

## SOMACLONAL PUTATIVE MUTANTS OF RICE TOLERANT TO SALINITY

### Putatif Mutan Somaklon Padi Toleran Salinitas

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#### ABSTRACT

Soil salinity could significantly reduce rice yield, therefore, varieties tolerant to salinity are urgent to be developed. Mutation induction could be used to create rice mutants tolerant to salinity. The study aimed to evaluate the tolerance of somaclonal mutants of rice to NaCl salinity in a greenhouse condition and characterize their tolerance mechanism. A total of 45 putative mutants were generated by a gamma ray mutation induction followed with *in vitro* selection in the growth media containing different NaCl concentrations in the greenhouse experiment. The study consisted of two-factor treatments, namely three levels of NaCl concentrations and 45 rice mutants suspected to be tolerant to salinity, arranged in a completely randomized design. Proline, cations (K, Na, Ca, and Mg) content, and stomata density were evaluated. The results showed that eight mutants were tolerant to 150 mM NaCl, namely CH30, CH-4-2, II-13-42, II-13-7, II-13-10, II-13-13, II-13-2, and IA-3-21. These tolerant mutants had a higher Na content compared to the check parent. The tolerant mutants had a high proline content, lower Na, and stable K, Mg and Ca cations as well as had a greater number of stomata and higher stomata length-width ratio. Some of the identified tolerant mutants demonstrated the tolerant mechanism against salinity stress. Further studies are required to evaluate these tolerant mutants in the field conditions under salinity stress.

[**Keywords:** NaCl, proline, rice, salinity, somaclonal mutants]

#### ABSTRAK

Salinitas pada tanah dapat menurunkan hasil padi secara nyata. Oleh karena itu, varietas yang toleran terhadap salinitas perlu dikembangkan. Induksi mutasi dapat digunakan untuk merakit varietas padi toleran terhadap salinitas. Penelitian bertujuan untuk mengevaluasi toleransi mutan putatif somaklon padi terhadap salinitas (NaCl) di rumah kaca dan karakteristik toleransinya. Sebanyak 45 mutan putatif yang telah dihasilkan dengan menggabungkan induksi mutasi sinar gamma dan seleksi *in vitro* dievaluasi menggunakan konsentrasi NaCl yang berbeda di rumah kaca. Penelitian dirancang dalam dua faktor, yaitu tiga tingkat konsentrasi NaCl dan 45 mutan padi yang diduga toleran terhadap salinitas, diatur dalam rancangan acak lengkap. Kandungan prolin, kation (K, Na, Ca, dan Mg), dan kerapatan stomata dievaluasi. Hasil penelitian menunjukkan delapan mutan diduga toleran terhadap perlakuan 150 mM NaCl, yaitu CH30, CH-4-2, II-13-42, II-13-7, II-13-10, II-13-13, II-13-2, dan IA-3-21. Mutan toleran ini memiliki kandungan Na yang lebih tinggi dibandingkan dengan tetuanya. Mutan toleran terpilih memiliki kandungan prolin tinggi, Na

rendah, dan kation K dan Mg yang stabil serta mempunyai densitas stomata yang lebih besar, dan rasio lebar-panjang stomata yang lebih tinggi. Beberapa mutan toleran menunjukkan mekanisme toleransi terhadap cekaman salinitas. Penelitian lebih lanjut diperlukan untuk mengevaluasi mutan toleran ini dalam kondisi lapangan di bawah cekaman salinitas.

[**Kata Kunci:** Mutan somaklon, NaCl, padi, prolin, salinitas]

#### INTRODUCTION

Saline soil affects growth and productivity of plants and becomes a major environmental stress on rice crop in Asia, including Indonesia. Worldwide, a total of 60.08 million hectares of saline soil had been indentified, especially on lands along the coastal areas. This area is expected to increase due to the increase in sea levels and the practice of intensive farming (Hariadi et al. 2015; Van Nguyen and Ferrero 2006). In some places, soil salinity is also found in the irrigated lands due to the intensive cropping of rice. Pingali and Rosegrant (1996) reviewed the distribution of saline soil on irrigated farmlands worldwide, and they reported that the decrease in rice productivity due to salinity was considerably high.

In Indonesia, saline soil is found mainly along the coastal area and in some places of the eastern Indonesia, where the lands were formed from the uplifted marine soil (Hariadi et al. 2015). The practice of eliminating salinity effect on rice crop in the coastal areas is by irrigation combined with flushing to delete NaCl concentration in the soil. However, this practice may be prohibitive for rice crop grown in the rain-fed coastal plain. The agronomic treatment to eliminate the negative effect of NaCl on rice crop has not been applied due to the lack of the technique.

