

## SALINITY TOLERANCE OF SEVERAL RICE GENOTYPES AT SEEDLING STAGE

### *Toleransi Beberapa Genotipe Padi terhadap Salinitas pada Fase Bibit*

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### ABSTRACT

Salinity is one of the most serious problems in rice cultivation. Salinity drastically reduced plant growth and yield, especially at seedling stage. Several rice genotypes have been produced, but their tolerance to salinity has not yet been evaluated. The study aimed to evaluate salinity tolerance of rice genotypes at seedling stage. The glasshouse experiment was conducted at Cimanggu Experimental Station, Bogor, from April to May 2013. Thirteen rice genotypes and two check varieties, namely Pokkali (salt tolerant) and IR29 (salt sensitive) were tested at seedling stage. The experiment was arranged in a randomized complete block design with three replications and two factors, namely the levels of NaCl (0 and 120 mM) and 13 genotypes of rice. Rice seedlings were grown in the nutrient culture (hydroponic) supplemented with NaCl at different levels. The growth and salinity injury levels of the genotypes were recorded periodically. The results showed that salinity level of 120 mM NaCl reduced seedling growth of all rice genotypes, but the tolerant ones were survived after 14 days or until the sensitive check variety died. Based on the visual injury symptoms on the leaves, five genotypes, i.e. Dendang, Inpara 5, Inpari 29, IR77674-3B-8-2-2-14-4-AJY2, and IR81493-BBB-6-B-2-1-2 were tolerant to 120 mM salinity level, while Inpara 4 was comparable to salt sensitive IR29. Hence, Inpara 4 could be used as a salinity sensitive genotype for future research of testing tolerant variety. Further evaluation is needed to confirm their salinity tolerance under field conditions.

[**Keywords:** rice, salinity, screening, tolerance]

### ABSTRAK

Salinitas merupakan masalah yang sangat serius dalam budi daya padi. Salinitas secara drastis menurunkan pertumbuhan dan hasil gabah, terutama pada fase bibit. Beberapa genotipe padi telah dihasilkan, namun toleransinya terhadap salinitas belum pernah diuji. Penelitian ini bertujuan mengevaluasi toleransi genotipe-genotipe padi terhadap salinitas pada fase bibit. Percobaan dilaksanakan di Rumah Kaca Cimanggu Bogor pada bulan April hingga Mei 2013. Materi yang digunakan adalah 13 genotipe padi dengan dua varietas pembanding yaitu Pokkali (toleran salinitas) dan IR29 (peka salinitas) yang diuji pada fase bibit. Percobaan menggunakan rancangan acak kelompok dengan tiga ulangan dan dua faktor. Faktor pertama adalah kadar NaCl (0 dan 120 mM) dan faktor kedua adalah 13 genotipe padi. Benih

padi ditanam dalam media hara (hidroponik) yang ditambah NaCl dengan kadar yang berbeda. Tingkat pertumbuhan dan kerusakan tanaman akibat salinitas dicatat secara berkala. Hasil penelitian menunjukkan bahwa kadar salinitas 120 mM NaCl menyebabkan penurunan pertumbuhan bibit semua genotipe padi, namun genotipe yang toleran mampu bertahan selama 14 hari atau hingga varietas pembanding yang sensitif mati. Berdasarkan gejala kerusakan yang tampak pada daun, lima genotipe yaitu Dendang, Inpara 5, Inpari 29, IR77674-3B-8-2-2-14-4-AJY2, dan IR81493-BBB-6-B-2-1-2 toleran terhadap salinitas 120 mM. Sementara Inpara 4 sangat peka salinitas seperti halnya IR29. Oleh karena itu, Inpara 4 dapat digunakan sebagai varietas peka salinitas pada penelitian selanjutnya tentang pengujian toleransi varietas. Evaluasi lebih lanjut diperlukan untuk mengetahui toleransinya terhadap salinitas pada kondisi lapangan.

[**Kata kunci:** padi, salinitas, skrining, toleransi]

### INTRODUCTION

Salinity is one of the main problems in rice cultivation worldwide (Hosseini et al. 2012; Abbas et al. 2013). Rice plant is sensitive to salinity but rice is one of the recommended crops grown in saline soil, because rice has the ability to grow in waterlogged soil (Sankar et al. 2011; Aref dan Rad 2012). Rice yield loss in saline soil conditions ( $>6 \text{ dS.m}^{-1}$ ) reaches 50-100% (Rad et al. 2012).

