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MORPHO-PHYSIOLOGICAL RESPONSE OF RICE GENOTYPES GROWN UNDER SALINE CONDITIONS

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ABSTRACT

Salt accumulation in irrigated soil is one of the main factors limiting rice productivity. A greenhouse experiment was conducted at Cimanggu Experiment Station, Bogor, Indonesia from May to September 2014 using a completely randomized design in a factorial arrangement with three replications. Four salt tolerant and two salt sensitive genotypes of rice were used in this experiment. The treatments were five concentrations of NaCl (0, 20, 40, 60 and 80 mM) applied on 21 day-old seedlings and maintained until harvest time. The study sought to evaluate the response of several rice genotypes to various NaCl concentrations through observation of morphological and physiological characters. The study showed that the increase of NaCl concentrations in soil significantly reduced plant height, number of panicles plant^{-1}, panicle length, leaf length, 1000 grain weight, and grain yield. On physiological characters, salinity increased [Na^+], decreased [K^+] and [Ca^{2+}] concentrations, and reduced K^+/Na^+ and Ca^{2+}/Na^+ ratios in the leaf tissue. The addition of 40 mM NaCl can distinguish tolerant and sensitive genotypes. The grain yield of sensitive genotypes decreased 90-100% at 40 mM NaCl, while the tolerant genotypes showed <70% in grain yield reduction.

Key words: morpho-physiological characters, NaCl, salt tolerance, saline soil

INTRODUCTION

Salinity is one of the main obstacles to increase rice (*Oryza sativa* L.) production in the world (Hosseini *et al*., 2012; Abbas *et al*., 2013; Ali *et al*., 2014). Salinity affects one-third of all irrigated land in the world, impairs normal growth and limits the realization of yield potential of modern cultivars. Salt accumulation in irrigated land affects physiology, morphology and biochemistry in rice plants (Sankar *et al*., 2011). Rice yield loss on land conditions with low salinity (2-6 dS m^{-1}) reached 40%, moderate salinity (6-10 dS m^{-1}) reached 75%, and high salinity levels (> 10 dS m^{-1}) reached 100% (Zeng and Shannon, 2000). Sankar *et al*. (2011) reported that the sensitivity of the rice plant to salinity ranges from 0 dS m^{-1} to 8 dS m^{-1}. Rice is classified as sensitive to salinity, but rice is one of the recommended crops to be grown in saline soil, because rice has the ability to grow in waterlogged soil (Sankar *et al*., 2011; Aref and Rad, 2012). Availability of water in irrigated rice systems enables the salt molecules to dissolve and transport these as run-off and leaching such that salt levels can be reduced (Asch and Wopereis, 2001).

Rice plant response to salinity varies according to the growth stage. In the vast majority of rice cultivars, plants at the early seedling phase are most sensitive to salinity (Zeng and Shannon, 2000; Zeng *et al*., 2001; Haq *et al.,* 2009). According to Zeng *et al*. (2001), salinity stress during seedling phase can reduce plant dry weight by two-fold compared to when stress occurs in the ripening phase. In the irrigated rice planting in coastal areas, salinity may occur at any stage of plant growth. Therefore, it is important to determine the response to salinity in rice plants throughout the growth stage.

The screening and breeding of rice varieties for tolerance to salinity have been carried out for over three decades and various methodologies have been used to screen tolerant varieties (Flower 2004; Egdane *et al*. 2007; Rao *et a*l. 2008). This study sought to evaluate the response of several rice genotypes at various NaCl concentrations through observation of morphological and physiological characters in saline soil. The differences in response will be useful index when salinity screening of rice genotypes is carried out in saline soil under greenhouse conditions to select suitable genotypes or useful breeding materials.

MATERIALS AND METHODS

Six rice genotypes with different levels of tolerance to salinity were used in this study. These genotypes were selected based on screening using nutrient solution at seedling phase containing 120 mM NaCl. Four genotypes were tolerant, namely IR77674-3B-8-2-2-14-4-AJY2, IR81493-B-B-B-6-B-2-1-2, Dendang and Pokkali, while the other two were sensitive, namely Inpara 4 and IR 29. The greenhouse experiments were conducted at the Cimanggu Experimental Station, Bogor, Indonesia from May until September, 2014 using completely randomized design in factorial arrangement with three replications. The treatment in this study was application of four concentrations of NaCl (20, 40, 60 and 80 mM). These NaCl concentrations were equivalent to EC (electrical conductivity) of 4.8 dS m^{-1}, 6.2 dS m^{-1}, 8.8 dS m^{-1} and 12.3 dS m^{-1}, respectively. In this experiment, the rice plants did not survive at 1-2 weeks after 80 mM NaCl treatment, so that only four NaCl concentrations remained in this experiment.

