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THE EFFECT OF GAMMA IRRADIATION AND SODIUM AZIDE ON GERMINATION OF SOME RICE CULTIVARS

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ABSTRACT

Efforts to increase rice production through genetic improvement are often limited by the availability of natural diversity. That natural diversity can be improved through induced mutation. Selected characters can be observed since the germination phases, which may also indicate the plants survival under field conditions. Experimental design was arranged in split plot, with cultivars as main plot and mutagen doses as sub plot. The experiment consisted of 36 treatment combinations, with each treatment consisting of 10 replications. Data were recorded on percentage of first count, final count and germination rate. The data were analyzed using F-test by SAS 9.0 and mean separation was carried out by employing DMRT at 95 % (α = 5 %) of confidence level. The results showed that the best cultivar response for germination traits was Inpari 13, the best mutagen to build cultivar for germination traits was Gamma 150 Gy, and the best combination between cultivar and mutagen for germination traits was Inpago Unsoed 1 that was treated with Gamma 150 Gy.

Keywords: gamma irradiation, germination, rice, sodium azide

INTRODUCTION

In Indonesia, the consumtion of rice as the staple food is very high and it will continue increasing around 2 %, in line with the growth rate of population that reaches 1.49 % per year (BPS, 2012). There have been efforts to increase the national productivity of rice. However, the results have not shown significant changes (Sumardi, 2010). Therefore, it is necessary to conduct genetic improvement through plant breeding programs. The crossing of parent plants with high genetic diversity is more possible to enhance better genetic characteristics (Alam *et al*., 2011). However, these efforts are often limited by the availability of natural diversity (Shu, 2009).

Natural diversity can be improved through induced mutation (Rustikawati *et al.*, 2012). Mutations are changes in the composition of genes, which is reported to be able to improve the traits of plants. Gamma irradiation treatment can improve the rice yield by 9-40 % (Shehzad *et al*., 2011), whereas applying sodium azide can improve the drought resistance up to -0.0077 MPa (Aurabi *et al*., 2012). Moreover, the combination of the two mutagens is also able to increase the yield by 7-15 % (Siddiqui and Singh, 2010), and to improve the drought resistance up to -0.0021 MPa (He *et al*., 2009). Those superior traits are obtained after passing various and rigorous selection processes.

Mutant selection can be done since the beginning of the germination phase (Harding *et al*., 2012). According to Wang *et al.* (2010), the traits of germination, as measured in the laboratory, are able to reflect the ability of plants to live under field conditions. This study aims to analyze the best cultivar response on germination traits, the best mutagen to build cultivars for the germination traits, the best combination between cultivar and mutagen for germination traits. This study is expected to provide information that can be used in rice cultivar improvement programs.

MATERIALS AND METHODS

The research has been conducted at the Center for the Application of Isotopes and

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Radiation Technology - National Nuclear Energy Agency (PATIR - BATAN), Pasar Jumat, South Jakarta; and Screen House of Seed Technology Laboratory, Vocational Education Development Center of Agriculture (VEDCA), Cianjur. The research objects included cultivar rice seed of Inpago Unsoed 1 (upland aromatic), Rojolele (lowland aromatic), Inpari 13 (lowland non-aromatic), and Cirata (upland non-aromatic), sodium azide (SA), and $HgCl_2$ 0.2% . The used tools included gamma chamber 4000 A, germination box, tweezers, sprayer, and thermohygrometer.

The research was arranged based on split plot design. Cultivars were placed in the main plot, while the doses of mutagen were placed in the sub plot. The tested cultivars included Inpago Unsoed 1 (K1), Rojolele (K2), Inpari 13 (K3), and Cirata (K4); while the doses of mutagen were included without mutagen (X1), gamma 100 Gy (X2), gamma 150 Gy (X3), SA 2 hours (X4), SA 6 hours (X5), gamma 100 Gy + SA 2 hours (X6), gamma 100 Gy + SA 6 hours (X7), gamma 150 Gy + SA 2 hours (X8), gamma 150 Gy + SA 6 hours (X9). Thus, the entire treatments contained 36 treatment combinations, with each treatment consisting of 10 replications.

Treatment was attempted in the physical mutagenesis by giving different doses of gamma irradiation, 100 Gy (Shehzad *et al*., 2011) and 150 Gy (Shereen *et al*., 2009) respectively. The seeds of some rice cultivars were used as experimental objects, they were put into a plastic bag labeled with the irradiation doses to be administered, then they were put in a gamma chamber 4000 A with ^{60}Co as a source of gamma irradiation. For chemical mutagenesis, the tested treatment was the difference of soaking time in SA (NaN_3) 10^{-3} M, which was dissolved in 0.1 M phosphate buffer solution, adjusted at pH 3.0. The seeds of some rice cultivars were used as experimental material, and then treated (soaked) in a solution of SA for 2 hours (Liu *et al.*, 2007) and 6 hours (Siddiqui and Singh, 2010), at a temperature of ± 25 ℃. Soon after the treatment, the seeds were washed with flowing water at a temperature of ± 15 ℃ (Shin and Jeung, 2011). Besides, the tested treatment was the combination of physical and chemical mutagenesis.