In Indonesia the effect of salinity on rice production in Indonesia can be as high as 50% of the rice fields along the north coastal area of Java (Hariadi et al. 2015). The use of salinity tolerant rice variety is, therefore, required to increase rice production in saline soil.

The effect of salinity on rice plant begins when salt accumulation in the old leaves reaches a toxic concentration (which causes the leaves no longer expanding and diluting the salt as younger growing leaves do), and finally they die. If the rate of old leaves die is greater than the rate of new leaves produced, the photosynthetic capacity of the plant cannot supply carbohydrate requirement of the young leaves, which further reduces their growth rate (Munns and

Tester 2008). Effect of salinity on rice resulted in failed or inhibited germination, decreased growth, leaf area, dry matter production and seed formation (Khatun and Flowers 1995) and increased empty rice grain (Asch et al. 1999).

Na toxicity was characterized by drying the side of the leaf tip, as well as Cl toxicity. The symptoms are very difficult to be distinguished from the symptoms of drought. Severity of the salinity effect depends on the intensity of salinity stress, climatic conditions, and the level of genotype tolerance (Suwarno 1985).

Rice plant responses to salinity vary according to the growth stage. In most rice cultivars, early seedling is the most sensitive stage to salinity (Suwarno 1985; Zeng and Shannon 2000; Zeng et al. 2001; Haq et al. 2009). Therefore, crop tolerance to salinity at seedling stage can be used to determine final growth and tolerance to salinity on plant species (Haq et al. 2009). Hosseini et al. (2012) suggested that screening for salinity tolerance at seedling stage was done because variations on this stage are genetically controlled. According to Zeng et al. (2001), salinity stress at seedling stage reduced plant dry weight by two fold compared to that occurred at ripening stage.

The early seedling and reproductive stages are more sensitive to salinity than the tillering stage. According to Suwarno (1985), at germination stage, rice varieties were tolerant to salinity and become very sensitive during the early seedling stage, and then the tolerance increased during the vegetative stage. Nevertheless, there is no correlation between tolerance at germination and seedling stages. The study aimed to determine tolerance of thirteen rice genotypes to salinity at seedling stage.

## MATERIALS AND METHODS

Greenhouse experiment was conducted in Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor, from April to May 2013. The materials used comprised of 15 genotypes of rice originating from IRRI and the Indonesian Center for Rice Research, including two check varieties, i.e. Pokkali (salinity tolerant) and IR29 (salinity sensitive) (Table 1). The experiment was arranged in a randomized complete block design with two factors and three replications. The first factor was the level of NaCl (0 and 120 mM), and the second factor was the rice genotypes. Each of the experimental units consisted of four seedlings of rice plants.

Screening of salt tolerance followed the method of Egdane et al. (2007) using the nutrient culture of Yoshida et al. (1976) containing 120 mM NaCl or electrical conductivity (EC) of  $\pm 12$  dS.m<sup>-1</sup>. Seven-day old

**Table 1. Fifteen rice genotypes used in this study.**

Genotypes	Origin or crossing
Banyuasin	Cisadane/Kelara
Cilamaya Muncul	Pelita I-1/B2388
Dandang	Osok/IR5657-33-2
Inpara 4 (Swarna sub-1)	IRRI (IR05F101)
Inpara 5 (IR64 sub-1)	IRRI (IR07F102)
Inpari 29	IR69502/KAL9418 // Pokkali/Angke
Inpari 30 (Ciherang sub-1)	Ciherang/ IR64Sub1/Ciherang
IR64	IRRI (IR5657/IR2061)
IR77674-3B-8-2-2-14-4-AJY2	IRRI (IR71730-51-2/IRRI 128)
IR78788-B-B-10-1-2-4-AJY1	IRRI (BR41/IRRI 128)
IR81493-B-B-6-B-2-1-2	IRRI (IR75000/IR71684)
Mendawak	Mahsuri/Kelara
Siak Raya	Batang Ombilin/Kelara
Pokkali	Salinity tolerant check
IR29	Salinity sensitive check