An increase of  $1\text{dS}^{-1}\text{ m}$  in salinity in the soil could decrease rice yield by 10% (Rad et al. 2011). The young seedlings and the flowering stage plants were the most sensitive to salinity, whereas during tillering stage it

was less sensitive (Joseph 2013). Other study showed that plant in the germination stage was more tolerant to salinity (Rad et al. 2011). Tavakkoli et al. (2011) reported that salinity stress affected ion imbalance in the cell, especially decreasing  $K^+$  concentration on sensitive plants.

Osmotic regulation is an important mechanism for plant cellular homeostasis in saline conditions. Proline accumulation is an important mechanism for osmotic regulation by plant cells under salt stress. One mechanism of salinity stress tolerance shown by plants is by using an osmotic regulator, such as proline, to prevent the entry of Na into the plant cells (Munns and Tester 2008). Under salt stress, plant accumulates several compatible solutes in the cytosol, such as proline (Huang et al. 2013). Proline accumulation in rice depends on the varieties. Tolerant plants accumulated higher proline than did sensitive plants (Gupta and Huang 2015; Nounjan and Theerakulpisut 2012).

Tolerance to iron toxicity in rice was reported through several mechanisms, namely (1) escape exclusion, (2) inclusion, (3) tolerance inclusion, and (4) membrane selectivity (Nugraha and Rumanti 2017). Such mechanisms may also occur on salinity tolerance. Munns and Tester (2008) reported that on rice, tolerance to salinity was based on exclusion and inclusion mechanisms. The exclusion mechanism excluded the salt from entering the tissue through osmotic regulation and membrane selectivity. This osmotic regulation was influenced by the entrance of proline formed in the root cells. On the inclusion mechanism, root cells absorbed Na ion and  $Na^+$  was influxed in to the vacuole so that it did not toxic to the plant.

The availability of rice varieties tolerant to salinity was only two varieties, namely Inpari 34 and Inpara 35 (Wahab et al. 2017). Therefore, research is needed to develop rice varieties tolerant to salinity stress. *In vitro* culture selection technique combined with mutation induction using the existing germplasm resources may offer an alternative technique for obtaining new rice varieties tolerant to salinity (Kim et al. 2012). The technology had been applied in rice breeding in India and it had successfully produced rice varieties tolerant to soil salinity (Saleem et al. 2005; Zinnah et al. 2013).

Recently, Yunita et al. (2014) generated putative mutants of rice which were presumed to be tolerant to salinity under *in vitro* culture by combining mutation induction and *in vitro* selection on NaCl media, using Ciherang, Inpari 13 and Inpara 3 varieties as the parent material. The seeds of those varieties were irradiated with gamma rays at a dose of LD50 and then selected on NaCl media at a concentration of  $LC_{50}$ . Fifty-five putative mutants of rice seedling tolerant to salinity were

identified (Yunita et al. 2014). These tolerant mutants need to be assessed in a greenhouse before being tested in the field conditions.

The study aimed to evaluate the tolerance of somaclonal putative mutants of rice to salinity in a greenhouse and to characterize the nature of their tolerance mechanism.

## MATERIALS AND METHODS

The study was carried out at the greenhouse of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) in Bogor, Indonesia. Forty-five putative mutants of rice tolerant to salinity and their original parents (Ciherang, Inpari 13 and Inpara 3) obtained from the previous research (Yunita et al. 2014) were tested. A total of 45 mutants, salt sensitive parent (IR29) and tolerant variety Pokkali were used in the study (Table 1).

Rice seeds of the putative mutants were germinated on sand media for one week, then the seedlings were transferred into plant media of pots containing Yoshida solution (Yoshida et al. 1976 in Gregorio et al. 1997). After four days, solutions of 75 mM and 150 mM NaCl were added. The experiment consisted of 45 rice genotypes (mutants, original parents and checks) and three levels of NaCl concentrations as growth media, arranged in a completely randomized design with five replications. The three levels of NaCl concentrations were 0 mM, 75 mM and 150 mM.

The 14-day old seedlings were assessed for their salinity tolerance responses based on the Standard Scoring System of Gregorio et al. (1997) (Table 2). Rice leaf samples were harvested for the analysis of proline content using the method of Bates; Na, K, Ca and Mg contents, stomata density, and the ratio of stomata length and width. The stomata density was observed using a light microscope with a magnification of 400 times.

**Table 1. Putative mutants of rice and the original germplasm parents.**

Original germplasm	Putative mutant
Ciherang	CH-4-1, CH-4-3, CH-6-1, CH-6-2, CH-13-1 CH -13-2, CH-16-1, CH-16-2, CH-21, CH- 27, CH-28, CH-29, CH-30, CH-4-2, CH-4-4
Inpari 13	II-13-46, II-13-43, II-13-45, II-13-1, II-13-4, II-13-5, II-13-7, II-13-8, II-13-9, II-13-10, II- 13-11, II-13-13, II-13-2, II-13-17, II-13-20
Inpara 3	IA-3-1, 3-3-IA, IA-3-4, 3-6 IA, IA-3-10, IA- 3-11, IA-3-13, IA-3-16, IA-3-17, IA-3-20, IA-3-21, IA-3-26, IA-3-27, IA-3-18, IA -3-30

