Salinity tolerance level of rice mutants (M₃) derived from Ciherang and Inpari 13 varieties at seedling phase

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Abstract. Salinity negatively affects rice production, therefore tolerant varieties are required to plant in an area with high salinity salinity. This especially will inhibit growth and trigger death at the seedling phase. The objective of this research is to obtain salinity tolerant rice mutants, from previously developed line by gamma ray irradiation and in-vitro selection, through a hydroponic testing in a greenhouse. Plant materials used were third generation (M₃) of rice mutants derived from Ciherang and Inpari 13 (each 45 lines), Ciherang and Inpari 13 as control parents, and Pokkali and IR20 as tolerant and sensitive salinity control, respectively. The experiment was conducted using methods developed by IRRI. This study was arranged in a completely randomized factorial design. The first factor was two concentration of NaCl (0 and 150 mM) and the second factor was the plant Rice varieties. The result of the study revealed that tolerant lines had higher plant height and root length than susceptible lines. This research yielded 64 rice mutants lines tolerant to salinity, i.e. 23 mutant lines of Ciherang and 45 mutant lines of Inpari 13.

1. Introduction
High salinity provides osmotic stress at the stage of plant growth. At a later stage, the salinity stress will effect to ionic stress [1]. The osmotic effect, i.e. decreasing potential osmotic pressure of the soil solution, therefore reduce water availability thus reduces the availability of water [2]. High concentrations of salt in the soil interfere with the capacity of roots to absorb water, causes partial dehydration of cells and loss of cell turgor [3]. A further effect is increasing ion concentration which causes growth inhibition. In rice, salinity can be characterized by stunted growth, reduced seedling, and reduce grain production by 50%.[4] [5]. One approach to the salinity problem is using tolerant varieties with high potential yield.

The utilization of saline soil for rice farming requires varieties that are tolerant of stress conditions. Local varieties tolerant are generally had low productivity, so it is important to generate of new Important to generate with high productivity. In vitro selection techniques combined with induction mutation is one of the alternative methods for obtaining new characters, which are not available in existing germplasm sources [6]. Which could increase plant tolerance to salinity. The method has been applied to several rice varieties [7] [8].

In the previous research, saline tolerant mutants using combining induction mutations and in vitro selection. Ciherang and Inpari 13 calluses were irradiated with gamma rays at doses of LD50 (22.468 Gy for Ciherang and 23.124 Gy for Inpari 13) [9] then selected on a medium containing NaCl in LC50 concentration (85.79 mM NaCl For Ciherang and Inpari 13 is 90.91 mM,) [10]. The study
yielded 45 line of putative tolerant mutant. The objective of this research is to screen 45 obtained mutant lines for salt tolerance using hydrophonic methods.

2. Materials and Methods
This study was conducted from March to December 2016 in the greenhouse Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIOGRAD). Plant material used was Ciherang variety and 45 mutants of third generation (M₃) from Ciherang, Inpari 13, and 45 mutants from Inpari 13 varieties.

Salinity screening use greenhouse was done by seeds are added to the sand media until the age of 1 week. Seeds that have been one week old grown in Yoshida solution. After four days, NaCl added in concentrations of 0 and 150 mM. The scoring system refers to the assessment standards made by Gregorio et al. 1997. Each treatment consisted of 20 replications. Observations were made on the 14th day, after the seeds were treated with saline conditions. Observation of response to salinity stress of tested lines each based on Standard Evaluation Score (SES) by IRRI (Table 1) [11].

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

3. Result and Discussion
Scoring for the salinity tolerance of rice seedling was done after symptom visible at 4 days after treatment to seedlings due to treatment of NaCl start to look after 4 days. Results showed that Ciherang mutants lines had different response to NaCl stress challenge: 12 mutan lines had very tolerant response, compare to the parent which had very sensitive to the stress condition. 2 putative mutants having highly tolerant properties, 21 putative mutants tolerant, 12 putative moderate mutants and 10 putative somaclonal mutants (Table 2). This shows that the third generation derivative still occurs segregation so that its character still varies.