Rice seeds were sown in a box filled with soil under non-saline conditions (without the addition of NaCl, equivalent to EC of 1.2 dS m^{-1}) until 21 days. The seedlings were then transplanted into pots containing soil and water with ratio of 7:3, one seedling for each pot. NaCl was previously added into the soil according to treatments. The water condition was maintained in the same level of volume throughout the experimental period. The plant growth was observed until harvest time. The variables observed were morphological and physiological characters, i.e. days to flower, plant height, panicle number plant^{-1}, flag leaf length, panicle length, 1000 grain weight, grain yield plant^{-1}, Na^+, K^+, and Ca^{2+} contents in the leaf tissue. The morphological and physiological characters were observed at the reproductive stage, i.e. at mature grain phase and dough grain phase, respectively. The rice leaves were oven-dried at 70 ^{0}C for three days. About 0.5 g of each dried powdered sample was digested with 5 mL of nitric acid at 300^{0}C, 0.5 mL of perchloric acid at 200^{0}C and 20 mL of 6 M hydrochloric acid. The ion concentrations were analyzed using atomic absorption spectroscopy (Perkin Elmer 1100 B).

Data Analysis

All data were analyzed using SAS 9.1 for the analysis of variance (ANOVA). Significance of differences of treatment means were analyzed by F-test at 95% probability level and Duncan’s Multiple Range Test (DMRT) at 95% probability level.

RESULTS AND DISCUSSION

Effect of salinity on plant growth

Rice plant is relatively sensitive to soil salinity (Jamil *et al*., 2012). The accumulation of a lot of salt in the soil generates a blockage for normal growth and development of plants (Ali et al., 2014). Salinity screening in greenhouses is usually done during the seedling phase. Salinity tolerance of the rice plant from vegetative to reproductive phases is important to be known. Salinity screening under greenhouse conditions using soil media is still performed rarely because it is relatively difficult in implementation. In this study, through an analysis of selected morphological and physiological variables, the salt tolerance of six rice genotypes in saline soil has been determined. An understanding of plant response under varied level of salt stress is important to determine the critical NaCl concentration that could be used for screening the rice genotypes for salinity tolerance in saline soil, especially under green house conditions. None of the genotypes survived at 80 mM NaCl concentration, therefore the results are not presented. Inpara 4 and IR29 did not survive at more than 20 mM NaCl. The plants died at 1-4 weeks after transplanting. The variances of plant growth and yield variables were analyzed and the results are presented in Table 1. The overall effects of NaCl concentrations (S), genotypes (G) and NaCl*genotype (S*G) interaction were highly significant (P<0.01) for all variables observed.

Table 1. Mean square for growth and yield parameters at different genotypes and NaCl concentrations.

Source of Variation	**df**	**Means Square**						
		Days to flower	**Plant Height**	**Panicle (plant^{-1})**	**Leaf length**	**Panicle length**	**A 1000 grain weight**	**Yield (plant^{-1})**
Genotype (G)	5	1060.62**	2550.40**	5.30**	741.23**	40.23**	111.04**	39.66**
NaCl (S)	3	71.29**	902.87**	122.15**	350.04**	66.59**	61.03**	1340.12**
S * G	12	31.10**	224.97**	4.19**	54.87**	11.17**	12.56**	50.03
Error	42	7.68	51.65	0.96	1.89	1.32	0.38	1.95
CV (%)		3.32	7.53	11.40	4.26	5.01	2.85	10.65

** Significant at p< 0.01 in *F*-test.

Salinity prolonged the number of days to flower (Fig. 1), changed morphological trait such as reduction in plant height (Fig. 2), panicle number plant^{-1} (Fig. 3), leaf length (Fig. 4), panicle length (Fig. 5), and in reduction of yield component such as a 1000 grain weight (Fig.6), and grain weight plant^{-1} (Fig. 7). The decreased in osmotic potential was the first effect of NaCl addition to the growth medium. It disrupted the absorption of water and nutrient by plants. The accumulation of toxic ions such as Na^+ is harmful to the plant, so that the plant spends a lot of energy to compartmentalize them in specific plant tissues which leads to the reduction of plant growth (Nemati *et al.*, 2008).

Salinity increased the number of days to flower of the genotypes, compared to control (Fig. 1). The exception of this variable was shown by Pokkali. The reduction of the number of days to flower was demonstrated by Pokkali at 40 mM NaCl concentration (63 days), while at 20 mM NaCl concentration the days to flower of Pokkali was equal to control (66 days). The highest increase of this variable was shown by IR81493 where it took 102 days to flower at 60 mM NaCl concentration, while control took only 87 days.

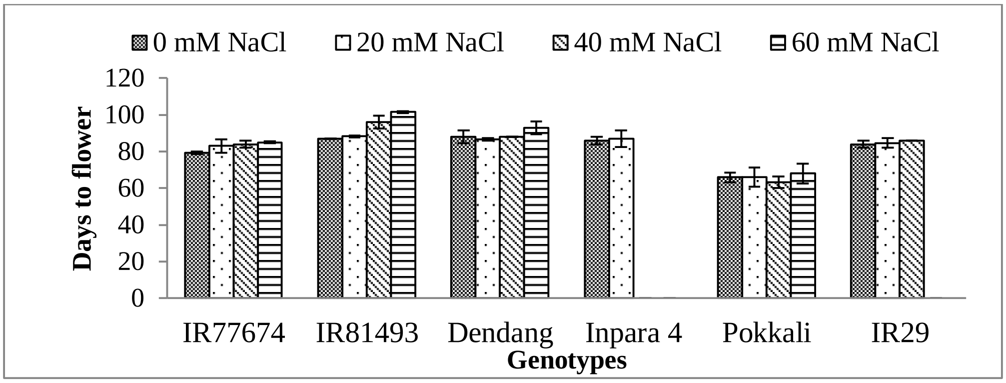

Fig. 1. Effect of varied levels of salinity on number of days to flower of six rice genotypes. Inpara 4 and IR29 did not survive at 40 mM and 60 NaCl, respectively.