## RESULTS AND DISCUSSION

### Salinity Tolerance

All plants including checks grew normally and showed uniform green leaf color and plant height on the no NaCl pots at four days after planting. However, in the saline media, both on 75 mM and 150 mM NaCl concentrations, mutant plants showed different responses, ranging from high tolerant (score 1) to high susceptible (score 9). In general, treatment of 75 mM NaCl was not able to separate the responses among mutants to salinity, of which susceptible variety (IR29) was not affected. However on the 150 mM NaCl media it could distinctively separate the tolerant, moderately tolerant and susceptible mutants. The highly susceptible mutants showed wilting leaves

**Table 2. A modified standard visual evaluation using the score for salt tolerance on rice at seedling stage (Gregorio et al. 1997).**

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth injury, but leaf tips of few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants died	Susceptible
9	Almost all plants died or dying	Highly susceptible

and the plant died at 14 days after planting. The original parents, i.e. Inpari 13, Ciherang and Inpara 3, showed very susceptible responses to 150 mM NaCl treatment. Subsequently, media containing 150 mM NaCl was used for evaluating salt tolerance among rice putative mutants.

The nine putative mutants derived from Ciherang were very susceptible, two were moderate and two were tolerant (Table 3). Likewise, on 150 mM NaCl media, eight putative mutants of Inpara 3 were susceptible, three were moderate and one was tolerant. Among putative mutants of Inpari 13 on 150 mM NaCl media, three mutants were susceptible, two were very susceptible, five were moderate, and five were tolerant. These results indicated that the previously identified tolerant mutants on M1 to salinity under *in vitro*, showed different responses to salinity on pot media. Among parent varieties, Inpari 13 produced the highest number of plants tolerant to NaCl. Inpara 3 and Ciherang each yielded the smallest number of NaCl tolerant mutants. The tolerant plant grew normally on Yoshida media containing 150 mM NaCl, showing green normal leaf, similar to that on zero NaCl media. Conversely, the susceptible mutants died at 14 days after NaCl treatment.

### Proline Content in Leaves

Proline is an important biochemical compound associated with salt tolerance on plants. The present study showed

**Table 3. Responses to NaCl media among the putative mutants of rice.**

Genotype/putative mutants	Score			Genotype/putative mutants	Score			Genotype/putative mutants	Score		
	0	75	150		0	75	150		0	75	150
Ciherang (P)	1a	3b	9e	Inpari 13 (P)	1a	3b	7d	Inpara 3 (P)	1a	3b	9e
IR29 (S)	1a	5c	9e	IR29 (S)	1a	5c	9e	IR29 (S)	1a	5c	9e
Pokkali (T)	1a	1a	3b	Pokkali (T)	1a	1a	3b	Pokkali (T)	1a	1a	3b
CH-4-1	1a	3b	5c	II-13-42	1a	3b	3b	IA-3-1	1a	3b	9e
CH-4-3	1a	3b	7d	II-13-43	1a	3b	5c	IA-3-3	1a	1a	9e
CH-6-1	1a	3b	9e	II-13-45	1a	3b	5c	IA-3-4	1a	1a	9e
CH-6-2	1a	3b	9e	II-13-1	1a	3b	7d	IA-3-6	1a	3b	9e
CH-13-1	1a	3b	7d	II-13-4	1a	1a	7d	IA-3-10	1a	3b	9e
CH-13-2	1a	3b	9e	II-13-5	1a	1a	5c	IA-3-11	1a	3b	9e
CH-16-1	1a	3b	9e	II-13-7	1a	1a	3b	IA-3-13	1a	3b	9e
CH-16-2	1a	3b	9e	II-13-8	1a	1a	7d	IA-3-16	1a	3b	9e
CH-21	1a	3b	9e	II-13-9	1a	1a	5c	IA-3-17	1a	3b	5c
CH-27	1a	3b	9e	II-13-10	1a	3b	3b	IA-3-20	1a	1a	5c
CH-28	1a	3b	9e	II-13-11	1a	1a	5c	IA-3-21	1a	1a	3b
CH-29	1a	3b	9e	II-13-13	1a	1a	3b	IA-3-26	1a	3b	5c
CH-30	1a	1a	3b	II-13-2	1a	1a	3b	IA-3-27	1a	1a	7d
CH-4-2	1a	1a	3b	II-13-17	1a	3b	9e	IA-3-18	1a	1a	7d
CH-4-4	1a	1a	5c	II-13-20	1a	1a	9e	IA-3-30	1a	1a	7d

P = Ciherang, Inpari 13 and Inpara 3 parent varieties, S = IR29, salt susceptible standard variety, T = Pokkali, salt tolerant standard variety

Score: 1 = very tolerant; 3 = tolerant, 5 = moderate, 7 = susceptible, 9 = very susceptible.

