Research Article

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Agronomic performances of aromatic and non-aromatic M₁ rice under drought stress

<https://doi.org/10.1515/opag-2019-0055>

received February 19, 2019; accepted June 24, 2019

Abstract: Flooded rice paddy fields act as a source of greenhouse gas emissions. So, the efforts to increase the drought tolerance of rice represent a much more environmentally friendly solution, and may bring a significant contribution to prevent global warming. The experiment was laid out in a split-plot design with ten replicates. Rice cultivars treated with different levels of γ irradiation and sodium azide (SA) soaking time were allocated in the main plots, and mutagen groups in sub-plots. We use the generalized linear models, as implemented in the GLIMMIX procedure of SAS University Edition, to analyze agronomic performances. These results showed that the genotypes in M₁ generation present diversity under the drought stress level of -0.03 MPa, and the best combination between cultivar and mutagen is Inpago Unsoed 1 that was irradiated with γ 100 Gy and then soaked in SA for 2h. These genotypes can be used as a sources of drought tolerance in future rice breeding programmes.

Keywords: gamma-ray, induction mutation, *Oryza sativa*, rice yield, sodium-azide

1 Introduction

The flooded rice fields have been known as important sources of anthropogenic methane (CH₄) emission, greenhouse gas (GHG) (Chun et al. 2016). CH₄ emission that come from flooded rice fields is estimated to reach 60 Tg yr⁻¹ and contribute to around 11% of total CH₄ emission

globally (Chowdhury and Dick 2013). It is known that the increase of CH₄ emission happens along with the expanding production area and intensification of rice cultivation, especially the flooded rice fields (Burney et al. 2010; Sasai et al. 2017). Therefore, the effort to reduce CH₄ emission on flooded rice fields can bring significant contribution to control the global warming (Feng et al. 2012). A 39 to 52% significant decrease in production of total CH₄ emission due to drainage of fields was proven (Zhang et al. 2011). The development of drought-resistance rice cultivar is an environmentally friendly solution (Pray et al. 2011), and it needs support with the improvement of drought-resistance rice characters (Luo 2010).

The crossing of drought-resistance rice is a conventional way to improve the drought-resistance characters (Dixit et al. 2014). However, high-yielding rice breeding program of the 1960s and 1970s reduced crop genetic diversity to a narrowing of the genetic base of the crops (van de Wouw et al. 2010). Narrow genetic diversity is a main problem restricting the progress of breeding (Kusmiyati et al. 2018). In the last decade, several methods of induction mutation have been developed and widely used, and they resulted in optimum of genetic diversity and minimum of viability decrease. Moreover, the rigorous selection method has been helpful for the breeders to choose the characters that are previously difficult to breed (Sikora et al. 2011). The induction mutation with the treatment of gamma irradiation, sodium azide, and their combination were reported to improve the rice yield by 7-40% (Siddiqui and Singh, 2010; Shehzad et al. 2011), and to improve the resistance under drought stress from -0.0021 to -0.0077 MPa (He et al. 2009; Aurabi et al. 2012).

Rice with drought-resistance and high-yielding characters are definitely achieved through the rigorous selection methods (Mohapatra et al. 2014). The agronomic performances as the criterion of evaluation on drought-resistance character have been used to show the visual indications on the water limitation, and it is the strategy to obtain the rice with characters that provide higher yield with little water or by minimizing the loss of production with the limited water (Yao et al. 2012). The drought-resistance character can be measured through the agronomic

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performances including survival percentage, plants height, root length, leaf area, culm diameter, number of tillers, number of productive tillers, number of seeds per panicle, filled seed percentage, 1,000 seeds mass, and harvest index. Previous peroxidase isozyme study of rice in M_1 generation have been conducted under -0.03 MPa and -0.41 MPa drought stresses (Herwibawa *et al.* 2014a; Herwibawa *et al.* 2018). Here we performed agronomic analysis to find the best combination between cultivar and mutagen in M_1 generation under drought stress level of -0.03 MPa. This study is expected to provide useful information in the improvement of drought-resistance characters on rice.