| Table 2. Results of scoring some rice varieties and numbers of putative mutants. |
|-------------------------------|-------------------------------|-----------------|-----------------|
| Code                         | NaCl concentration (mM)       | Code            | NaCl concentration (mM) |
|                              | 0    | 150 | 0   | 150               |
| Ciherang                     | 1    | 1   | 9   | 9                 |
| IR29                         | 1    | 9   | 1   | 9                 |
| Pokkali                      | 1    | 1   | 1   | 1                 |
| CH-28-1                      | 1    | 3   | 1   | 1                 |
| CH-28-3                      | 1    | 3   | 1   | 1                 |
| CH-28-7                      | 1    | 3   | 1   | 1                 |
| CH-28-8                      | 1    | 3   | 1   | 1                 |
| CH-28-9                      | 1    | 3   | 1   | 1                 |
| CH-28-17                     | 1    | 3   | 1   | 1                 |
| CH-28-18                     | 1    | 3   | 1   | 1                 |

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| Mutant Line   | Tolerance | Parent Inpari 13 Tolerance |
|--------------|-----------|---------------------------|
| CH-28-19     | 1         | 3                         |
| CH-28-21     | 1         | 3                         |
| CH-29-2      | 1         | 3                         |
| CH-29-3      | 1         | 3                         |
| CH-29-5      | 1         | 3                         |
| CH-29-6      | 1         | 1                         |
| CH-29-7      | 1         | 3                         |
| CH-29-10     | 1         | 3                         |
| CH-29-16     | 1         | 1                         |
| CH-29-18     | 1         | 3                         |
| CH-29-23     | 1         | 3                         |
| CH-29-25     | 1         | 3                         |
| CH-9-19      | 1         | 3                         |
| CH-9-21      | 1         | 3                         |
| CH-9-25      | 1         | 3                         |
| CH-13-1-1    | 1         | 3                         |
| CH-13-1-10   | 1         | 5                         |
| CH-13-1-25   | 1         | 7                         |
| CH-12-46     | 1         | 5                         |
| CH-12-48     | 1         | 5                         |
| CH-31-3      | 1         | 7                         |
| CH-31-12     | 1         | 5                         |
| CH-31-13     | 1         | 5                         |
| CH-22-16     | 1         | 5                         |
| CH-22-17     | 1         | 7                         |
| CH-22-18     | 1         | 5                         |
| CH-22-20     | 1         | 5                         |
| CH-22-22     | 1         | 7                         |
| CH-22-31     | 1         | 5                         |
| CH-22-32     | 1         | 5                         |
| CH-22-33     | 1         | 7                         |
| CH-25-34     | 1         | 7                         |
| CH-25-36     | 1         | 5                         |
| CH-25-37     | 1         | 7                         |
| CH-25-38     | 1         | 5                         |
| CH-38-2      | 1         | 7                         |
| CH-38-3      | 1         | 7                         |
| CH-38-38     | 1         | 7                         |

1 = very tolerant, 3 = tolerant, 5 = moderate, 7 = sensitive, and 9 = very sensitive (Gregorio et al. 1997).

Mutants lines derived from Inpari 13 shows that 11 lines were tolerant, 34 lines were very tolerant while parent Inpari 13 was highly susceptible after stress challenge.
Figure 1. The crop performances of salinity selection in greenhouses.

Table 3. Observation of agronomic characteristics of some rice varieties and putative mutant selection in greenhouses