0; 75; 150 denoted NaCl (mM) concentration in the media.

Numbers followed by the same letter are not significantly different at 5% probability level.

that the three parent varieties (Ciherang, Inpari 13 and Inpara3) and the putative mutants showed differences in proline contents in the leaves. Some of the mutants on 150 mM NaCl media indicated higher proline content in the leaves than that of the parents. High proline accumulation in the leaves showed a positive response to salinity stress. High proline content indicated tolerance to salinity.

Some of the putative mutants grown on 150 mM NaCl media showed an increase in proline content. Tolerant putative mutants obtained from Ciherang (CH4-2 and CH30) increased their proline content reaching 38.58 and 40.26 mg g<sup>-1</sup>, respectively. Those derived from Inpari 13 (I-13-42, II-13-7, II-13-10, II-13-13 II-13-2) had proline content of 10.19 to 11.75 mg g<sup>-1</sup> and those from Inpara 3 (IA-3-21) 17.10 mg g<sup>-1</sup> (Table 4). Apparently, in each group of mutants the level of proline contents to be effective as an osmotic regulator was different. It was high in Ciherang, moderate in Inpara 3 and much lower in Inpari 13.

#### Potassium, Sodium, Calcium and Magnesium Contents

In general, on a high concentration of NaCl media, Na content in the plant tissue increased but the K, Ca and Mg content decreased. However, among tolerant genotypes, they responded differently. Potassium accumulation in the leaves was also influenced by rice genotypes and salinity levels. Tolerant plants had the ability to maintain and increase K content in the leaves, while on susceptible mutants K content in the leaves decreased

(Figure 1). The lowest accumulation of K was shown by the susceptible IR29 variety. Potassium accumulation in the plant tissue decreased with the increased salinity level. Genotype Pokkali and tolerant mutants produced the highest accumulation of K compared with other genotypes in each salinity level.

Ciherang variety showed K decrease on 150 mM NaCl media, CH13-2 (susceptible mutant) and IR29 decreased its K content, while Pokkali and CH4-2 (tolerant mutant) increased their K content on the same media. Likewise, Inpara 3 variety showed a similar pattern, where increasing NaCl concentration resulted in decreasing K content in the leaves of Inpara 3 and IA-3-1 mutants. The IA-3-21 mutant was more tolerant to salinity hence K content in the leaves tended to rise. These data showed that tolerant genotype had a greater ability to absorb K than the susceptible one, as had also reported by Horie et al. (2012). Inpari 13 on 150 mM NaCl media reduced its K content in the tissue, but on the tolerant mutant II-13-42, the decreasing K was not too large.

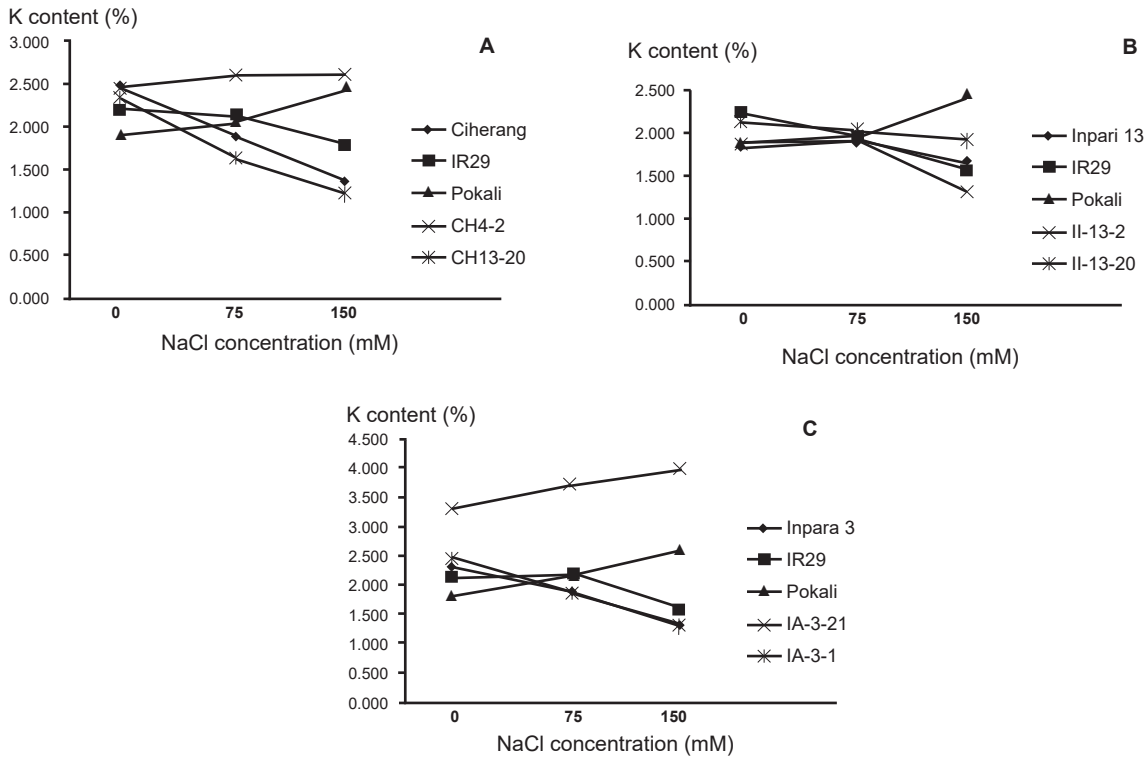
In general, on 150 mm NaCl media, Na content in the leaves increased (Figure 2). For susceptible variety Ciherang, increasing NaCl content on the media increased Na content in the plant, but for the tolerant genotype (Pokkali and CH4-2), the increase in Na content was not too large, meaning that the plant was able to maintain the low Na content in the cells. On susceptible genotypes (Ciherang, IR29 and CH-13-2), the Na content was relatively high.