2 Materials and methods

Four rice cultivars used in this study were obtained from Totok Agung Dwi Haryanto, (the breeder of Inpago Unsoed 1), and Indonesian Center for Rice Research, Sukamandi, Indonesia. These cultivars consist of Inpago Unsoed 1 (aromatic upland cultivar), Rojolele (aromatic lowland cultivar), Inpari 13 (non-aromatic lowland cultivar), and Cirata (non-aromatic upland cultivar). The seeds of each cultivar were categorized into nine groups. The first group did not get any treatment and was used as control, while other eight groups were irradiated with gamma (γ) at 100 and 150 gray (Gy) and soaked in sodium azide (SA) 10^{-3} M (Herwibawa *et al.* 2014b). Second group was irradiated with γ 100 Gy, third group was irradiated with γ 150 Gy, fourth group was soaked in SA for 2 h, fifth group was soaked in SA for 6 h, sixth group was irradiated with γ 100 Gy and then soaked in SA for 2 h (100 Gy + SA 2 h), seventh group was irradiated with γ 100 Gy and then soaked in SA for 6 h (100 Gy + SA 6 h), eighth group was irradiated with γ 150 Gy and then soaked in SA for 2 h (150 Gy + SA 2 h), and ninth group was irradiated with γ 150 Gy and then soaked in SA for 6 h (150 Gy + SA 6 h).

The study was arranged in split-plot design with ten replicates and ten plants for each replicate. There were four rice cultivars as the main plot and nine mutagen groups as the sub-plot, so there were 36 treatment combinations. The location was Screen House of Seed Technology Laboratory, Vocational Education Development Center of Agriculture, Cianjur, Indonesia. The two-week-old rice seedlings were grown hydroponically (Wang *et al.* 2013), and the source of nutrient was from Hoagland solution (Hoagland and Arnon 1950). The solution was replaced once a week and the water level was adjusted on the alternate days (Sandhu *et al.* 2012). Besides, pH

of solution was also monitored every two days. After adapting for three weeks, the Hoagland solution with 5 % (w/v) of polyethylene glycol 6000 was used to achieve the drought stress (osmotic) at the level of -0.03 MPa (Mexal *et al.* 1975). The drought stress was given for six weeks, and the rice was then maintained in optimum condition for 11 weeks. The collected data were based on the agronomic performances including survival percentage (number of the surviving plants / number of plants x 100 %), vegetative performances, and reproductive performances.

The vegetative performances included the plant height (cm) that was measured from the clump base to the highest leaf tip when it was stretched to the top, root length (cm) that was measured from the clump base to the longest root tip, leaf area (cm^2) that was measured with gravimetric method (mass of leaf replica paper / mass of standard paper x standard paper area), culm diameter (mm) that was measured on its bottom, and number of tillers that was counted directly and visually. The reproductive performances included number of productive tillers that were calculated with the number of tillers that produced panicle and seed, number of grains per panicle that was measured in accordance with the number of seeds on every panicle, filled seeds percentage (number of filled seeds / number of seeds x 100 %), 1,000 seeds mass (mass of filled seeds / number of filled seeds x 1,000), and harvest index (total filled seeds dry mass / total plants dry mass). The data were tabulated and analyzed with the generalized linear models in PROC GLIMMIX procedure of SAS University Edition software. Least square (LS) means for cultivar and mutagen were generated and compared through ADJUST=SIMULATE LINES option of LSMEANS statements, and the significance level for tests of significance among means was set at the 0.05 probability level.

3 Results and discussion

Rojolele (aromatic lowland cultivar) without treatment (control) was unable to survive under the drought stress at level of -0.03 MPa, while the control on Inpago Unsoed 1 (aromatic upland cultivar), Inpari 13 (non-aromatic lowland cultivar), and Cirata (non-aromatic upland cultivar) showed the survival percentages of 10%, 30%, and 40% respectively (Figure 1). This results with the control shows that the aromatic cultivars have the survival percentage that is lower than that of non-aromatic cultivars. According to Joshi *et al.* (2011), the aromatic cultivars are more sensitive to the drought stress than the non-aromatic cultivars, as total proline content of aromatic cultivar is

always lower on several levels of drought stress. Basu et al. (2010), also explained that the increase on peroxide (H_2O_2) levels, malondialdehyde (MDA) and lipoxygenase (LOX) activities, and the forming of carbonylated protein derivatives are higher in aromatic cultivars. Besides, in this research on the aromatic and non-aromatic groups, upland cultivars had survival percentage that were higher than that of lowland cultivars. The response of upland cultivar on the early drought stress was known to be more efficient related to the upregulation on more stress-related genes in which water-retention ability was stronger and water potentially was able to be taken more than the lowland cultivar (Ding et al. 2013).