Salinity caused significant reduction in plant height of the genotypes starting at 40 mM NaCl concentration (Fig. 2). This was observed in all of the genotypes except for Pokkali and IR81493. The plant height of Pokkali and IR81493 at 40 mM NaCl concentration were not significantly different from control. Plant height reduction was earlier demonstrated to be caused by the reduction in cell division and cell elongation (Yaghubi *et al.*, 2013). NaCl damages plants through ion toxicity and osmotic stress. The osmotic phase rapidly inhibits growth of young immature leaves by decreasing cell proliferation and delaying cell elongation, while the ionic phase gradually induces cellular senescence of mature leaves (Urano *et al.*, 2014).

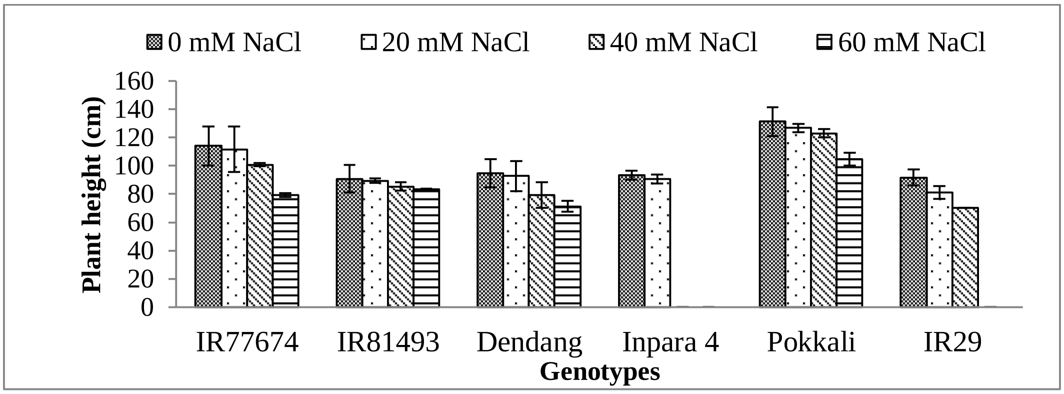

Fig. 2. Effect of varied levels of salinity on plant height of six rice genotypes. Inpara 4 and IR29 did not survive at 40 mM and 60 NaCl, respectively.

The number of panicles is related to tillering ability of the plant. In this experiment, salinity caused significant reduction in number of panicles compared to control. However, the tolerant genotypes, Pokkali and Dendang, showed lower reduction in number of panicles than other tested genotypes (Fig. 3). The inhibition of tillering ability was the main cause of yield loss under salt stress (Zeng *et al.* 2003; Haq *et al.* 2009). The reduction in tillering capacity might be due to the toxic effect of salt on plant growth and development.

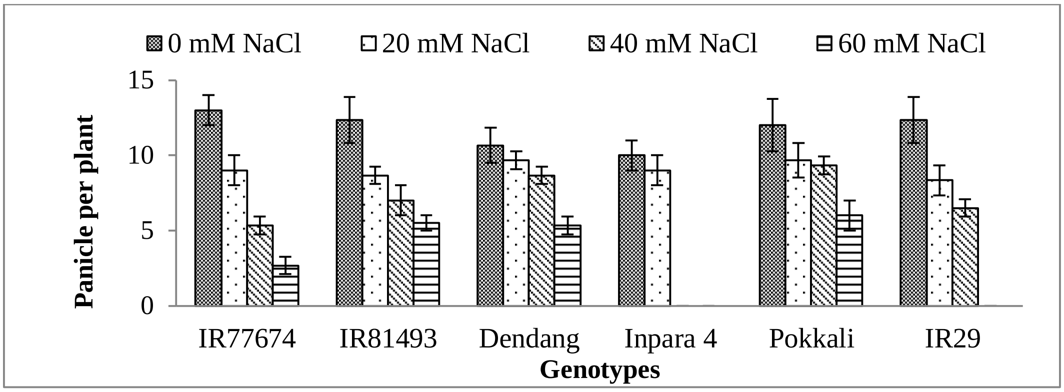

Fig. 3. Effect of varied levels of salinity on panicle number plant^{-1} of six rice genotypes. Inpara 4 and IR29 did not survive at 40 mM and 60 NaCl, respectively.

The leaf length of the genotypes was reduced by the increase of NaCl concentration, except for IR81493 at 40 mM NaCl concentration (Fig.4). The lowest reduction was observed in Dendang at 60 mM NaCl concentration (19.1 cm) compared to control (24.9 cm). In this experiment, the high significant reduction can be shown to start at 40 mM NaCl. Under saline conditions, the leaves tend to be smaller and thicker which observed adaptive mechanism of plants to reduce water loss by reducing their evaporation surface. Therefore, leaf elongation in rice declined after exposure to salinity (Yaghubi *et al.,* 2013).

