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The fourth industrial revolution era – which is denoted by automation in all fields including industry, financial, medical and education – is predicted to bring opportunities as well as disruption of relationships between human (society) and technology. This 4.0 Industrial Revolution will create new realms that humankind has never experience before. The readiness of human resources, technology and infrastructures are required to face the impacts of this revolution, as it will fundamentally change the way human lives, works and socializes. As such, it is important to ensure that the revolutionized technology will bring positive impacts to the society. This should be brought about by creating an integrated and comprehensive approach that involves stakeholders from all sections of society, including practitioners from public and private sectors, academia and researchers. Indonesia is a developing country which undergoes progressive changes towards a rapidly industrializing society. The country is characterized by its abundant natural resources and a demography dominated by parts of society at a productive age. It is a country on the verge of becoming a developed one. As with other developing countries, the shift towards the 4.0 industrial revolution demands a careful and measured management of existing opportunities and challenges. This requires human resources that are empowered with pertinent skills. Biotechnology, which moves around the interface between technology and biological sphere, holds a key role in assisting the country in navigating its course through the new industrial revolution. It utilizes biological resources for the welfare of human society and assists the progression of a nation towards prosperity. The advancement of biotechnology, supported by skilled human resources in the sector, is essential to ensure that Indonesia is well prepared in facing both the challenges and opportunities brought about by the 4.0 industrial revolution. By adopting a theme on "The Role of Biotechnology in the Era of 4.0 Industrial Revolution", The 2019 International Conference on Biotechnology and Life Sciences (IC-BIOLIS) aims to create a platform for relevant experts and stakeholders in the field of biology and biotechnology to discuss and share experience relating to the management and appropriate utilization of biological resources, as well as the recent advancement of biotechnology.

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Conference Paper

Salt Tolerance of Barak Cenana Rice (*Oryza sativa L cv. Barak Cenana*) EMS Derived M1 Putative Mutants

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Abstract

Barak Cenana (*Oryza sativa* L cv. Barak Cenana) is a native red rice cultivar of Tabanan regency, Bali. We have previously created first generation putative mutants (M1) lines of Barak Cenana using EMS. In this study, we aim to evaluate M1 lines tolerance to salt stress. Three-leaf seedlings of WT Barak Cenana and M1 lines were cultured in hydroponic system containing Yoshida solution and 100 mM NaCl for 7 days. The salt tolerance level of each seedling was evaluated using SES standard and the survived seedlings were transferred into the pot-containing soil growth for maturation. All WT were died when treated with 100 mM NaCl for 7 days. In contrast, some M1 lines were survived where 0.5% were highly tolerance (II2A-4) and 4.06% (1F-4, 1F-3, II2A-8, IID-1, IID-4, IID-6 and 1B-6) were tolerance to salt stress. This variation of salt tolerance level among M1 lines is likely due to the random mutation caused by EMS. Furthermore, all survived mutants were fertile and able to produce mature seeds. As characters in M1 generation are not stable, future studies are required to establish stable mutant lines.

Keywords: Rice, EMS, Mutants, Barak Cenana, salt tolerance

1. Introduction

Most people in Indonesia consume rice as their main carbohydrate source. In 2015, the average rice consumption in Indonesia reached approximately 22.2 million ton per year [2]. This number may increase in the future as the population of Indonesia is continuously growing. In 2045, the population of Indonesia has been predicted to reach 311 million people [1]. This suggests that the demand of rice as staple food would also increase as a result of the population growth.

Rice production is affected by several factors including the environmental stress. It is known that rice is sensitive to salt stress, especially during seedling stages [3, 4]. Salt stress is mainly caused by high concentration of sodium chloride (NaCl) that reduces the ability of rice to absorb water from the soil and induces chlorophylls degradation [5]. Both water and chlorophylls are key factors for plant to produce carbohydrate during photosynthesis. A study has demonstrated that rice grown under salt stress has a lower photosynthesis capacity compared to the one in normal condition [6]. This may lead to low productivity due to lack of carbohydrate as source of energy or building blocks for synthesis of other organic molecules [6].