The cultivar response, mutagen effect, and interaction (cultivar x mutagen) were significantly shown on several agronomic performances, with the exception to the culm diameter that showed the non significant cultivar response (Table 1). The analysis showed that the culm diameter of Inpago Unsoed 1, Rojolele, Inpari 13, and Cirata had the same responses under drought stress, and the variation was significantly affected by mutagen and interaction (cultivar x mutagen). Ibrahim et al. (2013) also reported that the performance of culm diameter of mutant as the result of gamma irradiation is not different under drought stress. However, Inpago Unsoed 1 that was soaked in SA for 6 h, and Rojolele that was irradiated with γ 150 Gy showed culm diameter that was significantly larger than the controls of Inpago Unsoed 1 and Cirata (upland cultivar). Besides, culm diameters of Rojolele 150 Gy + SA 6 h, Inpari-13 100 Gy + SA 2 h, Inpari-13 100 Gy + SA 6 h, and Cirata SA 2 h were significantly higher than the control of Inpago Unsoed 1 (Table 2). According to Sinniah

et al. (2012), the increase of culm diameter has positive correlation with stem bending resistance in which lignin or cellulose determines the physical strength. Besides, Kashiwagi et al. (2008) also explained that the increase of culm diameter is able to improve the lodging resistance with the plant height as its critical factor, in which the reduction of plant height has a significantly positive effect on the increase of yield.

However, in this research, there was no plant height that was significantly lower than the controls. On the contrary, the plant height of Inpago Unsoed-1 100 Gy + SA 2 h, and Inpari-13 100 Gy + SA 6 h were significantly higher than the controls of Inpago Unsoed-1 and Rojolele (aromatic cultivar) (Table 2). It is different from the result of research by Rao and Reddi (1986) showing that the treatment of sodium azide causes the reduction trend in general for the plant height. However, Fu et al. (2008) reported that the plant height significantly decreases at higher gamma irradiation dose (≥ 300 Gy), but it is not significantly different from lower dose (< 300 Gy). According to Manickavelu et al. (2006), the plant height also has positive correlation with the root length under the drought stress ($R^2 = 0.54$). Luo (2010) explained that the root length is one of main criteria in dehydration avoidance, in which it refers to the resistance mechanism under drought stress by increasing the capacity of plant to sustain high water status by water uptake or a reduction of water loss under drought stress.

Table 2 shows that the root length of Inpago Unsoed-1 100 Gy + SA 2 h, Inpari-13 100 Gy + SA 6 h, Cirata SA 6 h, Cirata 150 Gy + SA 2 h, and Cirata 150 Gy + SA 6 h were significantly longer than the controls of Inpago Unsoed 1

Figure 1: Survival percentage of four rice cultivars in M_1 generation grown under drought stress level of -0.03 MPa: C1. Inpago Unsoed 1 (aromatic upland cultivar), C2. Rojolele (aromatic lowland cultivar), C3. Inpari 13 (non-aromatic lowland cultivar), C4. Cirata (non-aromatic upland cultivar), M1. Control, M2. γ 100 Gy, M3. γ 150 Gy, M4. SA 2 H, M5. SA 6 H, M6. γ 100 Gy + SA 2 H, M7. γ 100 Gy + SA 6 H, M8. γ 150 Gy + SA 2 H, M9. γ 150 Gy + SA 6 H.

Table 1: Generalized linear model analysis of M_1 generation of four rice cultivars grown under drought stress level of -0.03 MPa