seedlings were transplanted in the perforated styrofoam trays (18 mm in diameter and the space between holes were 50 mm x 40 mm). The seedlings were placed on thin-foam sheets, rolled on, and put into the holes. The styrofoam with the seedlings were then floated in the Yoshida medium (17 L pot<sup>-1</sup>) and maintained for 14 days or until the seedlings had 3–5 leaves. Afterwards, NaCl was added into the styrofoam trays in gradual concentrations from zero to 60 mM, and pH was daily maintained at 5.0–5.1 by addition of 0.1 N HCl or NaOH. The treated seedlings were maintained for two days. The final concentration was increased to 120 mM NaCl. The gradual application of NaCl was done to avoid osmotic shock. Nutrient culture medium was replaced after one week. The 120 mM NaCl treatment was carried out for 14 days or until the sensitive check variety died.

Observations were performed on scoring of seedling tolerance to salinity according to the standard evaluation developed by IRRI (2003) as presented in Table 2. In addition, observations were also done on plant height, root length, shoot and root dry weight.

## RESULTS AND DISCUSSION

### Sensitivity to Salinity

Six out of 13 rice genotypes tested to salinity at seedling stage showed tolerant (Dandang, Inpara 5, Inpari 29, IR77674-3B-8-2-2-14-4-AJY2, IR81493-B-B-6-B-2-1-2 and Dandang), five moderately

**Table 2. Criteria for evaluation of salinity tolerance of rice at seedling stage.**

Score	Description	Tolerance
1	Normal growth, only the old leaves show white tips while no symptoms on young leaves	Very tolerant
3	Near normal growth, but only leaf tips burn, few older leaves become whitish partially	Tolerant
5	Growth severely retarded, most old leaves severely injured, few young leaves elongating	Moderately tolerant
7	Complete cessation of growth, most leaves dried, only few young leaves still green	Sensitive
9	Almost all plants dead or drying	Very sensitive

Source: IRRI (2003).

tolerant (Cilamaya Muncul, Inpari 30, IR64, IR78788-B-B-10-1-2-4-AJY1 and Siak Raya), two sensitive (Banyuasin and Mendawak), and one very sensitive (Inpara 4) (Table 3). Sensitivity to salinity was clearly shown from the damages of plant tissues. The longer the duration of stress, the severe the plant damage. At seven days after treatment, the sensitive salt tolerant genotype IR29 showed a moderate tolerance, however, after 14 days, the plant died. Growth performances of the tolerant, moderately tolerant, sensitive and very sensitive genotypes to salinity treatments were shown in Figure 1. In this study, Inpara 4 showed to be the most sensitive variety among the 13 genotypes tested and similar to the sensitive control IR29.

### Morphological Characters

Salinity reduced plant height of all genotypes tested (Table 4). The lowest reduction (28.02%) was shown by IR81493 as one of the tolerant genotypes, similar to the tolerant control Pokkali. The highest plant height reduction (41.60%) was observed on IR29 as a sensitive check variety. The tolerant genotypes such as Dendang, Inpara 5, Inpari 29 and IR77674 showed the least plant height reduction, presumably because they are able to do photosynthetic activity better than the sensitive genotypes. Islam and Karim (2010) reported that the treatment of salinity at 15 dS.m<sup>-1</sup> (equivalent to 150 mM NaCl) reduced seedling height of rice plant. This study indicates that growth reduction is a good indicator for sensitivity of rice genotypes to salt stress.

The average root length of all genotypes were reduced by salinity treatments, ranging from 18.95%

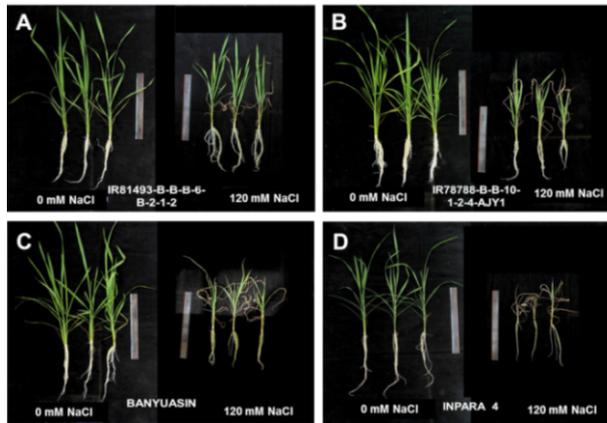
**Table 3. Visual tolerance score of fifteen rice genotypes to salinity at seedling stage.**