On Inpari 13 and Inpara 3, increasing NaCl concentration in the media increased Na content in plant tissues, especially on susceptible plants (II-13-17,

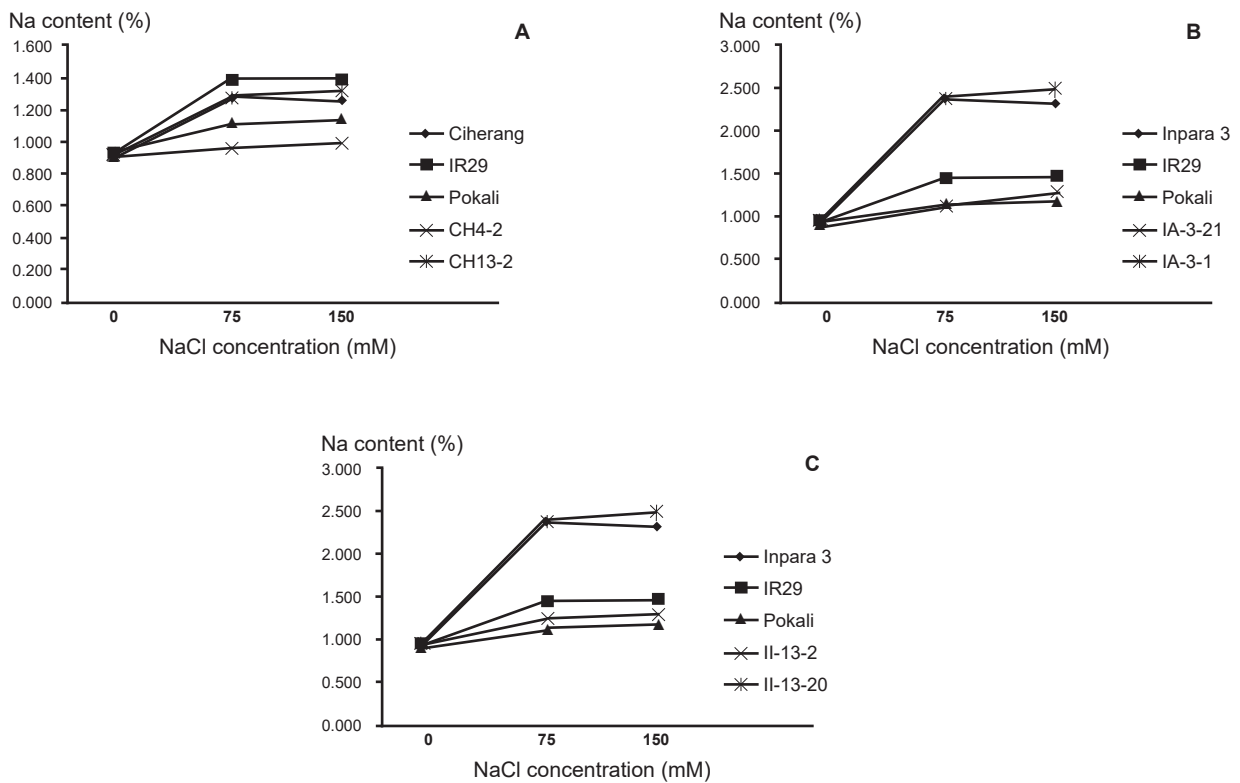
**Table 4. Proline content in rice leaves of putative mutants and their parents (Ciherang, Inpari 13, and Inpara 3).**