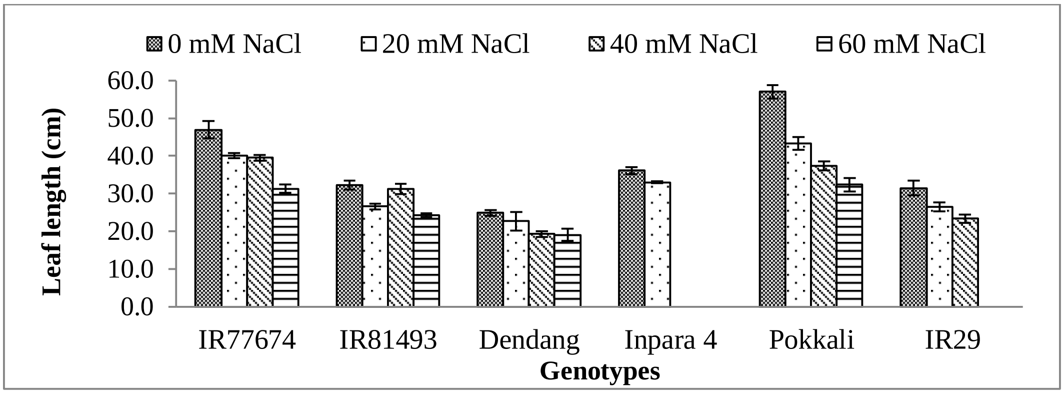

Fig. 4. Effect of varied level of salinity on leaf length of six rice genotypes. Inpara 4 and IR29 did not survive at 40 mM and 60 NaCl, respectively.

The panicle length is one of the important yield components under salinity because it determines the grain bearing panicles. In this experiment, salinity caused significant reduction in panicle length compared to control starting at 40 mM NaCl concentration (Fig. 5). The panicle length of the sensitive genotype (IR29) was reduced significantly from 22.5 cm to 18.1 cm, whereas the reduction of tolerant genotype (Pokkali) from 25.8 cm to 23.2 cm which was not significant. The panicle length of IR77674 and IR81493 was not reduced significantly as the NaCl concentration was increased from 0 mM to 40 mM, whereas the panicle length reduction of Dendang and Inpara 4 was significant. Zeng *et al*. (2001) observed that salinity influenced panicle initiation of rice by inducing reduction of primary and secondary rachis-branches and flower primordial, so that salinity also reduced the number of spikelets on the panicle.

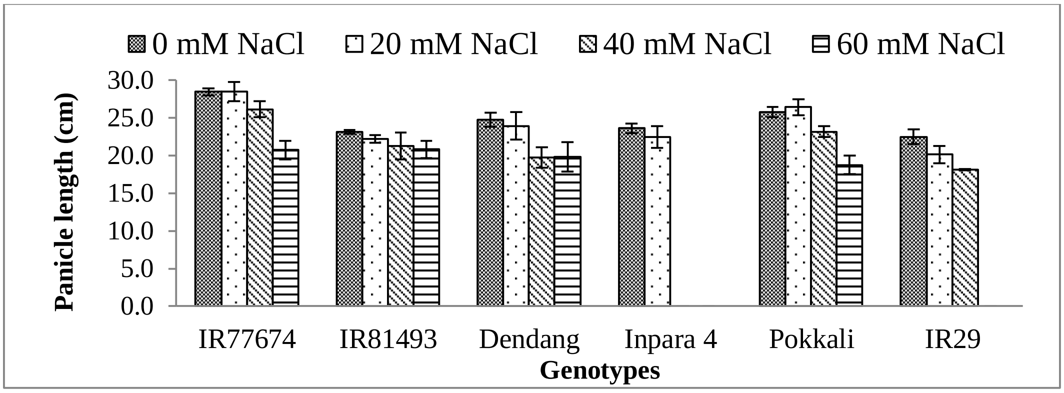

Fig. 5. Effect of varied levels of salinity on panicle length of six rice genotypes. Inpara 4 and IR29 did not survive at 40 mM and 60 NaCl, respectively.

Salt stress reduced a 1000 grain weight in all genotypes (Fig. 6). The significant reduction was observed starting at 40 mM NaCl concentration compared to control in IR81493, Dendang and IR29. The reduction of a 1000 grain weight was not significant for IR77674 and Pokkali. Rao *et al.* (2008) reported that a 1000 grain weight was reduced significantly under salinity condition, indicating that salinity reduced the size of the rice grains.

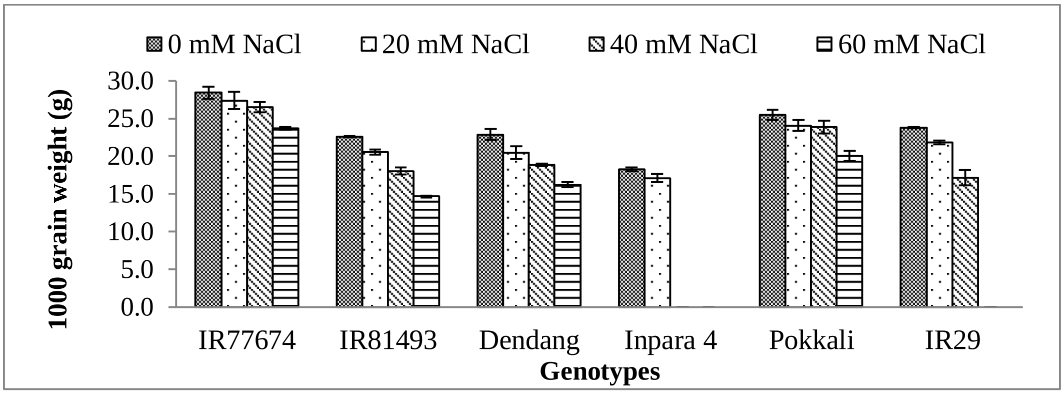

Fig. 6. Effect of varied levels of salinity on 1000 grain weight of six rice genotypes. Inpara 4 and IR29 did not survive at 40 mM and 60 NaCl, respectively.