Improving rice productivity in marginal land, such as salt affected land, can be used as a solution to anticipate increasing rice demand in Indonesia. Genetic alteration using chemical and physical mutagens have been applied to improve rice tolerance to salt stress. Song et al. [7] utilized gamma irradiation to improve salt tolerance of Donganbyeo rice up to 171 mM NaCl. Another study conducted by Baloch et al. [8] showed an increased productivity of 40% of Shua-92, a rice mutant generated by gamma irradiation, compared to the traditional salt tolerance cultivar when grown under salt stress. Furthermore, KDML 105, a rice mutant created using a combination of gamma irradiation and ethyl methylsulfonate (EMS), exhibits salt tolerance of 150 mM NaCl [9].

Barak Cenana (*Oryza sativa* L cv. Barak Cenana) is a native rice cultivar of Tabanan regency, Bali. This cultivar produces red rice rich in nutritional contents [10]. In this research, we aim to evaluate Barak Cenana EMS-derived first generation putative mutants (M1) tolerance to salt stress.

2. Methods

2.1. Plants

All plant materials were obtained from Laboratory of Plant Biotechnology, Faculty of Biotechnology, University of Surabaya. Seeds of twelve M1 lines (i.e., 2F, II2A, 2C, 3E, II3A, II2F, 1F, 1B, IID, 3G, II3E and 2K) as well as wild type (WT) were germinated on the rockwool, irrigated with Yoshida solution [11] until three leaf-stage of seedling. Each seedling was code as X-Y with X indicate M1 line and -Y indicated number of the seed. For example seedling from 2F will code as 2F-1, 2F-2,..........2F-Y.

2.2. Determination of NaCl LD_{100} in WT

Forty-five three-leaf seedlings of WT were randomly assigned into five groups (N=9 each) and cultivated in hydroponic system recommended by International Rice Research Institute (IRRI) [12] containing Yoshida solution with 0, 60, 70, 80, 90, 100 mM NaCl for 7 days. The morphology and survival rate of each group were observed in the end of treatment. LD_{100} in WT will be used for the salt tolerance selection of M1 lines.

2.3. Salt tolerance selection of M1 lines

Three-leaf stage of M1 putative mutant lines were transferred to hydroponic system containing modified Yoshida solution with 100 mM NaCl for 7 days. The salt tolerance level of survived M1 lines was evaluated using modified standard evaluation system (SES) from IRRI (Table 1).

Table 1: *Modified Standard Evaluation System for Rice* (SES) [12].

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

The percentage of tolerance level was calculated using the following equation:

$$\text{Percentage of tolerant putative mutants} = \frac{\text{number of tolerant putative mutants}}{\text{total putative mutants}}$$

2.4. Recovery of tolerant M1 lines

All tolerant plants were recovered by irrigating with Yoshida solution for three weeks, followed by transfer to the pot-containing soil. All plants were cultivated until mature.

3. Result

3.1. Determination of NaCl LD_{100} for salt tolerance selection.

WT Barak Cenana was tolerant to salt stress up to 60 mM NaCl for 7 days (Table 2). Stress symptoms started to appear on seedlings treated with 70 mM NaCl and worsen at 80 mM NaCl. Seedlings were completely died when treated with 100 mM NaCl for 7 days, indicated by dryness of all leaves. Thus, 100 mM NaCl were chosen to evaluate salt tolerance of M1 lines.

Table 2: The effect of different concentration of NaCl on survival and morphology of WT Barak Cenana seedling.

NaCl (Mm)	Survival (%)	Morphology
60	100 (9/9)	All leaves were green and new leaf were emerged.
70	100 (9/9)	The tip of old leaf was withies and new leaf were emerged.
80	100 (9/9)	Plant growth was inhibited, old leaf completely dried
90	11,11(1/9)	Most of the plants were dead or dying.
100	0 (0/9)	All plants were dead

3.2. Salt tolerance selection of M1 lines

The majority of M1 lines were susceptible to salt stress produced by 100 mM NaCl for 7 days (Fig. 1). Only 0.58% of putative mutant lines were highly tolerant and 4.069 % were tolerant to the exposure of 100 mM NaCl. Putative mutant line II2A-4 showed highly tolerance to 100 mM NaCl, while putative mutant line 1F-4, 1F-3, II2A-8, IID-1, IID-4, IID-6 and 1B-6 were tolerance to the exposure of 100 mM NaCl for 7 days (Table 3).