Genotypes	Control		120 Mm NaCl		Tolerance status
	7	14	7	14	
	DAT	DAT	DAT	DAT	
Dendang	1	1	1	3	Tolerant
Inpara 5	1	1	3	3	Tolerant
Inpari 29	1	1	3	3	Tolerant
IR77674-	1	1	1	3	Tolerant
IR81493-	1	1	1	3	Tolerant
Dendang	1	1	1	3	Tolerant
Cilamaya Muncul	1	1	3	5	Moderately tolerant
Inpari 30	1	1	3	5	Moderately tolerant
IR64	1	1	3	5	Moderately tolerant
IR78788-	1	1	3	5	Moderately tolerant
Siak Raya	1	1	3	5	Moderately tolerant
Banyuasin	1	1	7	7	Sensitive
Mendawak	1	1	7	7	Sensitive
Inpara 4	1	1	5	9	Very sensitive
Pokkali (tolerant check)	1	1	1	3	Tolerant
IR29 (sensitive check)	1	1	5	9	Very sensitive

DAT = days after treatment.

to 46.39% (Table 5). At 120 mM NaCl treatment, the tolerant genotypes such as Inpara 5 and IR81493 showed the longest root, similar with the moderately tolerant genotypes, i.e. Cilamaya Muncul and IR64. The root length reduction between tolerant and sensitive genotypes were similar. For example, IR64 (a moderately tolerant genotype) showed the lowest reduction of root length similar to the very sensitive one (IR29) and the tolerant genotype IR81493. Similar findings were reported by Hariadi et al. (2015). This means that root length reduction is not an indicator for tolerance of rice seedlings to salt stress.

Salinity reduced shoot dry weight of the genotypes tested. The highest reduction was shown by IR29 (66.05%) and the lowest reduction was observed on tolerant genotype IR81493 (32.05%). At 120 mM NaCl treatment, Pokkali had the highest shoot dry weight among the tested genotypes, while the sensitive genotypes IR29 and Inpara 4 had the lowest shoot dry weight. However, shoot dry weight was not correlated



**Fig. 1.** Performance of tolerant (A), moderately tolerant (B), sensitive (C) and very sensitive (D) rice genotypes to salinity at seedling stage.

**Table 4.** Effect of salinity on seedling height of fifteen rice genotypes at seedling stage.

Genotypes	Seedling height (cm)		Reduction (%)
	Control	NaCl 120 mM	
Banyuasin	61.10 efg	40.04 de	34.39 abcd
Cilamaya Muncul	61.60 def	38.92 e	36.85 abcd
Dandang	59.55 efgh	37.57 e	36.49 abcd
Inpara 4	51.43 i	31.83 f	37.91 abc
Inpara 5	58.30 fgh	37.27 e	36.10 abcd
Inpari 29	68.00 bc	43.77 cd	35.56 abcd
Inpari 30	57.66 fgh	37.34 e	35.23 abcd
IR64	56.83 gh	38.76 e	31.76 bcd
IR77674-	70.03 b	47.11 bc	32.68 abcd
IR78788-	65.70 cd	40.62 de	38.16 ab
IR81493-	62.88 de	45.28 bc	28.02 d
Mendawak	56.58 h	38.19 e	32.45 abcd
Siak Raya	69.68 bc	48.35 b	30.59 bcd
Pokkali (tolerant check)	99.88 a	71.42 a	28.42 cd
IR29 (sensitive check)	43.14 j	25.17 g	41.60 a

Means within a column followed by the same letter are not significantly different at 5% by DMRT.

with sensitivity of rice genotypes to salinity. Shoot dry weight reduction in Pokkali (tolerant check) was not significantly different from that of IR29 (sensitive check) (Table 6). Nonetheless, the tolerant genotypes were able to maintain their growth by reducing leaf damage, whereas the sensitive genotypes did not. Seedling dry weight reduction under salt stress may be due to the diversion of some quantum of energy from growth and

