YIELD AND AGRONOMIC PERFORMANCE OF SALINE-TOLERANT RICE MUTANT LINES

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Abstract. The purpose of this study was to determine the yield stability, adaptability, and agronomic traits of 10 saline-tolerant mutant rice lines at six locations with diverse saline conditions in Indonesia. This research was conducted in six saline soil locations, with the electrical conductivity (EC) ranging from 6.2 to 12.7 dSm^{-1}). The field experiments were arranged in a randomized complete block design (RCBD) with rice genotype as the factor. Each experimental unit was 4 m x 5 m of plots. The genotype treatment consisted of ten lines of salinity-tolerant mutant rice and four control varieties. Inpari 34 and Inpari 35 Salin agritan were the tolerant control varieties. The parent-control varieties were Ciherang (sensitive) and Inpari 13 (moderate). Each treatment was replicated three times. The results showed five mutant lines, namely CH-1, CH-2, CH-3, II-13-14, and II-13-78, were confirmed as being adaptive to saline environments. Two mutant lines (CH-1 and II-13-78) had high average productivity (>7 tons ha^{-1}) and potential yields (>8.5 tons ha^{-1}) that were significantly different from the parents and control saline-tolerant varieties. Supported by good agronomic characteristics, those two mutant lines have the potential to be released as new rice varieties tolerant to salinity.

Keywords: *electrical conductivity, Oryza sativa L., saline soil, mutation breeding, multilocation yield trial*

Introduction

Rice (*Oryza sativa* L.) production in Indonesia can be increased through the extensification method, which entails expanding the planting area (Ihsan et al., 2016). However, due to massive land conversion to other uses, the availability of fertile agricultural land has decreased and moved to suboptimal areas, such as saline prone areas. There are 440 thousand hectares of potential saline soil in Indonesia, the majority of which is located on the islands of Sumatra, Java, Madura, Sulawesi, Maluku, and Papua (Alihamsyah, 2004). It is anticipated that soil salinization in Indonesia will continue to increase, caused by sea level rise due to climate change and seawater intrusion into coastal rice fields (Rad et al., 2012; Karolinoerita and Yusuf, 2020). Among abiotic threats, salinity is the second most devastating constraint in rice production after drought (Hussain et al., 2017).

High salinity affects plants' osmotic and ionic stresses, dominated by sodium (Na^+) and chloride (Cl^-) ions, and refers to an increase in the concentration of plant-toxic ions.

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This effect of ion stress is the primary factor that can inhibit rice growth (Roy et al., 2014; Thu et al., 2017). The effects of salt poisoning on rice plants include stunted growth, fewer productive tillers, and a 50% decrease or more in grain yield, depending on the duration and type of salt stress (Rad et al., 2012; Aref and Rad, 2012; Hariadi et al., 2012).

Utilizing salinity-tolerant, high-yielding varieties is one of the measures taken to increase production on salt-affected land. This strategy is more promising, cost-effective, and socially acceptable (Emon et al., 2015; Reedi et al., 2017; Leake et al., 2020). Due to the vast potential of saline soil in Indonesia, the cultivation of high-yielding saline-tolerant rice can be utilized to replace low yielding local varieties in order to increase national rice production. Therefore, it is necessary to develop new salinity-tolerant rice varieties with high yields.

Combining *in vitro* culture with mutation induction is one of the alternatives for obtaining new traits that are unavailable from existing germplasm, such as high-yielding plant resistant to pests and disease or tolerance to abiotic stress (Viana et al., 2019; Khan et al., 2016). The method includes an irradiation treatment that was given to callus explants. Callus is an actively dividing cell, so the chances of producing mutants are greater than using explants in the form of organs. Application of this technique in sorghum resulted in the release of three high-yielding sweet sorghum varieties (Bioguma 1, Bioguma 2, and Bioguma 3) and the acquisition of several promising mutant lines originating from Suri variety (Djarot et al., 2021). In the previous research, using the same method Yunita et al. (2020) successfully obtained mutants tolerant to salinity. Then, after conducting preliminary and advanced yield trials, 10 rice lines tolerant to salinity levels of 12 dSm^{-1} were chosen. Those lines must be evaluated in multi-location yield trials in diverse environment with saline condition. Therefore, the aims of this research were to determine yield stability, adaptability and agronomic performance of 10 mutant lines of salinity-tolerant rice at six saline soil locations in Indonesia.

