Mutagenic Effects of Sodium Azide on The Germination in Rice (Oryza sativa L cv Inpago Unsoed 1)

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Submission date: 10-Sep-2018 10:59AM (UTC+0700)
Submission ID: 999339999
File name: 2759-9953-1-PB_2.pdf (178.4K)
Word count: 2979
Character count: 15467

MUTAGENIC EFFECTS OF SODIUM AZIDE ON THE GERMINATION IN RICE (*Oryza sativa* L. cv. INPAGO UNSOED 1)

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ABSTRACT

The available natural diversity highly limits the effort to improve the production of upland-rice with delicious and aromatic flavors. However, the mutagenesis can help improving the natural diversity. The aromatic-upland rice seeds, cv. Inpago Unsoed 1, were soaked in solution of NaN3 with the doses of 0 mM, 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, and 10 mM, in four replications in completely randomized design. The observation included LD50, germination percentage at first-count and final-count. Data were tabulated and analyzed with CurveExpert 1.4 software for LD50, and generalized linear model in the PROC-GLM procedure of SAS 9.1 software. The means were generated and compared through Dunnett option, at probability level of 5%, in order to determine the difference in means between mutated and non-mutated seeds. The results showed that the attributes of rice physiology affected by mutagenesis were sensitivity of rice to NaN3 with LD50 at 8.84 mM, and significantly effect of NaN3 on the decreasing capacity of seed germination at > 6 mM for first count, and > 7 mM for final count.

Keywords: germination, inpago unsoed 1, median lethal dose, rice, sodium azide.

INTRODUCTION

Rice is the staple food for more than 95% of Indonesian people, in which the number of people is predicted to reach 263 million in 2020, so the need for rice will increase to 35.97 million ton (Anindita *et al.*, 2016). Besides, current society prefers specific rice type for a number of reasons, like delicious and aromatic flavors (Calingacion *et al.*, 2014). In 2011, Inpago Unsoed 1 successfully met the need of people for rice with delicious and aromatic flavors, through the breeding of upland-rice which is adaptive in the dryland (Gusmiatun, 2015).

In Indonesia, the width of dry land reaches 22,307,120 ha, so it is very potential to increase the production of upland rice (Dariah *et al.*, 2012). Besides, the effort to increase rice production can be performed through crossing, and it is very potential to improve the nature of heredity (Luo, 2010). However, the available natural diversity often becomes limit in the crossing (Shu, 2009). One of ways to improve natural diversity is through mutagenesis (Rustikawati *et al.*, 2012). Mutagenesis with the treatment of sodium azide (NaN_3) has been reported to improve the resistance under drought stress from -0.0021 MPa to -0.0077 MPa (He *et al.*, 2009; Aurabi *et al.*, 2012).

The resistance traits under the drought stress are obtained after the specific selection for some generations (Mustikarini *et al.*, 2017), in which the selection can be performed since the germination phases (Harding *et al.*, 2012). The germination is a physiological process which is very complex with comprehensive genetic background and reflects the quality of seed related to the tolerance of plant under unfavorable-bad condition (Wang *et al.*, 2010). Therefore, this research aims to know the effect of sodium azide on the germination physiology of rice, cv. Inpago Unsoed 1. This study is expected to give initial information in the production-increasing program of upland rice with delicious and aromatic flavors.

MATERIALS AND METHODS

Plant material

The aromatic-upland rice seeds (*Oryza sativa* L. cv. Inpago Unsoed 1) were obtained from Laboratory of Biotechnology and Plant Breeding, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Indonesia. The seeds were submitted to treatment of NaN_3.

Mutagenesis

Seeds mutagenesis were performed in Laboratory of Physiology and Plant Breeding, Faculty of Animal and Agricultural Sciences, Diponegoro University. The healthy seeds were soaked in solution of NaN_3 with doses of

0 mM, 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, and 10 mM, for 6 hours, at a temperature of around 25 ^{0}C, in 0.1 M KH_2PO_4 (pH=3.5). After mutagenesis, the seeds were washed for 1.5 hours in the running water at a temperature of around 15 ^{0}C (Shin and Jeung, 2011).

Germination

The seeds from mutagenesis were germinated under the natural condition in soil in plastic seedling tray, in four replications in Completely Randomized Design (CRD). All trays were supplied with water every day in order to maintain soil moisture on the field capacity during experiment period.