**Table 5.** Effect of salinity on root length of fifteen rice genotypes at seedling stage.

Genotypes	Root length (cm)		Reduction (%)
	Control	NaCl 120 mM	
Banyuasin	29.54 bcde	19.29 def	34.48 abcd
Cilamaya Muncul	30.36 bcd	22.14 ab	26.43 cdef
Dandang	34.50 a	18.48 defg	46.39 a
Inpara 4	31.30 abc	20.50 bed	34.52 abcd
Inpara 5	29.49 bcde	23.13 a	21.62 ef
Inpari 29	25.61 fgh	20.53 bed	19.61 ef
Inpari 30	23.48 gh	17.72 efgh	24.39 def
IR64	26.47 efg	21.50 abc	18.95 f
IR77674-	20.11 i	15.75 h	21.38 ef
IR78788-	26.93 efgh	19.81 cde	26.36 cdef
IR81493-	29.79 bcde	23.18 a	21.55 ef
Mendawak	29.17 bcde	19.84 cde	31.87 bcde
Siak Raya	32.14 ab	18.68 def	41.81 ab
Pokkali (tolerant check)	28.28 cdef	17.55 fgh	37.85 abc
IR29 (sensitive check)	22.50 hi	16.48 gh	25.85 cdef

Means within a column followed by the same letter are not significantly different at 5% by DMRT.

metabolism. The decline may also be as a consequence to the enhancement in maintenance cost of growing cells under stress (Ali et al. 2014).

Salinity also reduced root dry weight of all genotypes tested. IR29 showed the highest reduction in root dry weight (74.60%) and the lowest was observed on IR81493 (39.75%). Pokkali showed quite high reduction in root dry weight (57.77%) and was not significantly different from IR29. The data showed that reduction in root dry weight in tolerant and sensitive genotypes were not significantly different (Table 7). The overall reduction in root dry weight could be due to the toxic effect of salt and reduced nutrient availability for root growing (Iqbal et al. 2007).

Total dry weights of tolerant genotype Pokkali and sensitive variety IR29 were significantly different. The lowest reduction in total dry weight was shown by IR81493 (33.55%). The data showed that application of 120 mM NaCl reduced total plant dry weight by >40%. Inpara 4 (sensitive genotype), Inpari 30, IR78788 and Siak Raya (moderately tolerant), and IR77674 (tolerant) showed no difference in total dry weight reduction compared to IR29 (Table 8). This suggests that high reduction in total dry weight not only occurred in sensitive genotypes, but also in moderate and tolerant genotypes. Jamil et al. (2012) reported that

**Table 6. Effect of salinity on shoot dry weight of fifteen rice genotypes at seedling stage.**

Genotypes	Shoot dry weight (g)		Reduction (%)
	Control	NaCl 120 mM	
Banyuasin	0.83 cde	0.49 b	40.58 cd
Cilamaya Muncul	0.68 efg	0.34 ef	49.22 bc
Dandang	0.55 gh	0.33 efg	41.07 cd
Inpara 4	0.43 hi	0.17 h	59.48 ab
Inpara 5	0.75 efg	0.38 cde	49.04 bc
Inpari 29	0.76 ef	0.36 de	52.83 abc
Inpari 30	0.65 efg	0.25 g	60.31 ab
IR64	0.58 fgh	0.27 fg	52.64 abc
IR77674-	1.02 b	0.45 bc	56.26 ab
IR78788-	0.81 de	0.33 efg	57.03 ab
IR81493-	0.68 efg	0.45 bc	32.05 d
Mendawak	1.00 bc	0.47 b	52.59 abc
Siak Raya	0.97 bcd	0.43 bcd	55.38 ab
Pokkali (tolerant check)	1.41 a	0.66 a	53.12 abc
IR29 (sensitive check)	0.33 i	0.11 h	66.05 a

Means within a column followed by the same letter are not significantly different at 5% by DMRT.