Materials and methods

Material used in this study consisted of 14 genotypes: 10 salinity-tolerant mutant lines (*Table 1*), derived from gamma-ray irradiation of Ciherang and Inpari 13 varieties, and four control varieties. Control varieties consisted of two parents (Ciherang and Inpari 13), and two control varieties for tolerance to salinity (Inpari 34 and Inpari 35 Salin Agritan). This research was carried out at six locations with diverse saline conditions (*Table 2*). Planting was conducted under irrigation during the dry season of 2019-2020.

The field experiments were arranged in a randomized complete block design (RCBD) with rice genotype as the factor (*Table 1*). Each treatment was replicated three times. Each experimental unit was 4 m x 5 m of plots. Prior to transplanting, seeds were germinated by soaking them in water for 24 h and sown in the nursery. Transplanting was done by planting 21-day-old rice seedlings, three seedlings for each hill. Plant spacing was 25 cm by 25 cm, so that in each plot there were 17 rows and 21 hills for each row. The space between treatment plots was 0.50 m, and the space between replicates was 1 m. The total area of the experiment was 1,862.5 m^2.

Maintenance includes fertilization, weeding, and controlling plant pests and diseases. Fertilization was done by giving NPK in the form of urea (200 $kg.ha^{-1}$), KCl

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(200 kg.ha^{-1}), and SP36 (100 kg.ha^{-1}). Fertilizer was given in two stages. The first fertilization was carried out by giving half of the recommended rate of urea (45 percent N), all of the P fertilizer (36 percent P), and all of the K fertilizer (60 percent K) as a basal fertilizer one day prior to planting. The remaining 45 percent of nitrogen is applied 60 days after planting.

Table 1. *Rice genotype utilized in the experiment and its salinity tolerance level*

No.	Genotype	Parent	Salinity tolerance level*
		Mutant lines	
1	CH-1	Ciherang	Tolerant
2	CH-2	Ciherang	Tolerant
3	CH-3	Ciherang	Tolerant
4	CH-23	Ciherang	Tolerant
5	CH-24	Ciherang	Tolerant
6	II-13-10	Inpari 13	Moderate
7	II-13-12	Inpari 13	Tolerant
8	II-13-14	Inpari 13	Tolerant
9	II-13-17	Inpari 13	Tolerant
10	II-13-78	Inpari 13	Tolerant
		Control varieties	
11	Ciherang	Parent of CH mutants	Sensitive
12	Inpari 13	Parent of II mutants	Moderate
13	Inpari 34 Salin Agritan	Control for tolerance to salinity	Tolerant
14	Inpari 35 Salin Agritan	Control for tolerance to salinity	Tolerant

*Observation of response to salinity stress of mutant lines each based on Standard Evaluation Score (SES) by International Rice Research Institute IRRI (Gregorio et al., 1997)

Table 2. *Characteristics of the experimental site in the multilocation yield trials*

Location name	Village/GPS	District	Regency	Electrical conductivity (EC in dSm^{-1})*	Average rainfall (mm) per month	Temperature (°C)
1	Patimban -6.2502, 107.9038	Pusaka Nagara	Subang	8.90	232	21-31
2	Ujung Gebang -6,2539, 107,9248	Sukra	Indramayu	12.07	271	25-31
3	Eretan -6,3114, 108,0522	Kandang Haur	Indramayu	9.16	229	25-31
4	Segomeng 1.063, 102.6875	Rangsang Barat	Kepulauan Meranti	11.12	134	25-33
5	Kedabu Rapat 1,857, 102.45	Rangsang Pesisir	Kepulauan Meranti	7.12	109	25-33
6	Semampir -7,6745, 109,1008	Kesugihan	Cilacap	6.12	232	24-31

*Measurement of salinity level using a salinity test tool Hanna H199331

Weeding was done intensively when the plant was in the vegetative and reproductive phases. Pest and disease control were carried out by mechanical and chemical methods. In the mechanical method, pests were killed directly or the environment was made