Physiological attributes of rice as affected by mutagenesis

The seeds from mutagenesis were considered germinating when the length of radicle is > 2 mm. Median lethal dose (LD_{50}) was calculated based on the number of seeds surviving in different mutagen doses (Roslim *et al.*, 2015).

The germination percentage (GP) were calculated at 5th day (first count) and 14th day (final count) after germinated, following the formula of Marcu *et al.* (2013), in which NT = proportion of the germinating seeds in every treatment; N = number of seeds used in bioassay.

$$\text{GP (\%)} = \frac{\text{NT x 100}}{\text{N}}$$

Statistical analysis

Data were tabulated and analyzed with CurveExpert 1.4 software for LD_{50}, and generalized linear model in the PROC-GLM procedure of SAS 9.1 software. The means were generated and compared through Dunnett option, at probability level of 5%, in order to determine the difference in means between mutated and non-mutated seeds.

RESULTS AND DISCUSSION

NaN_3 is the chemical mutagen which is known to improve the genetic diversity, as its trait is very mutagenic (Dewi *et al.*, 2016). Effectiveness and efficiency of mutagenic in general increase with the increasing mutagen dose (Sharma *et al.*, 2010), in which the maximum amount of cell mutant is predicted in the range of LD_{50} (Gaswanto *et al.*, 2016).

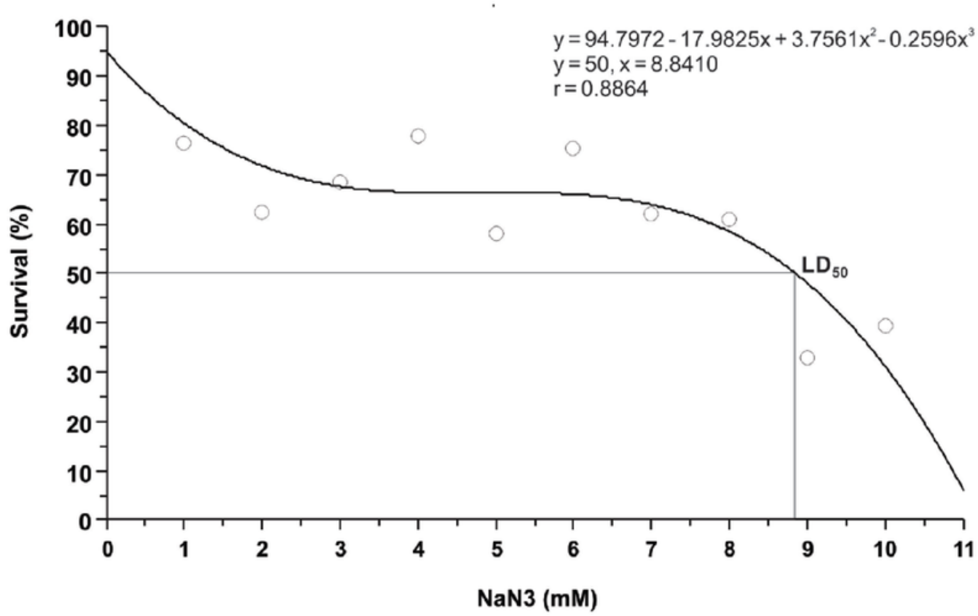

Figure 1. Dose – response curve with polynomial fit

Dose-response curve shows the sensitivity of rice cv. Inpago Unsoed 1 on NaN_3 with LD_{50} at 8.84 mM (Figure 1). Different from research of Omoregie *et al.* (2014), in which LD_{50} of rice cv. Faro 44, Faro 52, Faro 57, Nerica L-34 and Nerica L-47 are estimated at 4.92 mM. In other species, LD_{50} can be different, such as musk-okra (*Abelmoschus moschatus* L.) at 15.38 mM (Warghat *et al.*, 2011), onion (*Allium cepa* L.) at 76.90–139.95

mM (Joshi *et al.*, 2011), maize (*Zea mays* L.) at 40 mM (Gnanamurthy *et al.*, 2012), and garlic (*Allium sativum* L.) at 3.23-16.61 mM (Mahajan *et al.*, 2015).

The difference of LD_{50} among genotypes depends on its sensitivity on mutagen, related to production of free radicals (Roslim *et al.*, 2015), in which free radicals of oxygen from the accumulation of azide derivative of peroxidase is the secondary mutagen responsible for substituting DNA base (Gruszka *et al.*, 2012).