Rice yield is highly dependent upon the number of fertile tiller plant^{-1} and filled grain panicle^{-1} (Zeng *et al.*, 2003). In this experiment, the reduction in grain yield, i.e. grain weight plant^{-1} was observed when plants were stressed at 20 mM NaCl, except in Dendang. All of genotypes showed significant reduction in grain yield at 40 mM NaCl concentration compared to that of control. The grain yield reduction at 20 mM to 40 mM NaCl concentrations was observed in all genotypes except in Pokkali (Table 2).

Table 2. Means of grain yield plant^{-1} of six rice genotypes of rice under varied salinity levels.

NaCl	Grain Yield (g plant^{-1})					
	IR77674	**IR81493**	**Dendang**	**Inpara 4**	**Pokkali**	**IR29**
0 mM	27.11±4.07 a	23.27±3.32 a	22.50±4.97 a	21.78±2.68 a	26.58±4.58 a	23.05±5.47 a
20 mM	18.71±3.12 b	14.71±4.04 b	19.67±2.51 a	11.02±2.37 b	16.46±0.54 b	8.19±2.97 b
40 mM	9.54±2.31 c	9.87±1.43 c	7.16±1.91 b	0.00±0.00 c	14.30±3.87 b	2.10±0.05 c
60 mM	1.34±0.52 d	2.10±0.23 d	2.62±1.33 c	0.00±0.00 c	6.72±1.77 c	0.00±0.00 d

Means within columns followed by the same letter are not different at P = 0.05 according to Duncan's Multiple Range Test (DMRT).

The reduction in percentage of yield of the genotypes by the increase in salt concentration is shown in Fig. 7. The reduction in grain yield at 20 mM NaCl concentration relative to control was less than 40% for all rice tolerant genotypes (IR77674, IR81493, Dendang and Pokkali), while in sensitive genotypes (IR29 and Inpara 4), the yield reduction were 64.4% and 49.4%, respectively. The reduction in grain yield of sensitive genotypes at 40 mM salt level was 100% for Inpara 4 and 90.1% for IR29, while in Pokkali as the most tolerant genotype compared to other tested genotypes, the reduction was 46.2 %. The grain yield of IR77674, IR81493 and Dendang was reduced by 64.8%, 57.6%, and 68.2%, respectively, at 40 mM salt level compared to control. The grain yield reduction was most pronounced when plants were stressed at 40 mM NaCl. The reduction in grain yield was 90-100% for sensitive genotypes (IR29 and Inpara 4) and less than 70% for tolerant genotypes.

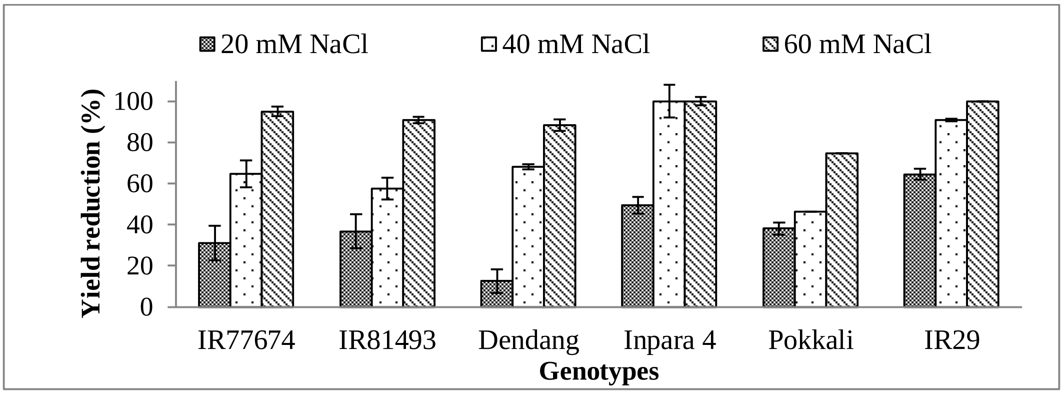

Fig. 7. Grain yield reduction (%) of rice genotypes under varied salinity levels relative to control (0 mM NaCl)

Effect of salinity on physiological characters

The influences of salinity on plant growth by ionic and osmotic ways sometimes differ between one and another and sometimes overlap (Hariadi *et al*., 2015). Analysis of variance revealed that NaCl concentration and genotype factors were highly significant (P<0.01), whereas NaCl*genotype interaction was significant ($P \leq 0.05$) on K^+ concentration (Table 3). In response to applied salinity there was reduction in all genotypes on K^+ concentration in leaves compared to control. The maximum K^+ concentration was observed in IR77674 (2.87%) followed by Pokkali (2.85%) in the control treatment, while at 40 mM salt level, it was recorded in Dendang (2.65%) followed by IR77674 (2.57%). The most sensitive genotype in this experiment, Inpara 4, had the lowest leaf K^+ concentration at 20 mM salt level (2.24%) compared to other genotypes, while IR29 which known to be used as standard sensitive control, had the lowest K^+ concentration at 40 mM NaCl concentration (1.86%). The K^+ reduction in tolerant genotypes, at 40 mM NaCl, was less than 10%, while it was more than 25% for the sensitive genotypes (Fig. 8).