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unsuitable for them. For example, traps for pest animals and insects; or barriers such as screens or fences to keep animals and insects out are used in biological control, while in chemical control, chemical pesticides such as Decis 25 EC (Deltametrin 25 g.L^{-1}) and Curacron 500 EC (Profenofos 500 g.L^{-1}), are applied to protect plants from pests and diseases. Observations and measurements of the yield and yield components were carried out on 3 hills for all lines tested. The agronomic characteristic observed according to Standard Evaluation System for Rice from IRRI (2013) were as follows:

1. Yield (tons.ha^{-1}) was calculated using the formula: (160,000 hills.ha-^{1} / number of hills harvested per plot) × (grain yield per plot (kg)/1,000).
2. Plant height (cm) was measured from ground level to the longest panicle tip. This character was observed before harvest.
3. Number of productive tillers (tillers/plant) was measured before harvest by counting tillers that produce panicles.
4. The day to harvest was calculated from the days after sowing (DAS). The harvest begin when 90% of rice panicles in one plot turned yellow.
5. Number of grains (grains/panicle) was observed by counting the number of grains in each panicle.
6. Weight of 1,000 grains (g) was calculated by weighing 1,000 filled grains with ± 14% moisture content.

All data was subjected to a combined analysis of variance using SAS 9.0, STAR, and PBSTAT-GE. If there was a significant difference, an LSD test was performed at the level of 5% significance. For grain yield, if the G x E interaction was significant, then stability analysis was performed. Stability analysis was applied for yield trait using the stability parameters based on linear regression model as proposed by Finlay and Wilkinson (1963) as follows:

Coefficient of regression (*bi*) for the genotype *i* was calculated by the following formula:

$$b_i = \frac{\sum_{j=1}^{m} Y_{ij} X_J - \frac{(Y_{i.})(X_.)}{m}}{\sum_{j=1}^{m} X_j^2 - \frac{X_.^2}{m}}$$

In this equation of regression coefficient, i and j indices explain genotype (i = 1....14) and environment (j = 1....6), respectively. In addition, m was the number of environments.

Results and discussion

Yield and productivity

The average yield of 10 lines and four control varieties in six locations at various levels of soil salinity is shown in *Table 3* (*Fig. 1*). Kedaburapat village had the highest yield, while Semampir village had the lowest yield. The coefficient of variation in each location is acceptable, varying between 9.9 and 14.1 (*Table 3*). The ideal coefficient of variation for yields in rice research is less than twenty percent (Krismawati and Arifin, 2011).

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Table 3. *Yield productivity (ton ha^{-1}) of mutant lines and their controls in multilocation trials*

No	Genotype	Locations						Yi
		1	2	3	4	5	6	
1.	CH-1	6.91ab	6.33ab	6.56a	7.82abc	8.75ab	6.57ab	7.16 a
2.	CH-2	4.77de	4.95cd	5.19bcd	6.2de	7.12cd	5.43bc	5.61bc
3.	CH-3	5.58cd	5.07cd	5.37bcd	5.69e	8.05abc	5.12c	5.81bc
4.	CH-23	5.95bbc	4.9cd	4.61cd	7.08bcd	8.01abc	3.79de	5.73bc
5.	CH-24	5.28c	5.01cd	5.02bcd	8.43a	8.53ab	4.31cde	6.1b
6.	II-13-10	5.8bcd	5.21bbcd	4.87bcd	5.95de	8.65ab	3.74e	5.7bc
7.	II-13-12	4.83cde	4.99cd	4.58cd	6.36de	7.79bc	3.40e	5.33bc
8.	II-13-14	5.74cd	5.14cd	4.37d	7.98abc	5.65e	5.01c	5.65bc
9.	II-13-17	5.31cd	5.17bcd	4.43d	6.82cde	7.14cd	3.63e	5.42bc
10.	II-13-78	7.03a	7.27a	6.7a	8.20ab	9.09a	7.02cde	7.62a
11.	Ciherang	5.19cde	5.25bcd	4.72cd	6.55de	7.32cd	4.53cde	5.59bc
12.	Inpari 13	4.05e	4.4d	4.55cd	5.85e	7.22cd	3.75e	4.97c
13.	Inpari 34	5.97bc	5.36bcd	5.65abc	6.34de	6.56de	4.93cd	5.8bc
14.	Inpari 35	5.52cd	5.58bc	6.01ab	6.08de	7.06cd	5.20c	5.91bc
	Mean	5.59	5.33	5.19	6.81	7.64	4.75	5.88
	$LSD_{0.05}$	1.18	0.76	0.9	1.62	1.27	1.41	0.47
	CV (%)	12.52	8.5	10.5	14.1	9.9	15.9	10.6
	P value	**	**	**	*	**	**	**