The substitution can be expressed as a variation of germination percentage (Table 1). According to Milosevic *et al.* (2010), the germination percentage is an indicator of seed quality, which can be used to predict the growth of plant when the condition in the field is nearly ideal.

Table 1. Effects of NaN_3 on germination percentages (GP) were taken at 5th day (first count) and 14th day (final count) of rice (*Oryza sativa* L. cv. Inpago Unsoed 1)

NaN_3 (mM)	First Count (%)	Final Count (%)
0	100.00	100.00
1	76.50	93.00
2	62.50*	80.50
3	68.50	76.50
4	78.00	94.50
5	58.00*	66.00
6	75.50	78.50
7	62.00*	71.50
8	61.00*	68.00*
9	33.00*	60.00*
10	39.50*	52.50*

Data were transformed by arcsine prior to analysis; nontransformed data are presented; * Significant difference ($p<0.05$)

Seed germination after mutagenesis (1-10 mM) states that maximum percentage of germination can be observed at control (0 mM). As shown in Table 1, the first-count decreases with the increasing mutagen dose, in which in the maximum decrease, 33.00 %, the observed germination percentage is at 9 mM. It shows that the increasing dose of mutagen reduces the germination percentage, but the decrease is not in line with the increasing dose of mutagen. Statistical analysis stated that dose higher than 6 mM significantly causes the decreasing capacity of seed germination at 5th day after germination.

The potency of seed germination expressed through final-count (Table 1), states the decreasing pattern similar with the dose increase, like first-count. Dose of 1-7 mM causes the decreasing percentage of germination insignificantly, but the inhibition is significant statistically on the potency of germination noted at higher dose, in which final-count decreases by 68.00 % for 8 mM, and 60% for 9-mM, compared with control. The highest inhibition from germination process, 52.50 %, is noted at 10 mM.

The decreasing percentage of germination as result of the increasing mutagen dose with nonlinear decrease was also reported by Sasikala and Kalaiyarasi (2010). However, different research was reported by Anbarasan *et al.* (2013), in which the germination percentage decreases along with the increase of mutagen dose. According to Shah *et al.* (2008), the decreasing percentage of germination as the effect of mutagen treatment, is caused by the increasing activity of free radicals encouraging the death of seeds.

The effect of mutagen causes quantitative and qualitative deviations. The qualitative deviation is indicated by the albino seedling at 5 mM (Figure 2). NaN_3 is actually reported effective to make rice seedling albino (Ando and Montalvan, 2001). The albino condition is caused by the mistakes in the chloroplast genome replication, so it makes chlorophyll abnormal (Lin *et al.*, 2008).

Albino is the chlorophyll deficiency seen as a white colour and indicates that there is no pigment, so plant will die 7-14 days after germination (Warghat *et al.*, 2011). However, albino rice seedling in this study can live more than 14 days after germination. According to Herwibawa *et al.* (2014), the chlorophyll deficiency which does not cause the death more than 14 days after germination is not a perfect albino. However, many seedlings from seeds treated at high dose (≥ 8 mM) can survive, so it is very possible for further investigation.

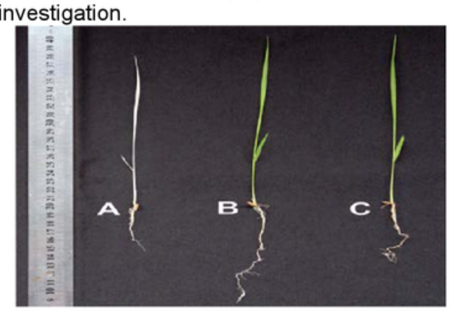

Figure 2. Rice seedlings at 14th day after germination. A = white rice seedling (albino) (NaN_3 5 mM); B = green rice seedling (after mutagenesis) (NaN_3 5 mM); C = green rice seedling (control) (NaN_3 0 mM)

CONCLUSIONS

The physiological attributes in rice cv. Inpago Unsoed 1 affected by mutagenesis, shows the sensitivity on NaN_3 with LD_{50} at 8.84 mM. NaN_3 significantly affects the decreasing capacity of seed germination at > 6 mM for first-count, and > 7 mM for final-count. The surviving seedlings are very possible for further investigation.

ACKNOWLEDGEMENTS

Authors thank Professor Totok Agung Dwi Haryanto, Ph.D. (the breeder of rice cv. Inpago Unsoed 1) for providing seeds. Support and laboratory facility provided by Dr. Florentina Kusmiyati, Head of Laboratory of Physiology and Plant Breeding, Diponegoro University.

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