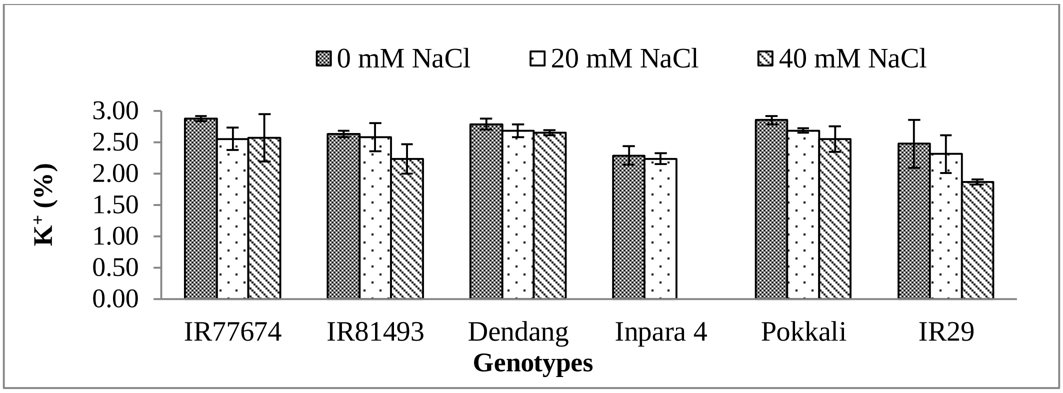

Fig. 8. Effect of varied levels of salinity on K^+ concentration in leaf of six rice genotypes. Inpara 4 did not survive at application of 40 mM NaCl.

Fig. 9 showed that the genotype differences in leaf Na^+ concentration under saline and non-saline conditions. For Na^+ concentration, the analysis of variance showed that the effect of genotype, salt levels and NaCl*genotype interaction were highly significant (Table 3). Salinity caused the increase of leaf Na^+ concentration in the genotypes. IR77674 and IR81493 had the lowest leaf Na^+ concentration compared to the other genotypes. The highest increase of leaf Na^+ concentration was shown in sensitive genotype IR29, i.e. 0.06%, 0.11% and 0.13% under control, 20 mM and 40 mM NaCl concentrations, respectively.

Table 3. Mean square for physiological parameters at different genotypes and NaCl concentrations.

Source of variation	**df**	**Means Square**				
		K	**Na**	**Ca**	**K/Na**	**Ca/Na**
Genotype (G)	5	0.654**	0.009**	0.085**	17726.667**	922.948**
NaCl (S)	2	0.687**	0.006**	0.146**	7912.777**	707.751**
S * G	10	0.048*	0.001**	0.021**	620.880*	38.950**
Error	36	0.023	0.000	0.004	223.036	13.429
CV		6.070	15.893	8.440	23.977	20.435

*, ** Significant at p<0.05 and 0.01 in *F*-test, respectively.

Salinity exhibited significant effects on physiological traits such as ion concentrations of rice plants (Jamil *et al.*, 2012). Increased soil salt concentrations decrease the ability of a plant to take up water and Na^+ and Cl^- are taken up in large amounts by roots, both Na^+ and Cl^- negatively affect growth by impairing metabolic processes and decreasing photosynthetic efficiency (Deinlein *et al.*, 2014). The result of the experiment showed that sodium (Na^+) in leaf increased under salinity stress in all rice genotypes (Fig. 9). In contrast, leaf potassium (K^+) content in salt-stressed plants was significantly reduced (Fig. 8). The diminution of K^+ concentration in leaf tissue may be due to direct competition between K^+ and Na^+ at plasma membrane, inhibition of Na^+ on K^+ transport process and/or Na^+ induced K^+ efflux from the root (Jamil *et al.*, 2012). In this study, the highest increase of leaf Na^+ concentration was recorded by IR29 which is sensitive genotype. Pokkali and Dendang had a lower leaf Na^+ concentration than IR29 at 40 mM NaCl concentration, although it was still high if it was compared to the other tolerant genotypes. It means that Pokkali and Dendang could maintain growth even when leaf Na^+ concentration was high. In this experiment, IR77674 and IR81493 were categorized as salt tolerant due to less accumulation of Na^+ and high accumulation of K^+ in leaves under salt stress.