Agronomic performances	d.f.	F	P	
Plant height				
Cultivar response	3, 27	6.21	0.0024	**
Mutagen effect	8, 28	5.69	< 0.0001	**
Interaction (cultivar x mutagen)	24, 28	2.04	0.0033	**
Root length				
Cultivar response	3, 27	15.07	< 0.0001	**
Mutagen effect	8, 28	5.28	< 0.0001	**
Interaction (cultivar x mutagen)	24, 28	2.22	0.0012	**
Leaf area				
Cultivar response	3, 27	11.49	< 0.0001	**
Mutagen effect	8, 28	3.99	0.0002	**
Interaction (cultivar x mutagen)	24, 28	2.56	0.0001	**
Culm diameter				
Cultivar response	3, 27	1.27	0.3042	ns
Mutagen effect	8, 28	4.46	< 0.0001	**
Interaction (cultivar x mutagen)	24, 28	2.23	0.0011	**
No. of tillers ⁽¹⁾				
Cultivar response	3, 27	11.68	< 0.0001	**
Mutagen effect	8, 28	7.83	< 0.0001	**
Interaction (cultivar x mutagen)	24, 28	1.92	0.0069	**
No. of productive tillers ⁽¹⁾				
Cultivar response	3, 27	11.49	< 0.0001	**
Mutagen effect	8, 28	4.71	< 0.0001	**
Interaction (cultivar x mutagen)	24, 28	2.32	0.0006	**
No. of seeds per panicle				
Cultivar response	3, 27	17.51	< 0.0001	**
Mutagen effect	8, 28	2.96	0.0034	**
Interaction (cultivar x mutagen)	24, 28	1.89	0.0081	**
Filled seed percentage ⁽²⁾				
Cultivar response	3, 27	18.24	< 0.0001	**
Mutagen effect	8, 28	2.20	0.0276	*
Interaction (cultivar x mutagen)	24, 28	2.19	0.0014	**
1000 seeds mass				
Cultivar response	3, 27	9.97	0.0001	**
Mutagen effect	8, 28	4.09	0.0001	**
Interaction (cultivar x mutagen)	24, 28	2.58	0.0001	**
Harvest index ⁽¹⁾				
Cultivar response	3, 27	16.36	< 0.0001	**
Mutagen effect	8, 28	2.06	0.0396	*
Interaction (cultivar x mutagen)	24, 28	2.33	0.0006	**

d.f. is degrees of freedom; * significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; ns not significant at $P \leq 0.05$. Data were transformed by square-root ⁽¹⁾ and arcsine ⁽²⁾ prior to analysis. F-value indicating the level of significant difference within or between variate groups. P-value indicating statistical significance.

Table 2: Vegetative performances in M₁ generation of four rice cultivars grown under drought stress level of -0.03 MPa