Genotype/putative mutants	Proline content (mg g <sup>-1</sup> )			Genotype/putative mutants	Proline content (mg g <sup>-1</sup> )			Genotype/putative mutants	Proline content (mg g <sup>-1</sup> )		
	0	75	150		0	75	150		0	75	150
Ciherang	6.71	14.60	17.39	Inpari 13	1.85	2.76	4.83	Inpara 3	3.89	4.83	5.83
CH -4-1	6.65	14.66	23.56	II-13-42	2.24	2.05	10.83	IA-3-1	4.24	5.06	3.99
CH-4-3	6.50	11.15	10.74	II-13-43	1.78	2.80	5.42	IA-3-3	4.68	3.63	3.95
CH-6-1	6.02	10.24	9.18	II-13-45	1.97	2.27	6.19	IA-3-4	3.99	3.50	4.17
CH-6-2	6.12	12.89	12.10	II-13-1	1.97	2.01	5.74	IA-3-6	4.19	4.75	4.39
CH-13-1	6.98	13.80	14.72	II-13-4	1.81	2.17	4.47	IA-3-10	3.81	3.64	4.28
CH-13-2	6.75	11.53	12.59	II-13-5	1.91	2.28	4.35	IA-3-11	3.71	3.54	4.38
CH-16-1	4.59	11.99	11.68	II-13-7	1.99	2.42	11.75	IA-3-13	4.67	4.14	3.73
CH-16-2	6.60	15.05	13.58	II-13-8	1.86	2.51	4.73	IA-3-16	4.44	5.03	3.97
CH-21	5.81	13.74	11.99	II-13-9	1.89	2.21	6.55	IA-3-17	4.10	6.88	4.35
CH-27	6.67	10.09	12.67	II-13-10	2.06	2.95	13.56	IA-3-20	3.36	6.17	5.43
CH-28	7.13	20.58	22.08	II-13-11	1.90	3.16	6.63	IA-3-21	3.56	11.72	17.10
CH-29	4.91	21.56	14.39	II-13-13	1.78	2.33	10.19	IA-3-26	3.31	5.81	8.21
CH-30	6.47	24.64	40.26	II-13-2	2.13	2.29	10.81	IA-3-27	3.35	5.21	5.53
CH4-2	6.24	26.65	38.58	II-13-17	2.17	2.57	3.72	IA-3-18	3.17	3.44	5.48
CH4-4	5.66	26.65	29.95	II-13-20	2.14	2.63	3.98	IA-3-30	5.00	3.60	5.26

0; 75; 150 denoted the concentration of NaCl (mM) in the media.



**Fig. 1.** Potassium (K) content in the leaves of rice mutants and in their parent varieties, i.e. Ciherang (A), Inpari 13 (B) and Inpara 3 (C) under different NaCl concentrations on media.



**Fig. 2.** Sodium (Na) content in the leaves of rice mutants and in their parent varieties, i.e. (A) Ciherang, (B) Inpari 13, (C) Inpara 3. under different NaCl concentrations in the media.

IA-3-1). On tolerant plants (II-IA-13-42 and 3-21), Na increase in the tissue was relatively low, so it is not to be toxic to the plants. This means that these plants had the resistance mechanism by way of exclusion, where the plant was able to prevent the entry of Na into the plant tissue or prevent Na translocation in the leaves, as was described by Assaha et al. (2017).

Increasing NaCl concentration in the media lowered Ca content in the plant tissue. But on the tolerant plants, such as Pokkali, CH4-2, II-13-42 and IA-3-21, decrease in Ca content in the leaves was small, as compared to those on susceptible genotypes.

Calcium is essential for the integrity of cell membranes and is required for cell wall development. Moreover, decrease in Ca availability reduces plant growth. Decrease in Ca concentration in the plant tissues and high concentration of saline might further reduce plant growth over the salinity effect alone, due to the removal of Ca ion from the cell plasmalemma (Arshad et al. 2012). Rice cell membranes were damaged by Ca deficiency and increase in Na would interfere the plant metabolism. Calcium ion also plays a role in activating calcium-dependent protein kinases (CDPKs), a very important role in the mechanism of tolerance to biotic and abiotic stresses in plants. Excessive expression of these enzymes, however, may increase plant tolerance to saline conditions as stated by Asano et al. (2012). Plant which is able to maintain

Ca content in the tissue is expected to be more tolerant to saline conditions.