Yi: Yield means over all environments; The same letter in the same collum indicated not significantly different according to LSD 0.05; * and **: significant at 5% and 1% level, respectively. Location 1: Patimban, Pusaka Nagara-Subang; location 2: Ujung Gebang, Sukra-Indramayu; location 3: Eretan, Kandang Haur-Indramayu; location 4: Segomeng, Rangsang Barat- Kepulauan Meranti; location 5: Kedabu Rapat, Rangsang Pesisir-Kepulauan Meranti; location 6: Semampir, Kesugihan- Cilacap

Figure 1. *Cropping performance in 6 locations. Location 1: Patimban, Pusaka Nagara-Subang; location 2: Ujung Gebang, Sukra-Indramayu; location 3: Eretan, Kandang Haur-Indramayu; location 4: Segomeng, Rangsang Barat- Kepulauan Meranti; location 5: Kedabu Rapat, Rangsang Pesisir-Kepulauan Meranti; location 6: Semampir, Kesugihan- Cilacap*

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The average yields of the tested varieties at the six locations ranged from 5.33 to 7.62 tons per hectare (*Table 3*). There were two mutant lines, namely CH-1 and II-13-78, that excelled both in average yield productivity and yield potential, which were significantly different from those of the control parents at all locations. This research demonstrates that gamma irradiation causes genetic changes in the parent plants, resulting in new plants that are distinctly different from their parents, particularly in their tolerance to salinity.

From the CH mutant group, the CH-1 mutant line surpassed the average yield productivity of the parent (Ciherang) and other control varieties (Inpari 13, Inpari 34 Salin Agritan and Inpari 35 Salin Agritan). The CH-1 mutant line has a potential yield of 8.75 tons per hectare. The potential yield was obtained from location 5 (the village of Kedabu Rapat at Meranti Islands Regency), which had a salinity level of 7.12 dSm^{-1} (*Table 2*).

From II mutant group, the II-13-78 mutant line had an average yield productivity of 7.62 tons per hectare, the highest among the other mutants, its parent and the control varieties at the six locations. The yield potential of the II-13-78 mutant line is 9.09 tons ha^{-1}, and it was obtained from the same location as that of CH-1 mutant line. In the field, variations in rice plant growth and yield were caused primarily by genetic differences between the tested genotypes (Rana et al., 2014). Lestari et al. (2019) reported differences in seed production per hectare in sorghum mutants due to genetic changes caused by gamma irradiation treatment on callus explants.

Islam et al. (2007) reported that as soil salinity increased, plant height, tiller number, panicle length, 1000 grain weight, and rice yield per plant decreased. Molumpong et al. (2019) screened 9000 rice mutants with 150 mM NaCl (equivalent to 15 dSm^{-1}), and the results indicated that 5397 mutants were incapable of producing seeds under the given salinity stress. Interestingly, at salinities greater than 8 dSm^{-1}, both the CH-1 and II-13-78 mutant lines also showed a decrease in productivity, though the differences were not statistically significant (*Table 3*).

Yield stability

The combined analysis revealed a significant interaction between the examined lines and the location (*Table 4*). The presence of genotype - environment interaction suggests that grain yield response varies between genotypes and locations. This can lead to variations in the ranking of these genotypes at various locations (Xu et al., 2014).