Cultivar	Mutagen	Performances									
		Plant height (cm)		Root length (cm)		Leaf area (cm ²)		Culm diameter (mm)		No. of tillers ⁽¹⁾	
Inpago Unsoed 1	Control	18.01	c	4.24	de	10.76	bcd	3.04	d	1.70	ef
	γ 100 Gy	63.65	abc	16.28	abcde	22.27	bcd	4.03	abcd	6.10	abcdef
	γ 150 Gy	29.07	bc	6.22	bcde	12.04	bcd	4.07	abcd	2.70	def
	SA 2 H	91.34	abc	21.67	abcde	50.45	abcd	4.53	abcd	4.60	abcdef
	SA 6 H	90.99	abc	22.10	abcde	41.71	abcd	6.16	a	6.30	abcdef
	γ 100 Gy + SA 2 H	121.75	a	27.73	a	76.98	a	5.12	abcd	7.10	abcdef
	γ 100 Gy + SA 6 H	81.13	abc	17.45	abcde	38.74	abcd	3.98	abcd	6.10	abcdef
	γ 150 Gy + SA 2 H	56.73	abc	12.26	abcde	31.73	abcd	3.74	bcd	3.90	bcdef
	γ 150 Gy + SA 6 H	98.25	abc	22.73	abcde	34.64	abcd	4.54	abcd	10.10	abcd
Rojolele	Control	12.88	c	2.83	e	5.36	d	4.06	abcd	1.00	f
	γ 100 Gy	35.84	abc	5.51	bcde	14.67	bcd	3.95	abcd	1.90	ef
	γ 150 Gy	50.39	abc	13.71	abcde	15.68	bcd	5.47	ab	4.20	abcdef
	SA 2 H	82.05	abc	16.84	abcde	37.97	abcd	4.90	abcd	4.80	abcdef
	SA 6 H	27.33	bc	5.87	bcde	10.65	bcd	4.26	abcd	3.60	bcdef
	γ 100 Gy + SA 2 H	80.65	abc	13.21	abcde	27.38	bcd	4.74	abcd	4.70	abcdef
	γ 100 Gy + SA 6 H	29.76	bc	6.12	bcde	11.82	bcd	4.52	abcd	1.70	ef
	γ 150 Gy + SA 2 H	27.30	bc	5.26	cde	9.64	cd	4.84	abcd	2.20	ef
	γ 150 Gy + SA 6 H	58.75	abc	11.77	abcde	22.37	bcd	5.45	abc	4.40	abcdef
Inpari 13	Control	38.56	abc	10.25	abcde	25.12	bcd	3.97	abcd	2.40	def
	γ 100 Gy	44.69	abc	9.21	abcde	24.24	bcd	3.99	abcd	2.90	cdef
	γ 150 Gy	75.84	abc	20.86	abcde	34.78	abcd	4.64	abcd	7.10	abcdef
	SA 2 H	65.15	abc	19.57	abcde	42.24	abcd	4.10	abcd	3.60	bcdef
	SA 6 H	97.53	abc	25.12	abcd	51.09	abc	5.06	abcd	7.00	abcdef
	γ 100 Gy + SA 2 H	60.56	abc	17.05	abcde	27.09	bcd	5.45	abc	5.60	abcdef
	γ 100 Gy + SA 6 H	113.56	ab	26.39	ab	51.96	abc	5.40	abc	10.20	ab
	γ 150 Gy + SA 2 H	72.23	abc	17.93	abcde	46.17	abcd	4.30	abcd	5.00	abcdef
	γ 150 Gy + SA 6 H	83.75	abc	21.88	abcde	40.69	abcd	4.81	abcd	6.60	abcdef
Cirata	Control	42.95	abc	14.73	abcde	27.28	bcd	3.23	cd	3.10	bcdef
	γ 100 Gy	35.69	abc	11.03	abcde	18.71	bcd	5.03	abcd	4.00	bcdef
	γ 150 Gy	64.81	abc	21.20	abcde	36.25	abcd	4.28	abcd	5.50	abcdef
	SA 2 H	31.57	bc	8.01	abcde	12.30	bcd	5.45	abc	3.90	bcdef
	SA 6 H	91.16	abc	28.64	a	41.20	abcd	4.10	abcd	11.50	a
	γ 100 Gy + SA 2 H	78.68	abc	23.40	abcde	32.96	abcd	4.50	abcd	7.20	abcdef
	γ 100 Gy + SA 6 H	71.53	abc	20.90	abcde	33.03	abcd	4.80	abcd	6.30	abcdef
	γ 150 Gy + SA 2 H	95.23	abc	28.40	a	55.25	ab	4.57	abcd	8.00	abcde
	γ 150 Gy + SA 6 H	88.05	abc	25.79	abc	42.29	abcd	4.27	abcd	9.70	abc

Means followed by the same letters in a column are not significantly different ($P \leq 0.05$). Data were transformed by square-root⁽¹⁾ prior to analysis; nontransformed data are presented

and Rojolele (aromatic cultivar). Besides, the root length of Inpari-13 + SA 6 h was significantly longer than the control of Rojolele. Based on the research of Sandhu *et al.* (2012) that explained that under the drought stress, the drought-resistant genotype has root that is 54-73.8% longer than the drought-sensitive genotype. Devi *et al.* (2013) also reported that the maximum root length is indicator of drought-resistant genotype, and it is related to the forming of more root branches that are larger to support the water uptaking. Besides, Liu *et al.* (2012) explained that the water uptaking by root is not probably the main contributing factor to the resistance on drought stress in the genotype with the root length that is not statistically different, but it is caused by the reduction in the leaf area that affects the reduction of transpiration per plant to reduce the loss of water and improve the resistance on drought. However, Mohankumar *et al.* (2011) reported that the root length only has small correlation with the leaf area under the drought stress ($R^2=0.18$).