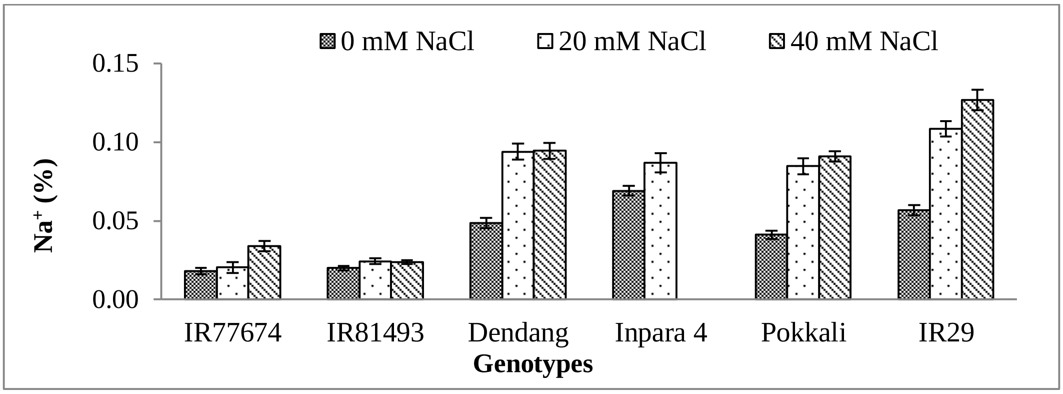

Fig. 9. Effect of varied levels of salinity on Na^+ concentration in leaf of six rice genotypes. Inpara 4 did not survive at application of 40 mM NaCl.

Table 3 showed that the NaCl concentrations, genotypes and NaCl*genotype interaction were highly significant ($P<0.01$) on Ca^{2+} concentration. The leaf Ca^{2+} concentration of different rice genotypes, under control and salinity is presented in Fig. 10. The Ca^{2+} concentration was prone to reduce by the increase of salt levels in all genotypes, except in IR77674 and IR81493. Among genotypes, Pokkali showed the highest level of leaf Ca^{2+} concentration under saline condition.

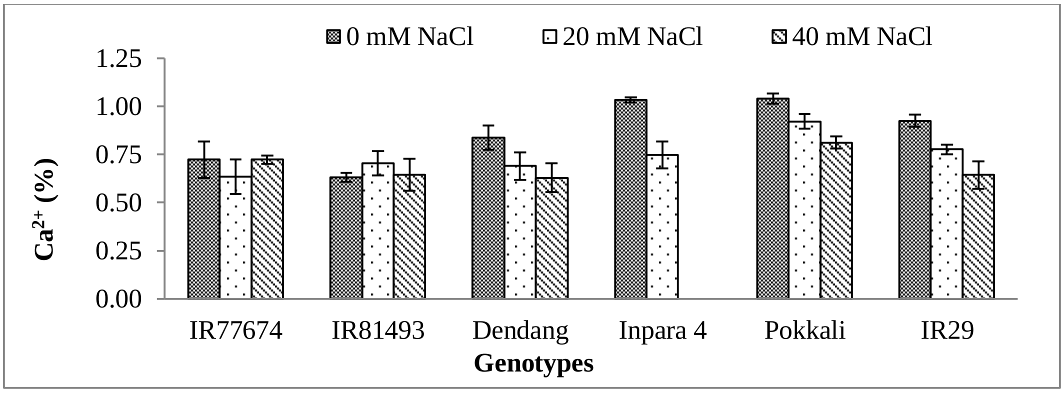

Fig. 10. Effect of varied levels of salinity on Ca^{2+} concentration in leaf of six rice genotypes. Inpara 4 did not survive at application of 40 mM NaCl.

Analysis of variance showed that the effect of NaCl concentration and genotypes was highly significant ($P<0.01$), while the NaCl*genotype interaction was significant ($P\leq0.05$) on K^+/Na^+ ratio (Table 3). There was significant reduction in K^+/Na^+ ratio in all genotypes under salt stress compared to control. The lowest K^+/Na^+ ratio under salt stress was achieved by IR29, i.e. 21 and 15 at 20 mM and 40 mM salt levels, respectively. IR77674 and IR81493 showed the high K^+/Na^+ ratio under salt stress conditions (Fig. 11).

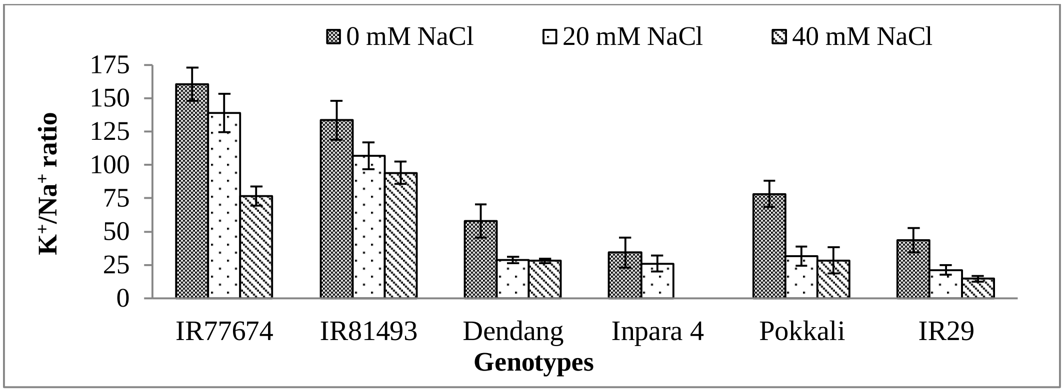

Fig. 11. Effect of varied levels of salinity on K^+/Na^+ ratio in leaf of six rice genotypes. Inpara 4 did not survive at application of 40 mM NaCl.