| Code  | Agronomic characteristics | Code  | Agronomic characteristics |
|-------|---------------------------|-------|---------------------------|
|       | PH (cm) | RL (cm) | NT | PH (cm) | RL (cm) | NT |
| CIHANG | 24      | 14      | 1  | Inpari 13 | 23  | 15  | 1  |
| CH-28-1 | 30      | 17      | 1  | II-13-43-1 | 27  | 20  | 1  |
| CH-28-3 | 27      | 18      | 1  | II-13-43-6 | 28  | 19  | 1  |
| CH-28-7 | 29      | 17      | 1  | II-13-43-11 | 28 | 19  | 1  |
| CH-28-8 | 28      | 18      | 1  | II-13-43-16 | 29  | 18  | 1  |
| CH-28-9 | 30      | 19      | 1  | II-13-43-17 | 27  | 12  | 1  |
| CH-28-17 | 29     | 21      | 1  | II-13-43-19 | 28  | 19  | 1  |
| CH-28-18 | 30     | 21      | 1  | II-13-43-22 | 27  | 21  | 1  |
| CH-28-19 | 29     | 18      | 1  | II-13-43-23 | 28  | 21  | 1  |
| CH-28-21 | 30     | 17      | 1  | II-13-43-25 | 30  | 23  | 1  |
| CH-29-2 | 28      | 20      | 1  | II-13-6-3  | 30  | 16  | 1  |
| CH-29-3 | 27      | 20      | 1  | II-13-6-6  | 32  | 17  | 1  |
| CH-29-5 | 27      | 21      | 1  | II-13-6-11 | 32  | 18  | 1  |
| CH-29-6 | 27      | 19      | 1  | II-13-6-17 | 33  | 19  | 1  |
| CH-29-7 | 28      | 19      | 1  | II-13-6-19 | 32  | 19  | 1  |
| CH-29-10 | 29     | 19      | 1  | II-13-6-22 | 33  | 18  | 1  |
| CH-29-16 | 29     | 19      | 1  | II-13-6-20 | 34  | 17  | 1  |
| CH-29-18 | 29     | 17      | 1  | II-13-6-23 | 31  | 17  | 1  |
| CH-29-23 | 29     | 16      | 1  | II-13-6-24 | 29  | 18  | 1  |
Report on gamma ray mutations. Important to generate can lead to changes in plant genetic properties towards better traits. It is suggested that the variation in tolerance score for tested mutant lines (Table 3) has resulted from their genetic alteration. Induced mutations combined with in vitro selection may alter plant tolerance characteristics in saline conditions as in the Bastami variety mutans lines (Table 3) has resulted from their genetic alteration. Induced mutations combined with in vitro selection may alter plant tolerance characteristics in saline conditions as in the Bastami variety mutans [12], ST-87 and ST-301 strains [13].

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| CH-29-25 | 24 | 15 | 1 | II-13-21-10 | 29 | 19 | 1 |
| CH-9-19 | 29 | 15 | 1 | II-13-21-11 | 32 | 17 | 1 |
| CH-9-21 | 30 | 16 | 1 | II-13-21-17 | 32 | 17 | 1 |
| CH-9-25 | 30 | 15 | 1 | II-13-21-18 | 31 | 18 | 1 |
| CH-13-1-1 | 24 | 16 | 1 | II-13-21-20 | 28 | 19 | 1 |
| CH-13-1-10 | 25 | 17 | 1 | II-13-21-23 | 29 | 17 | 1 |
| CH-13-1-25 | 24 | 16 | 1 | II-13-21-24 | 29 | 21 | 1 |
| CH-12-46 | 23 | 17 | 1 | II-13-21-25 | 32 | 22 | 1 |
| CH-12-48 | 24 | 14 | 1 | II-13-2-6 | 31 | 17 | 1 |
| CH-31-3 | 24 | 14 | 1 | II-13-2-7 | 29 | 19 | 1 |
| CH-31-12 | 25 | 14 | 1 | II-13-2-9 | 29 | 18 | 1 |
| CH-31-13 | 23 | 15 | 1 | II-13-2-14 | 29 | 19 | 1 |
| CH-22-16 | 21 | 16 | 1 | II-13-2-21 | 29 | 21 | 1 |
| CH-22-17 | 22 | 16 | 1 | II-13-2-24 | 28 | 20 | 1 |
| CH-22-18 | 23 | 17 | 1 | II-13-43-2 | 28 | 20 | 1 |
| CH-22-20 | 24 | 14 | 1 | II-13-43-6 | 28 | 18 | 1 |
| CH-22-22 | 25 | 14 | 1 | II-13-43-9 | 27 | 18 | 1 |
| CH-22-31 | 23 | 16 | 1 | II-13-43-10 | 26 | 19 | 1 |
| CH-22-32 | 23 | 15 | 1 | II-13-43-15 | 27 | 19 | 1 |
| CH-22-33 | 24 | 15 | 1 | II-13-43-17 | 28 | 18 | 1 |
| CH-25-34 | 23 | 17 | 1 | II-13-43-19 | 27 | 19 | 1 |
| CH-25-36 | 23 | 17 | 1 | II-13-4-3 | 29 | 18 | 1 |
| CH-25-37 | 24 | 16 | 1 | II-13-4-4 | 28 | 19 | 1 |
| CH-25-38 | 23 | 13 | 1 | II-13-4-6 | 29 | 17 | 1 |
| CH-38-2 | 25 | 14 | 1 | II-13-4-7 | 29 | 18 | 1 |
| CH-38-3 | 26 | 13 | 1 | II-13-4-11 | 28 | 19 | 1 |
| CH-38-38 | 26 | 13 | 1 | II-13-4-12 | 29 | 17 | 1 |

PH = plant height, RL = root length, NT = number of tillers
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