The reduction of leaf area may be considered as an avoidance mechanism, and improve drought resistance through the reduction of plant water use (Bimpong *et al.* 2011). However, in this research, there was no leaf area that was significantly narrower than the controls. In fact, the leaf area of Inpago Unsoed-1 100 Gy + SA 2 h was significantly wider than the controls on all cultivars. Besides, the leaf area of Inpari-13 SA 6 h, Inpari-13 100 Gy + SA 6 h, and Cirata 150 Gy + SA 2 h were significantly wider than the control of Rojolele. The wider leaf shows the effect of high osmotic adjustment under the drought stress (Fukai and Cooper, 1996). According to Ambavaram *et al.* (2014), the osmotic adjustment encourages the leaf to develop a more negative osmotic potential by accumulating solutes, so it maintains the plant turgor and contributes to the stress-protective functions, such as maintaining higher relative water content during a period of leaf water-potential reduction as the mechanism of drought tolerance.

Pandey and Shukla (2015) argued that drought stress reduces the number of tillers, in which it is related to the extending growths, and the obstacle in the cell growth that exceeds the cell division. However, in this research, number of tillers of Cirata SA for 6 h was significantly higher than the controls of all cultivars. Besides, number of tillers of Inpago Unsoed-1 150 Gy + SA 6 h was significantly higher than the controls of Inpago Unsoed 1 and Rojolele, while the Cirata 150 Gy + SA 2 h was significantly higher than the control of Rojolele. Meanwhile, number of tillers of Inpago Unsoed-1 100 Gy + SA 6 h, and Cirata 150 Gy + SA 6 h were significantly higher than the controls of Inpago Unsoed 1, Rojolele, and Inpari 13 (Table 2). These results are different from the research by Ali *et al.*

(2014) that shows that the sodium azide affects the reduction of number of tillers in general. However, Harding *et al.* (2012) reported that the number of tillers is not significantly different on the gamma irradiation dose of 50-300 Gy, and the number of tillers will be lower on the increase of gamma irradiation dose above 300 Gy.

On the contrary, Ramchander *et al.* (2015) reported that the gamma irradiation affects the increase of number of productive tillers in general. The report from Rao and Reddi (1986) also showed that the sodium azide has positive effect in increasing number of productive tillers in Jaya (indica cultivars) and Fujiminori (japonica cultivar), though there is the reduction in number of productive tillers on IET 5656 (indica cultivars). The increasing number of productive tillers was also shown in Inpari-13 100 Gy + SA 6 h, and Cirata SA 6 h with number of productive tillers that were significantly higher than the controls of Inpago Unsoed 1, Rojolele, and Inpari 13. However, number of productive tillers of Inpago Unsoed-1 150 Gy + SA 6 h, and Cirata 150 Gy + SA 2 h were significantly higher than the control of Rojolele. Besides, number of productive tillers in Cirata 150 Gy + SA 6 h was significantly higher than the controls of Inpago Unsoed 1 and Rojolele (Table 3). According to Vanisree *et al.* (2013), number of productive tillers has a positive correlation with number of seeds per panicle ($R^2 = 0.155$).

The drought stress was also reported to affect the decreasing number of seeds per panicle (Akram *et al.* 2013). Sarvestani *et al.* (2008) also explained in detail that the number of seeds per panicle does not decrease on the drought stress at vegetative stage and seed-filling period, but it is decreased significantly during the drought stress at the flowering stage. On the contrary, in this research, number of seeds per panicle of Inpago Unsoed-1 100 Gy + SA 2 h, Inpari-13 SA 6 h, and Inpari-13 100 Gy + SA 6 h were significantly higher than the controls of Inpago Unsoed 1 and Rojolele (aromatic cultivar). Besides, number of seeds per panicle of Cirata 150 Gy + SA 2 h was significantly higher than the control of Rojolele (Table 3). Haider *et al.* (2012) explained that the number of seeds per panicle has positive correlation with 1,000-seed mass under the drought stress ($R^2 = 0.343$). Ji *et al.* (2012) reported that the drought stress made 1,000-seed mass decrease on the drought resistant and sensitive genotypes. It is different from the research of Sellammal *et al.* (2014) that shows that 1,000-seed mass only decreases on the sensitive genotype. On the contrary, in this research, 1,000-seed mass increased in Inpago Unsoed-1 100 Gy + SA for 2 h, Inpari-13 SA 6 h, and Inpari-13 100 Gy + SA 6 h with 1,000-seed mass that were significantly higher than the controls of Inpago Unsoed 1 and Rojolele (aromatic cultivar). Besides, 1,000-

Table 3: Reproductive performances in M₁ generation of four rice cultivars grown under drought stress level of -0.03 MPa