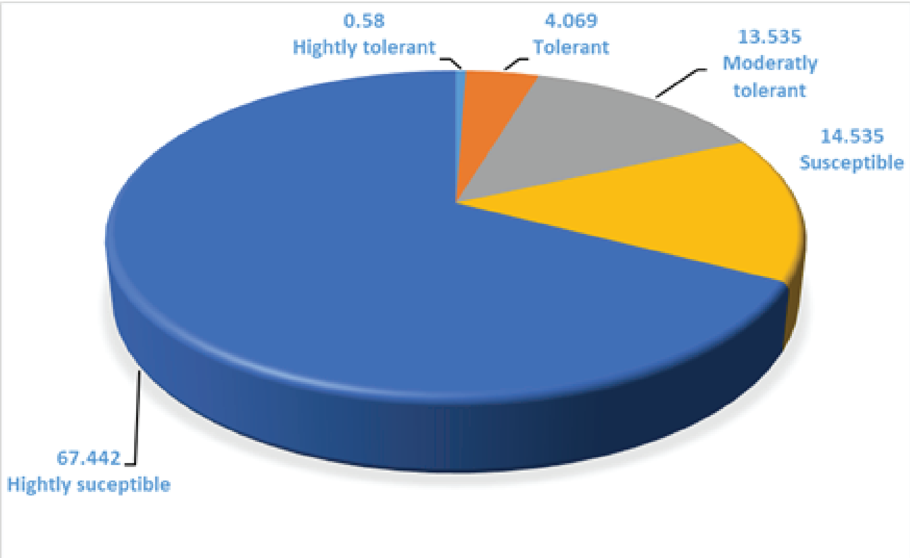

Figure 1: The percentage of tolerance level among M1 lines treated with 100 mM NaCl for 7 days.

Table 3: The number of putative mutant line with highly or tolerance to salt stress produced by exposure of 100 mM NaCl for 7 days.

Tolerance level	Number of plants	Putative mutant line
Highly tolerant	1	II2A-4
Tolerant	7	1F-4, 1F-3, II2A-8, IID-1, IID-4, IID-6 and 1B-6

3.3. Recovery of tolerant M1 lines

Highly tolerant and all tolerant M1 lines were able to recover and produced mature seed (Fig 2). In addition, those putative mutant lines were resembling the morphology of WT Barak Cenana which has tall shoot (>140 cm), droopy leaves and spikelet with horn.