Table 4. *The results of the combined analysis of variance for yield character across six locations*

Source of variation	df	SS	MS	F	Pr > F
Environment (E)	5	256.670	51.334	98.250	< 0,001
Genotype (G)	13	113.862	8.759	7.780	< 0,001
G x E	65	73.174	1.1 26	2.150	< 0,001
Error	156	81.508	0.522		
Total	251	541.690			

E = environment or locations; df = degrees of freedom; SS = sum of squares; MS = mean squares

Finlay and Wilkinson’s method was utilized to evaluate the stability and adaptability of the tested genotype. In this study, the regression coefficient (b_i) values ranged from

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0.66 to 1.65 for grain yield. This variation in the b_i value indicates that genotypes had different responses to environmental changes. The genotype is considered stable if the b_i value is not significantly different from 1, while a b_i value greater than 1 indicates that a specific genotype is considered responsive to good environments, has below-average stability, is sensitive to environmental changes, and should only be recommended for cultivation in favorable environments. A genotype with b_i less than 1 is considered unresponsive to different environments or has above-average stability, indicating it is adapted to marginal or suboptimal environments. Therefore, stability below or above average basically shows the pattern of adaptability of the genotypes. In this research, five lines, namely CH-1, CH-2, CH-3, II-13-14, and II-13-78, had a bi value less than 1, so they were confirmed as being adaptive to saline environments (*Table 5*).

Table 5. *The results of the stability analysis of salinity tolerant lines based on the regression of the results of the lines to the environmental index*

No	Genotype	Yi	bi	Prob	Status
1.	CH-1	7.16	0.82**	0.0016912	Unstable
2.	CH-2	5.61	0.70*	0.0232942	Unstable
3.	CH-3	5.81	0.88*	0.0249036	Unstable
4.	CH-23	5.73	1.41**	0.0007506	Unstable
5.	CH-24	6.10	1.65**	0.0012861	Unstable
6.	II-13-10	5.70	1.38**	0.0071808	Unstable
7.	II-13-12	5.33	1.36**	0.0004918	Unstable
8.	II-13-14	5.65	0.66*	0.0229813	Unstable
9.	II-13-17	5.42	1.18**	0.0019880	Unstable
10.	II-13-78	7.62	0.77**	0.0017678	Unstable
11.	Ciherang	5.59	0.99**	0.0000924	Unstable
12.	Inpari 13	4.97	1.15**	0.0017769	Unstable
13.	Inpari 34	5.80	0.51**	0.0084826	Unstable
14.	Inpari 35	5.91	0.53	0.0173051	Unstable

Y*i*: Yield means over all environments; *bi*: coefficient of regression; ns = non-significant. * and ** significantly to very significantly different from 1

The agronomic performance of salinity tolerant mutants

Plant height, the number of productive tillers, the day to harvest, the number of filled and empty grains per panicle, and the weight of 1000 grains were generally used as performance indicators. Short and rigid stems, upright flag leaves, a large number of tillers, an early day to harvest, and dense panicles with high fertility were characteristics observed when selecting high-yielding rice varieties (Souleymane et al., 2017).

One of the factors that can influence farmers' interest in new varieties is plant height. Plants that are too tall will collapse easily, whereas plants that are too short will make harvesting difficult for farmers (Souleymane et al., 2017). IRRI classifies the height of lowland rice plants into three categories: short (110 cm), medium (110-130 cm), and tall (>130 cm) (IRRI, 2004). The 10 tested mutant lines were categorized as short, while four control varieties were categorized as short to medium (*Table 6*). *Table 6* displays the results of the measurements of the selected lines' plant height variable. The selected lines, CH-1 and II-13-78, had slightly shorter plant heights (±4%-5%) than the parent

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and control varieties, but this was not statistically significant. This may be due to the effect of high salinity on plant growth (Wu et al., 2015; Thitisaksakul et al., 2015; Zang et al., 2022). The phenomenon occurs not only in rice but also in wheat (Gadimaliyeva, et al., 2020) and in other plant families, such as eggplant plants (Issa et al., 2020), and tomatoes (*Solanum lycopersicum*) (Sajyan et al., 2018), where salt stress stunts growth.