Cultivar	Mutagen	Performances								
		No. of tillers ⁽¹⁾	productive	No. of panicle	seeds per	Filled seed percentage (%) ⁽²⁾	1000 seeds mass (g)	Harvest index ⁽¹⁾		
Inpago Unsoed 1	Control	0.70	def	16.04	cd	5.65	cde	2.38	cd	0.046 b
	γ 100 Gy	2.40	abcdef	71.70	abcd	29.77	abcde	9.28	abcd	0.196 ab
	γ 150 Gy	1.30	bcdef	35.21	abcd	13.28	abcde	2.73	cd	0.078 ab
	SA 2 H	3.10	abcdef	98.34	abcd	44.01	abcde	11.52	abcd	0.410 ab
	SA 6 H	5.10	abcdef	89.58	abcd	33.71	abcde	12.91	abcd	0.240 ab
	γ 100 Gy + SA 2 H	4.60	abcdef	133.55	a	53.42	ab	23.39	ab	0.527 a
	γ 100 Gy + SA 6 H	3.60	abcdef	86.06	abcd	36.58	abcde	13.57	abcd	0.330 ab
	γ 150 Gy + SA 2 H	2.50	abcdef	43.81	abcd	20.15	abcde	6.39	abcd	0.224 ab
	γ 150 Gy + SA 6 H	6.60	abcd	79.05	abcd	32.55	abcde	16.01	abcd	0.219 ab
Rojolele	Control	0.00	f	0.00	d	0.00	e	0.00	d	0.000 b
	γ 100 Gy	0.70	def	12.40	d	7.22	cde	3.24	cd	0.050 b
	γ 150 Gy	2.70	abcdef	4.41	d	1.27	de	4.83	bcd	0.002 b
	SA 2 H	4.30	abcdef	23.94	bcd	10.48	abcde	14.60	abcd	0.062 b
	SA 6 H	0.50	def	5.72	d	0.00	e	0.00	d	0.000 b
	γ 100 Gy + SA 2 H	1.70	abcdef	45.40	abcd	11.32	abcde	11.00	abcd	0.047 b
	γ 100 Gy + SA 6 H	0.40	ef	12.95	d	6.47	cde	4.28	bcd	0.029 b
	γ 150 Gy + SA 2 H	0.60	def	6.38	d	0.18	e	3.00	cd	0.001 b
	γ 150 Gy + SA 6 H	1.80	abcdef	35.70	abcd	9.87	abcde	8.93	abcd	0.043 b
Inpari 13	Control	0.90	cdef	40.67	abcd	21.18	abcde	6.94	abcd	0.096 ab
	γ 100 Gy	1.40	bcdef	46.03	abcd	11.10	abcde	6.55	abcd	0.103 ab
	γ 150 Gy	2.70	abcdef	76.75	abcd	33.66	abcde	15.32	abcd	0.240 ab
	SA 2 H	2.30	abcdef	72.99	abcd	51.42	abcd	15.15	abcd	0.403 ab
	SA 6 H	3.70	abcdef	127.52	ab	58.11	a	23.37	ab	0.534 a
	γ 100 Gy + SA 2 H	2.60	abcdef	79.26	abcd	30.79	abcde	13.10	abcd	0.315 ab
	γ 100 Gy + SA 6 H	7.20	ab	125.90	ab	41.18	abcde	25.33	a	0.375 ab
	γ 150 Gy + SA 2 H	3.20	abcdef	81.86	abcd	27.88	abcde	14.17	abcd	0.264 ab
	γ 150 Gy + SA 6 H	3.00	abcdef	48.10	abcd	26.41	abcde	11.36	abcd	0.235 ab
Cirata	Control	2.20	abcdef	48.85	abcd	24.69	abcde	7.52	abcd	0.290 ab
	γ 100 Gy	2.30	abcdef	25.94	abcd	7.14	bcde	3.75	cd	0.103 ab
	γ 150 Gy	3.80	abcdef	71.58	abcd	28.82	abcde	13.99	abcd	0.350 ab
	SA 2 H	2.00	bcdef	26.20	abcd	9.06	abcde	3.68	cd	0.085 ab
	SA 6 H	7.80	a	83.98	abcd	22.61	abcde	21.35	abc	0.180 ab
	γ 100 Gy + SA 2 H	3.70	abcdef	42.81	abcd	7.09	abcde	9.49	abcd	0.116 ab
	γ 100 Gy + SA 6 H	2.80	abcdef	64.98	abcd	20.53	abcde	7.02	abcd	0.221 ab
	γ 150 Gy + SA 2 H	5.50	abcde	122.34	abc	50.47	abc	17.45	abcd	0.536 a
	γ 150 Gy + SA 6 H	7.00	abc	60.40	abcd	17.35	abcde	13.74	abcd	0.180 ab