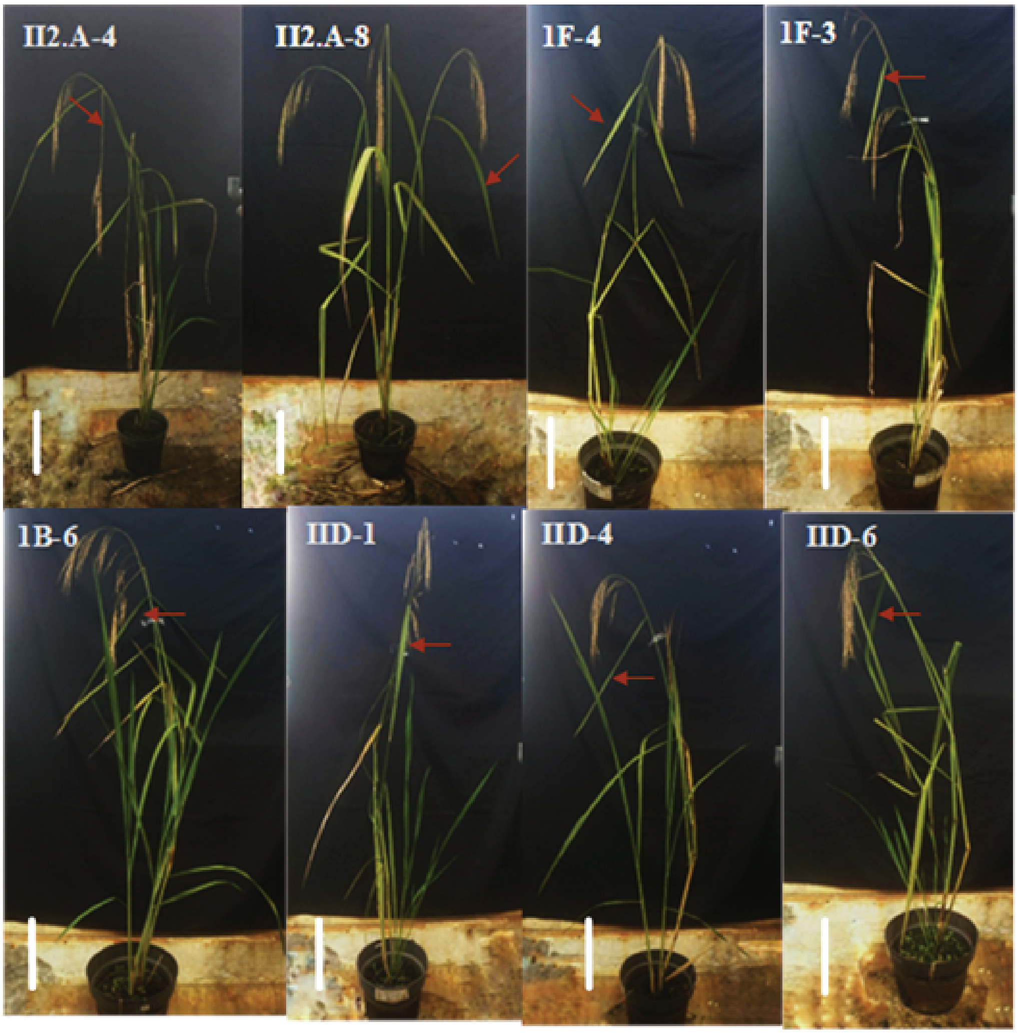

Figure 2: The morphology of salt tolerant M1 lines at mature stage. Red arrow: droopy leaf; white bar = 14 cm.

4. Discussion

Salt tolerance test during seedling stage is widely used as an early screening for salt tolerance in rice [12]. Our previous study using *in vitro* culture has shown that Barak Cenana (WT) is able to survive when exposed to 150 mM NaCl for two weeks [13]. However, this present study demonstrated that Barak Cenana was unable to survive when exposed to 100 mM NaCl for 7 days. This may due to the higher evaporation

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rate in *ex vitro* condition compared to the *in vitro*, where plants are exposed to a higher temperature and a lower humidity in *ex vitro* environment [14]. Water loss during evapotranspiration enhances osmotic stress produced by salt stress causing *ex vitro* plant more susceptible to salt stress compared to *in vitro* plant.

Our present study showed diversity in salt tolerance between M1 lines and between individual seedlings within M1 lines. Lee et al. has reported a similar result where gamma irradiation was used to generate rice mutants [15]. Rice response to salt stress is orchestrated by many genes [4, 16]. Mutagens such as EMS or gamma irradiation induce random mutation in rice genome [16, 17]. Furthermore, independent assortment of allele from different gene during gametogenesis in M0 (zero generation) and random meeting between sperm and ovule during fertilization in the flower of M0 generation [16] also increase the probability of gene combination. Altogether, these may lead to the observed diversity of salt tolerance in M1 lines.

In order to produce mutant line with stable characters, multiple sequential selection should be done until M4 generation [17]. For those reason, putative mutant lines should be able to produce seed. All highly tolerant and tolerant M1 lines in this study exhibited WT Barak Cenana morphology and were fertile. These lines produced second generation of putative mutant lines (M2) which would be used to evaluate the stability of salt tolerance in the future study.

5. Conclusion

Mutation breeding using EMS potentially used for production of mutant line that can be used to improve Barak Cenana's tolerance to salt stress. One M1 rice line that highly tolerant (II2A-4) and seven M1 rice line (1F-4, 1F-3, II2A-8, IID-1, IID-4, IID-6 and 1B-6) that tolerant to 100mM NaCl have been isolated.

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