Data regarding Ca^{2+}/Na^+ ratio in leaf of six rice genotypes under saline and non-saline condition are shown in Fig.12. Analysis of variance revealed that salt level, genotypes and NaCl*genotype interaction were highly significant ($P<0.01$) on Ca^{2+}/Na^+ ratio (Table 3). In response to applied salinity, there was reduction on Ca^{2+}/Na^+ ratio in all genotypes along with the increase of NaCl concentration. The lowest reduction was observed in IR81493 (29 and 27 at 20 mM and 40 mM salt level, respectively) compared to control (32). The lowest Ca^{2+}/Na^+ ratio (5) was observed in IR29 at 40 mM NaCl concentration.

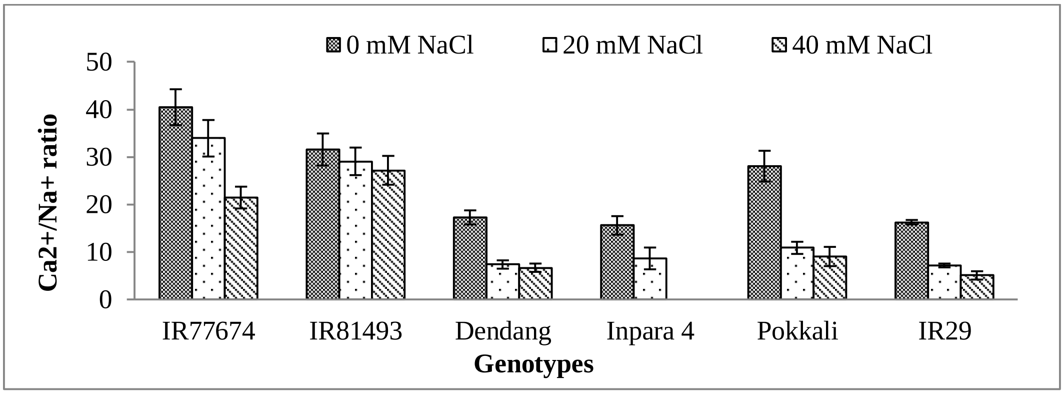

Fig. 12. Effect of varied levels of salinity on Ca^{2+}/Na^+ ratio in leaf of six rice genotypes. Inpara 4 did not survive at application of 40 mM NaCl.

A good supply of K^+ to plants can minimize injurious effects of high Na^+ under salinity. Influx of Na^+ in shoot tissue is often accompanied by the decrease in leaf K^+ concentration, leading to a decrease in K^+/Na^+ ratios. The decrease in K^+ contents occurred in plants grown in medium with excessive Na^+ (Haq *et al.*, 2009). One of the key features of salt tolerant plants is the ability of plant cells to maintain optimal K^+/Na^+ ratio in the cytosol when exposed to salt stress (Tester and Davenport, 2003; Haq *et al.*, 2009). The decreased in K^+/Na^+ ratios may relate directly to a decrease in yield in some conditions (Asch *et al.*, 2000). From this study, the tolerant genotypes, Pokkali and Dendang, showed the highest reduction of K^+/Na^+ ratio under salt stress, but could maintain growth with the result that the grain yield was not reduced excessively. Similarly, other tolerant genotypes, IR77674 and IR82493, showed high K^+/Na^+ ratio. These tolerant genotypes could maintain the grain yield under salt stress up to 40 mM NaCl, even better than the sensitive genotypes, IR29 and Inpara 4, which showed the low leaf K^+/Na^+ ratio.

K^+ and Ca^{2+} have been reported to be the major contributors to osmotic adjustment under stress conditions in several plant species (Jamil *et al*., 2012). Ca^{2+} is known to play a crucial role in maintaining the structural and functional integrity of plant membranes in addition to its considerable roles in cell wall stabilization, regulation of ion transport and selectivity and activation of cell wall enzymes (Ashraf, 2004). The maintenance of calcium acquisition and transport under salt stress is an important determinant of salinity tolerance. In this study it was shown that the NaCl*genotype interaction was significant. By increasing salt level treatments, the Ca^{2+} concentration and Ca^{2+}/Na^+ ratio in leaf tissue of the genotypes tend to decrease. Thus, enhancement of salinity affects the concentration of Ca^{2+} in the leaf tissues of the tested genotypes.

CONCLUSIONS

Salinity inhibited growth of the six rice genotypes from vegetative to reproductive stages, as shown by reduction of plant height, panicle number plant^{-1}, panicle length, leaf length, 1000 grain weight and grain yield. The growth reduction was caused by the increase of leaf Na^+, the reduction of leaf K^+ and leaf Ca^{2+} concentrations, and the reduction of leaf K^+/Na^+ and leaf Ca^{2+}/Na^+ ratios. The high Na^+ concentration in the soil solution reduces the absorption of K^+ and Ca^{2+} ions. The tolerant and sensitive genotypes can be distinguished at 40 mM NaCl (7-8 dS m^{-1}), showing that the addition of 40 mM NaCl to growth medium can be used as a useful tool to select tolerant and sensitive genotypes under greenhouse conditions. The relative grain yield reduction at 40 mM NaCl of tolerant genotypes was less than 70% and while it was more than 90% for sensitive genotypes.

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