Means followed by the same letters in a column are not significantly different ($P \leq 0.05$). Data were transformed by square-root⁽¹⁾ and arcsine⁽²⁾ prior to analysis; nontransformed data are presented.

seed mass of Cirata SA 6 h was significantly higher only than control of Rojolele (Table 3). On the contrary, Babaei et al. (2010) dan El-Degwy (2013) reported that several gamma irradiation doses have an effect that is not significantly different on 1,000-seed mass. However, Ikhajiagbe

et al. (2013) reported that the increasing dose of sodium azide affects the increase of 1,000-seed mass. According to Lin et al. (2007), 1,000-seed mass has positive correlation with the filled seed percentage under drought stress ($R^2 = 0.292$).

The drought stress was reported to decrease filled seed percentage on several drought-resistant genotypes, and it will keep decreasing along with the increasing drought stress level (Sikuku *et al.* 2010). Sakai *et al.* (2010) also reported that filled seed percentage decreases on the drought resistant and sensitive genotypes, but the decrease of filled seed percentage on the drought-resistant genotype are non significant. It is different from this research, which showed that filled seed percentage of Inpago Unsoed-1 100 Gy + SA 2 h, and Inpari-13 SA 6 h were significantly higher than the controls of Inpago Unsoed 1 and Rojolele (aromatic cultivar). Besides, the filled seed percentage of Inpari-13 SA 2 h, and Cirata 150 Gy + SA 2 h were significantly higher than control of Rojolele (Table 3). According to Pandey *et al.* (2014), the drought stress in the booting and grain-filling stages results in the reduction of filled seed percentage that is smaller than it in the flowering stage, but the seed-filling period that is shorter can reduce the seed mass. Uga *et al.* (2013) also explained that the drought-resistant genotype facilitates the photosynthesis and the seed filling that are better under the drought stress, so it supports the achievement of higher seed yield.

Sandhu *et al.* (2012) reported that the harvest index in drought-resistant genotype is not almost different with the drought-sensitive genotype since the seed dry mass and plant dry mass on the drought-resistant genotype are higher than drought-sensitive genotype under the drought stress. However, in this research, harvest index of Inpago Unsoed-1 100 Gy + SA 2 h, Inpari-13 SA 6 h, and Cirata 150 Gy + SA 2 h were significantly higher than the controls of Inpago Unsoed 1 and Rojolele (aromatic cultivar) (Table 3). Higher harvest index describes better plant capacity in allocating the assimilates into seed, and it is known as the efficiency measurement of the source-sink balance process (Wnuk *et al.* 2013). The efficient relation of source and sink is related to the increase of cytokinin synthesis that improves the seed yield under drought stress, while the effect of chlorosis and necrosis reduces the photosynthesis in drought-sensitive genotype (Degenkolbe *et al.* 2013). Therefore, the selection of M_1 genotypes with photosynthesis adjustment and source-sink relation under the drought stress, as the marker of the increasing expression of drought resistance character are expected to contribute in the improvement of rice with drought-resistance character.

4 Conclusions

Genotypes in M_1 generation under the drought stress at level of -0.03 MPa show high diversity based on several agronomical performance. The best combination between cultivar and mutagen is Inpago Unsoed 1 that was irradiated with γ 100 Gy and then soaked in SA for 2 h. The result is expected to be used as the reference, especially in determining the genotypes for the next drought-resistant rice breeding program.

Acknowledgements: The authors thank Totok Agung Dwi Haryanto (the breeder of Inpago Unsoed 1) and Indonesian Center for Rice Research (ICRR) for providing the seeds. Support and laboratory facilities provided by Vocational Education Development Center of Agriculture (VEDCA) are appreciated.

Conflict of interest: Authors state no conflict of interest.

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