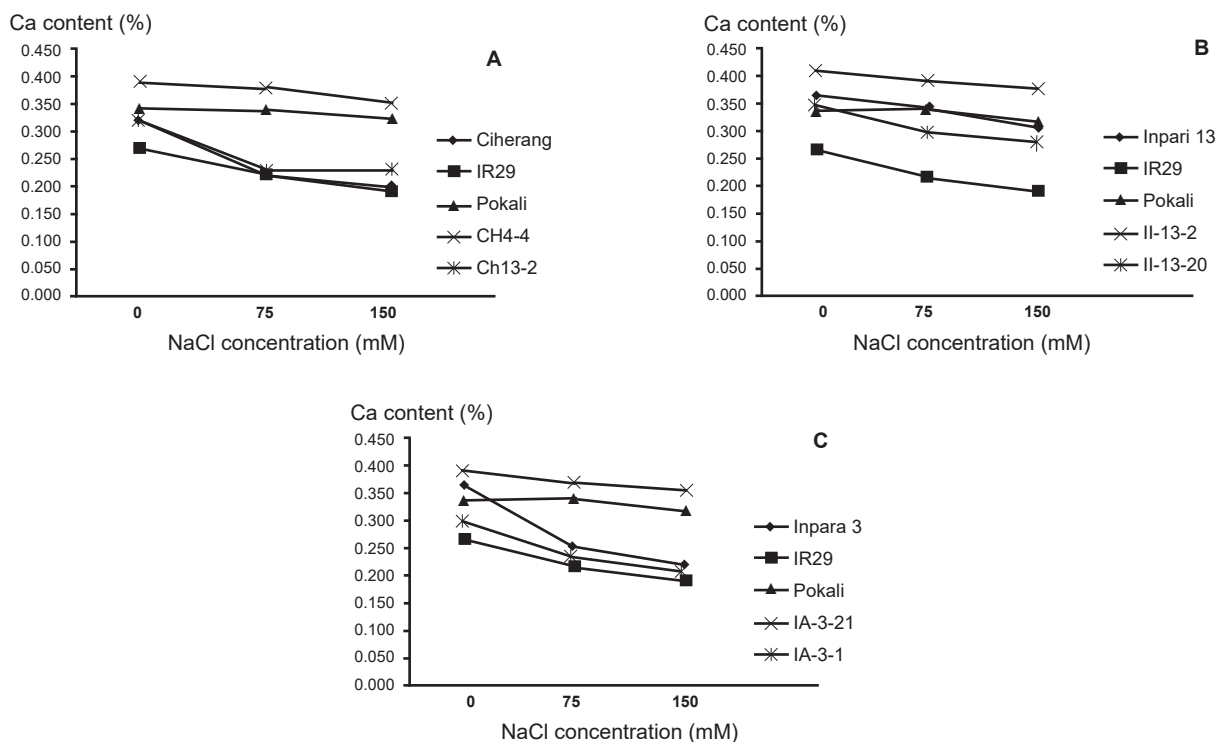
In the media of high NaCl content, Mg content in leaf tissue decreased (Figure 4). On the tolerant plants such as Pokkali, CH4-2, II-13-42, and IA-3-21, the decline of Mg content in the leaves was not too pronounced, as compared to those on sensitive plants.

### Stomata as Affected by NaCl in the Media

There were differences in stomata density among the mutants as compared to those of the parents, related to their responses to NaCl concentration in the media. Previous study by Lestari (2006) also showed a change in stomata density on rice mutant derived from Gajah Mungkur, Towuti and IR64 varieties when compared with those of their parents.

The lower density of stomata on the leaves indicates tolerance of the genotype to NaCl concentration in the media. Ciherang variety and some putative mutants (CH4-2 and CH-30) had less stomata densities compared with other varieties. On some mutants derived from Inpari 13, the tolerant mutants (II-13-42, II-13-7, II-13-10, II-13-13, and II-13-2) also had a lower stomata density than the sensitive mutants. Likewise, the tolerant mutant of Inpara 3 (IA-3-21) possessed lower stomata density.

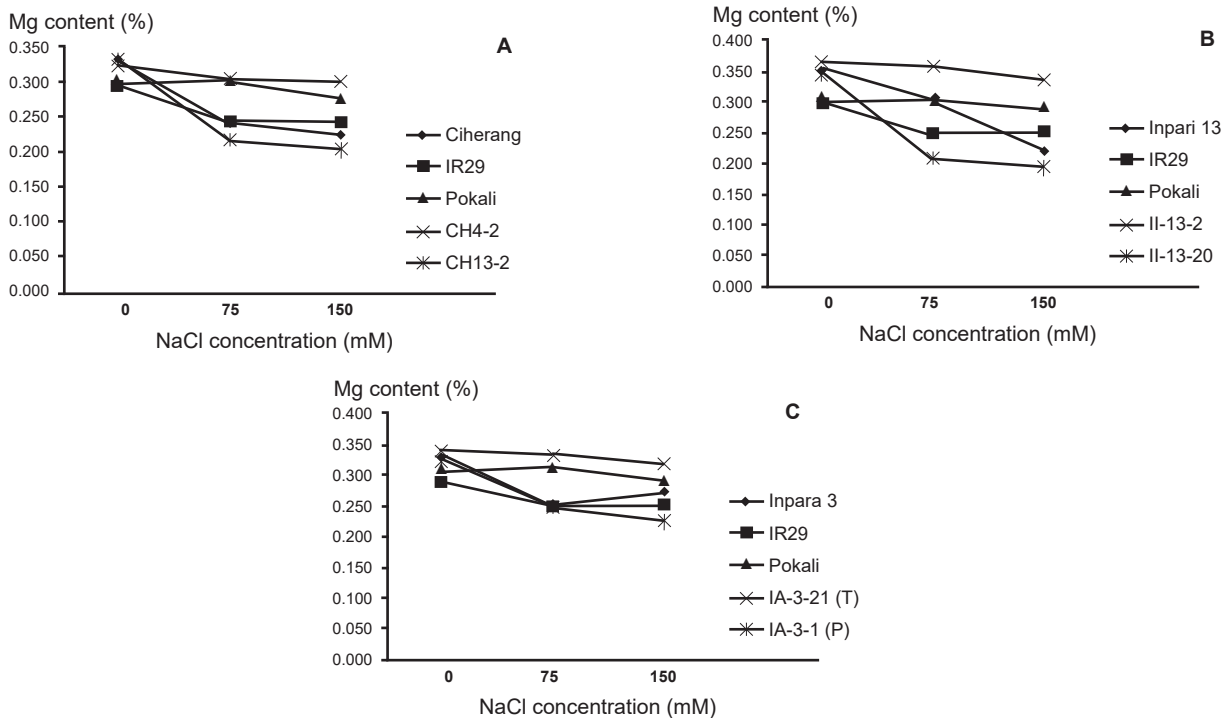
The mutants tolerant to NaCl (CH-30 and CH4-2) showed a higher ratio of stomata length and width