**Table 7. Effect of salinity on root dry weight of fifteen rice genotypes at seedling stage.**

Genotypes	Root dry weight (g)		Reduction (%)
	Control	NaCl 120 mM	
Cilamaya Muncul	0.16 efg	0.08 abcde	52.83 bcd
Dandang	0.13 fg	0.06 def	51.70 bcd
Inpara 4	0.08 g	0.04 fg	47.35 bcd
Inpara 5	0.17 def	0.07 cdef	55.55 abcd
Inpari 29	0.17 cdef	0.07 bcde	58.26 abcd
Inpari 30	0.15 fg	0.06 efg	61.10 abc
IR64	0.15 fg	0.09 abcd	42.06 cd
IR77674-	0.24 abc	0.10 a	58.08 abcd
IR78788-	0.23 abcd	0.07 bcde	65.60 ab
IR81493-	0.16 def	0.10 abc	39.75 d
Mendawak	0.26 a	0.10 ab	61.92 abc
Siak Raya	0.25 ab	0.08 abcde	65.67 ab
Pokkali (tolerant check)	0.23 abcde	0.10 abc	57.77 abcd
IR29 (sensitive check)	0.13 fg	0.03 g	74.60 a

Means within a column followed by the same letter are not significantly different at 5% by DMRT.

**Table 8. Effect of salinity on total dry weight of 15 rice genotypes at seedling stage.**

Genotypes	Total dry weight (g)		Reduction (%)
	Control	NaCl 120 mM	
Cilamaya Muncul	0.83 cd	0.42 d	49.92 bcd
Dandang	0.68 def	0.39 de	42.99 cde
Inpara 4	0.52 ef	0.21 f	57.88 ab
Inpara 5	0.91 cd	0.44 cd	50.27 bcd
Inpari 29	0.93 cd	0.43 cd	53.85 bcd
Inpari 30	0.79 cd	0.31 e	60.49 ab
IR64	0.73 de	0.36 de	50.15 bcd
IR77674-	1.26 b	0.55 b	56.61 abc
IR78788-	1.04 bc	0.40 de	58.96 ab
IR81493-	0.85 cd	0.55 b	33.55 e
Mendawak	1.27 b	0.57 b	54.52 bcd
Siak Raya	1.22 b	0.52 bc	57.51 ab
Pokkali (tolerant check)	1.64 a	0.76 a	53.79 bcd
IR29 (sensitive check)	0.46 f	0.14 f	68.51 a

Means within a column followed by the same letter are not significantly different at 5% by DMRT.

treatment of salinity up to 150 mM reduced shoot and root dry weight of rice plant at seedlings stage, while Chunthaburee et al. (2015) reported that treatment of 100 mM NaCl reduced shoot and root dry weight of 12 rice genotypes tested.

Salinity reduced rice seedling growth as indicated by the reduction in plant height, root length and seedling dry weight. In this study, salinity level at 120 mM NaCl reduced plant growth and viability. The sensitive genotypes (IR29 and Inpara 4) failed to survive at 14 days after salt treatment, whereas the tolerant genotypes showed good growth. Most of the leaves were green despite the old leaves have dried. Salt inhibited plant growth at seedling stage which resulted in a reduction in plant biomass (Munns 2002; Jamil et al. 2012).

The correlation coefficients between agronomic characters and leaf damage score on screening of rice genotypes are presented in Table 9. Seedling tolerance to salinity determined by leaf damage scores was negatively correlated with plant height, shoot dry weight, root dry weight and total dry weight. This means that the higher the leaf damage score the more sensitive the seedling. The negative correlation between leaf damage score and seedling dry weight was also reported by Haq et al. (2009) and Chunthaburee et al. (2015).

**Table 9. Pearson's correlation coefficients between agronomic characters and leaf damage score of 15 rice genotypes exposed to salt stress at seedling stage.**

Variable	Score	Plant height	Root length	Shoot dry weight	Root dry weight	Total dry weight
Score	1.000					
Plant height	-0.582**	1.000				
Root length	-0.143	-0.101	1.000			
Shoot dry weight	-0.517**	0.812**	0.011	1.000		
Root dry weight	-0.408**	0.538**	0.151	0.756**	1.000	
Total dry weight	-0.511**	0.798**	0.028	0.994**	0.817**	1.000

\*\*Correlation was significant at  $P < 0.01$ .

## CONCLUSION

Salinity at 120 mM NaCl decreased seedling growth of rice genotypes, but the tolerant ones were survived after 14 days or until the sensitive check variety died. Five genotypes, i.e Dendang, Inpara 5, Inpari 29, IR77674-3B-8-2-2-14-4-AJY2 and IR81493-BBB-6-B-2-1-2 were tolerant to salinity, whereas Inpara 4 was sensitive, similar to the control sensitive IR29. Further evaluation is needed to confirm tolerance of the genotypes to salinity under field conditions.

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