicals induced by the salt stress and accumulation of sodium ions in the shoot and roots of crops. The results were consistent with the results of this study. In this study, POD enzyme activity was increased in the tolerant cultivars faster than the susceptible ones.

The results of mean comparison indicated that the CAT enzyme activity was so high in Hassani, FL478, Shahpasand, Nemat. The changes in CAT enzyme activity under the salinity conditions in the rice, depends on varieties and the levels of stress. Catalase is considered as one of the main enzymes in ROS detoxification (especially H_2O_2); it is reported that the activity of this enzyme increases under environmental stresses (especially salinity) (Vaidyanathan et al. 2003; Kumar et al. 2009) and this increase is associated with its role in improving the environmental stress tolerance (Sairam et al. 2002). In this study, the activity of this enzyme increased in salt-tolerant varieties which suggest that this enzyme plays an important role in improving the stress tolerance (Sairam et al. 2002; Saha et al. 2010; Abu-Muriefah 2015). It has been found that the activity of catalase to increase under salt stress in cucumber (Lechno et al. 1997), soybean (Comba et al. 1998) and mulberry (Sudhakar et al. 2001) mustard. De Azevedo Neto et al. (2006) also found more catalase activity in two maize cultivars differing in the salt tolerance.

In plant stress physiology, it is generally considered that the accumulations of compatible solutes are involved in cellular osmotic balance (Valliyodan and Nguyen 2006). For example, proline accumulation in plant increases salinity tolerance (Kishor et al. 2005). Whatever, the amount of proline is higher in plant tissues, the plants will be more resistant to stress. In this study, NaCl treatments significantly increased the proline contents in all the rice varieties (Table 2). Based on the findings in our study, it is interesting to mention that the proline accumulation in rice under salt stress treatments completely correspond to the extent of improved salinity resistance. Our results were contrary to Chunthaburee et al. (2015). They believed that there is no relationship between proline content and salt tolerance. Like their study, Lutts et al. (1999) showed that the proline accumulation was related to the salt injury rather than an indicator of salt tolerance. Our findings were consistent with Ghosh et al. (2011) who reported that the salt-tolerant Pokkali and Nonabokra exhibited the increase in proline in the rice seedlings under salt stress conditions. In a similar way, Kong-ngern et al. (2012) showed that the proline was so high in the roots of the salt-sensitive KDML 105 followed by the salt-sensitive Pathumthani 60 and the moderately tolerant Luang Anan and the tolerant Pokkali under salinity stress. In addition, Kanawapee et al. (2013) also showed that under salt stress treatment, the highly susceptible cultivars had the higher levels of proline than the tolerant ones. However, Igarashi et al. (1997) found a relationship between proline accumulation and the degree of salt tolerance. The results show that glycine, proline, pinnitol etc. as the stress markers will increase in the plant organs with increasing salinity to adjust the osmotic potential of the cells. Proline also acts as source of carbon and nitrogen for post stress recovery in the plants, as a sink for energy to regulate redox potential and also serves to protect the protein against denaturation (Saha et al. 2010; Fariduddin et al. 2013).

In this study, the MDA content increased in the leaves of rice seedlings under the salt stress conditions. It shows that ROS production leading to oxidative stress will cause membrane damage due to peroxidation of lipids. Increasing in the amount of lipid peroxidation in other plants (under salt stress) was reported by the other researchers (Azuma et al. 2010; Abu-Muriefah 2015). Salinity induces water stress and causes stomatal closure, reduction of CO_2 concentration in the mesophilic cells and accumulation of NADPH in the chloroplast. In these circumstances, the amount of available $NADP^+$ for photosynthetic light reaction is reduced; therefore O_2 acts as an electron acceptor, produces superoxide radicals followed by other reactive oxygen species and finally causes the oxidative stress (Abdul-Jaleel et al. 2009; Sudhakar et al. 2001). Malondialdehyde (MDA) is the main product of the decomposition of unsaturated fatty acids in biological membranes, which increases under salt stress conditions (Meloni et al. 2003; Sudhakar et al. 2001). Significant increases in the levels of lipid peroxidation (malondialdehyde (MDA) and other aldehydes), as an

indicator of damage to the membrane, have been observed in salinity in some of the plant species (Stepien and Klobus 2005; Sudhakar et al. 2001). In this study, an increase in salinity increased the lipid peroxidation, which represents the membrane damage (resulting in increased oxidative stress from ROS) in sensitive varieties (Saha et al. 2010).

The results of molecular analysis showed that AP3206, RM1287, RM3412 and RM8094 are the most common markers, which have been found in the regression model of the majority of traits. Among the regression models, the highest coefficient of determination was related to CAT, K/Na and MDA respectively. Our results were in line with Mohammadi-Nejad et al. (2010) to some extent. They haplotyped 30 rice genotypes using SSRs tightly linked to *SalTol* and found two markers, RM8094 and RM10745, can be found in the regression model of the majority of traits related to the salinity tolerance. High coefficient of determination for most physiological and biochemical traits associated with salt tolerance in this study, reflecting the effective role of *SalTol* locus (on chromosome 1) in controlling of these traits. According to this analysis, *SalTol* can be considered as an effective locus for salt tolerance in Iranian rice varieties.

Conclusion

The results of the present study showed that salt stress can affect the early growth of the studied rice cultivars. Due to the reduced chlorophyll content, it can be concluded that the photosynthetic machinery were also damaged by the salinity stress. The results also showed that salt stress induced oxidative stress and membrane damage by lipid peroxidation. Rice, like other cops, has different mechanisms for salt tolerance; among them it can be pointed out antioxidant system. Catalase, superoxide dismutase and proline showed that they can be considered as important factors for salinity tolerance in rice. According to the results, RM8094, RM1287, RM493, RM3412, RM5, RM140, RM10793, RM490 and AP3206 were detected to be linked with important physiological and biochemical traits related to salt tolerance and Hassani, Shahpasand, Gharib, Binam, Ahlamitarom, Nemat, Hassansaraee, Domzard, Ghasroddashti, Sadri and Hassanjoo are introduced as the most salt tolerant varieties in this research.

Author contributions M. Kordrostami performed the experiments, analyzed the data and wrote the first draft of the paper. B. Rabiei and H. Hassani Kumleh conceived the project, performed the critical revision of the data and wrote the final version of the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Research involving human participants and/or animals The authors declare that the present study does not involve any human participants and/or animals.

Informed consent The authors declare that the present study does not involve any informed consent.

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