**Fig. 3.** The Calcium (Ca) content in the leaves in putative mutants and in their parent varieties, i.e. (A) Ciherang, (B) Inpari 13, and (C) Inpara 3. Under different NaCl concentrations in the media.

**Table 5. Stomata density and their length and width ratio of rice mutants and their parents (Ciherang, Inpari 13, and Inpara 3) grown in the salt media.**

Genotype/putative mutants	Stomata density (per mm <sup>2</sup> )			Stomata length and width ratio		
	0 mM NaCl	75 mM NaCl	150 mM NaCl	0 mM NaCl	75 mM NaCl	150 mM NaCl
Ciherang	6.385	6.375	6.305	1.160	1.150	1.160
CH-13-2 (S)	6.758	7.018	6.666	1.140	1.110	1.160
CH-30 (T)	5.544	5.403	5.122	1.440	1.480	1.520
CH-4-2 (T)	5.544	5.333	4.982	1.390	1.380	1.510
Inpari 13	4.712	4.703	4.703	1.513	1.590	1.550
II-13-17 (S)	4.772	4.631	4.691	1.770	1.760	1.800
II-13-42 (T)	4.070	4.070	4.089	2.250	2.360	2.390
II-13-7 (T)	4.211	4.289	4.219	2.450	2.660	2.570
II-13-10 (T)	3.789	3.509	3.649	2.440	2.710	2.710
II-13-13 (T)	4.000	3.649	3.719	2.210	2.430	2.350
II-13-2 (T)	4.000	3.789	3.859	1.770	1.760	1.800
Inpara 3	5.474	5.474	5.263	1.727	1.836	1.760
IA-3-1 (S)	6.035	5.684	5.404	1.837	1.886	1.765
IA-3-21 (T)	3.789	3.789	3.579	2.088	2.105	2.030

**Fig. 4.** Magnesium (Mg) content in the leaves of rice mutants and in their parent varieties, i.e. Ciherang (A), Inpari 13 (B), and Inpara 3 (C) as affected by NaCl concentrations in the media.

compared with that of their parent. The mutants of Inpari 13 (II-13-42, II-13-7, II-13-10, and II-II-13-2 13-13) and Inpara 3 (IA-3-21) also had a higher ratio of stomata length and width compared to those of the susceptible ones. This indicates that the tolerant plants had wider and flatter stomata so that it decreased transpiration process.

The decrease in stomata density and the increase in stomata length and width in the tolerant plant are

considered as a tolerance mechanism to protect cells from high transpiration. The dense stomata can prevent greater transpiration, so that excessive water loss can be suppressed.

Result of the study indicated that tolerant mutants generally had the structure and density of stomata lower than that of the susceptible mutants. This condition suggests that tolerant mutants are able reducing

excessive water loss from evaporation, hence to reduce the adverse effects of salinity stress. One of the effects of salinity stress is a water deficit due to potential water outside the root cells higher than that in the root cells because of the high soluble salts in the soil.

## CONCLUSION

A total of eight somaclonal mutants of rice derived from Ciherang, Inpari 13 and Inpara 3 varieties were tolerant to NaCl. These tolerant mutants accumulated more proline and contained lower Na and higher K ions in the leaves compared to those of the susceptible genotypes. These tolerant mutants had lower stomata densities, as well as greater stomata length and width ratio, indicating the presence of cells mechanism to high transpiration. Induction mutation combined with *in vitro* selection on NaCl media of the rice callus generated different levels of salt tolerant mutants. Further study is needed to evaluate the tolerant mutants under field conditions.

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