Table 6. *Agronomic characteristics of 10 salinity-tolerant mutant lines and control varieties*

No	Genotype	Plant height (cm)	Number of reproductive tillers per plant	Day to harvest (DAS)	Number of grains per panicle	1000 grains weight (g)
1.	CH-1	104.20cd	14.8a	113.8	132.1d	22.46d
2.	CH-2	105.85cd	15.3a	115.4	129.0d	22.92 cd
3.	CH-3	105.16cd	14.9a	114.2	129.2d	23.65bc
4.	CH-23	105.20cd	15.6a	115.0	138.3cd	23.46bc
5.	CH-24	115.22b	15.4a	113.8	142.5ab	22.79cd
6.	II-13-10	107.13bc	14.3a	115.6	136.5d	22.99cd
7.	II-13-12	108.09bc	14.4a	114.7	128.4d	23.37bc
8.	II-13-14	109.11bc	14.6a	114.2	135.1cd	23.46bc
9.	II-13-17	107.39bc	14.3a	114.7	132.5cd	23.17bc
10.	II-13-78	109.37bc	14.3a	106.5	145.0bc	24.24b
11.	Ciherang	109.79bc	13.3b	113.0	135.6cd	24.11b
12.	Inpari 13	113.94b	12.9bc	108.5	150.3a	23.21bc
13.	Inpari 34	127.95a	13.0b	113.2	132.8cd	24.31b
14.	Inpari 35	124.46a	13.1b	113.6	107.7e	26.25a
	Mean	110.92	14.4	113.3	133.9	23.70
	$LSD_{0.05}$	5.16	1.3	0.4	3.8	0.78
	P-value	**	**	ns	**	*

The same letter in the same column indicated not significantly different according to LSD 0.05; ns: non-significant; * and **: significant at 5% and 1% level, respectively

Table 6 displays the variance in the number of reproductive tillers per plant among the mutant lines. The number of reproductive tillers per plant for the two selected lines (CH-1 and II-13-78) was 15 and 14 tillers, respectively. Both Inpari 34 and Inpari 35 Saline Agritan had 13 productive tillers per plant, 13 percent fewer than those mutant lines. Plants suffering from salt poisoning can be identified by the diminished number of tillers that develop (Ganapati et al., 2020). *Table 6* demonstrates that the mutant lines possessed more productive tillers than the control varieties.

The day to harvest of the mutant lines ranged from 107 to 116 DAS, while control varieties ranged from 109 to 114 DAS. The CH-1 and II-13-78 mutant lines and the other genotypes in this study were categorized as having early days to harvest (IRRI, 2014).

The characteristics of the panicle were closely related to grain yield. Large panicles with more spikelets per panicle can increase grain density (Das et al., 2018). According to *Table 6*, the number of grains per panicle of the tested lines varied between 128 and 145 spikelets per panicle. The number of grains per panicle in the CH-1 line was 132 grains/panicle, which was non-significant to the parent (Ciherang), but significantly different from one of the salinity control varieties, Inpari 35 Saline Agritan. While for the II-13-78 mutant line, the number of grains per panicle (145 grains/panicle) was lower and significantly different from the parent (Inpari 13), although it was higher and significantly different from salinity control varieties, Inpari 35 Saline Agritan

Due to a lack of water and nutrients required by plants, salinity stress inhibits plant development and growth. The ability of plants to grow well indicates that they possess a

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tolerance mechanism for salinity stress. The CH-1 and II-13-78 mutant lines had a significantly greater number of productive tillers than their parents and controls (*Table 6*). It is suspected that the two mutants possess certain tolerance mechanisms that allow them to survive high soil salinity conditions. One of the mechanisms of plant tolerance to salinity stress is compartmentation and salt secretion into the vacuoles, which prevent them from inhibiting plant growth (Acosta-Motos et al., 2017; Anwar et al., 2022).

Conclusions

Five mutant lines, namely CH-1, CH-2, CH-3, II-13-14, and II-13-78, were confirmed as being adaptive to saline environments. Two mutant lines (CH-1 and II-13-78) had high average productivity (>7 tons ha^{-1}) and potential yields (>8.5 tons ha^{-1}) that were significantly different from the parents and control saline-tolerant varieties. Both mutant lines were high yielding and had good agronomic characters, such as a short plant, a medium number of productive tillers, early harvesting days, a high number of grains/panicle (>130 grains/panicle), and a medium seed index (22-24 g per 1000 grains). The CH-1 and II-13-78 mutant lines have the potential to be released as new rice varieties tolerant to salinity.

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