The mutant seeds were soaked in $HgCl_2$ 0.2 % for 1 minute, soon after the sterilization, the seeds were rinsed three times with aquadest (Santika, 2011). The sterilized seeds were soaked in aquadest for 24 hours (ISTA, 2010). Furthermore, the seeds were germinated in a germination box placed in a screen house under paranet 70 %. Nursery was maintained by using mist spray and it was monitored with thermohygrometer (25-30 ^{0}C; ± 80 %).

Germination percentage was calculated based on the number of seeds germinated on day 5 (first count) and day 14 (final count) with the following formula (Sutopo, 1993).

$$\frac{\Sigma \text{ normal germination}}{\Sigma \text{ germinated pure seeds}} \times 100\%$$

Remarks:
GP: Germination percentage

Test for germination rate (KCt) was done for 5 days, in which **t** is the germination period, and **d** is the additional percentage of normal germination per etmal, which was counted according to the following formula (Sadjad, 1993):

$$KCt = \sum_{0}^{t} d$$

The data were analyzed by F-Test, and if this test showed significant difference then it was followed by Duncan Multiple Range Test (DMRT) at 95 % (α = 5 %) of confidence level. Statistical analysis software in this research was *SAS 9.0.*

RESULTS AND DISCUSSION

Germination traits are very important, because it relates to the ability to grow and resistance to unfavorable conditions after the planting process. Cultivar response for the highest percentage of first count, final count, and germination rate (KCt) was shown by Inpari 13, while Cirata showed the lowest first count and KCt, but the lowest final count was indicated by Rojolele. Treatment without mutagen showed the highest percentage of germination of first count, final count, and KCt among other mutagens. But mutagen of gamma 150 Gy showed that KCt was not significantly different from those without mutagen. It usually appears that the provision of mutagen decreased the percentage of first count, final count, and KCt, but decreasing was not in line with the increasing doses of mutagen (Table 1).

The decrease in germination percentage was similar to research of Sasikala and

Kalaiyarasi (2010), where the germination percentage will decrease as the effect of mutagen treatment, however the decrease is not in line with the increasing doses of mutagen. On the other hand, Anbarasan *et al.* (2013) reported that the seed vigor would decrease with the increasing doses of mutagen. According to Shah *et al.* (2008), the decrease is caused by the increase of free radical activities that trigger the death of seeds.

Inpari 13 without mutagen showed the highest percentage in the first count. Besides, the whole cultivars without mutagen also show the percentage of first count more than 80% (Table 2). High percentage of germination explains that the source seeds have very good quality. However, Harding *et al.* (2012) reported that the rice germination after gamma irradiation of 100 and 150 Gy was able to reach more than 90 %. This fact explains that mutagenesis on different cultivars may cause physiological damage with various levels of the death of seed.

Good quality seeds are potential to have potentially high germination and a chance to appear on final count. Table 3 shows that Cirata was treated with gamma 150 Gy, and Inpari 13 treated with gamma 150 Gy + sodium azide 10^{-3} M 6 hours had the highest final count. However, in general, the interaction of non-aromatic rice (Inpari 13 and Cirata) treated with some mutagens shows the higher percentage of final count compared with the aromatic rice (Inpago Unsoed 1 and Rojolele).

Sadjad (1993) classifies the KCt to demonstrate the level of vigor into three classes, namely strong vigor when KCt is greater than 30% / etmal, moderate vigor when KCt is between 25-30% / etmal, and weak vigor when KCt is below 25% / etmal. Table 4 shows that the most strong vigor is seen in Inpari 13 without mutagen, while the weakest vigor is seen in Inpago Unsoed 1 treated with gamma 150 Gy + sodium azide 10^{-3} M 6 hours, and Cirata treated with sodium azide 10^{-3} M 6 hours. This is consistent with the research of Surya and Soeranto (2006) on sorghum, which shows that increasing doses of mutagen cause KCt to decrease, but at a dose of 150 Gy still shows the strongest vigor.

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Table 1. The percentage of first count, final count, and germination rate (KCt) of four cultivars on some mutagens

Treatment	First count on day 5 (%)		Final count on day 14 (%)		KCt for 5 days (%/etmal)	
Cultivar:						
Inpago Unsoed 1	59.92	b	74.63	c	31.02	c
Rojolele	57.32	c	73.00	d	30.99	b
Inpari 13	71.86	a	87.78	a	38.53	a
Cirata	54.87	d	78.68	b	24.49	d
Mutagen:						
Without mutagen	88.48	a	89.25	a	49.28	a
Gamma 100 Gy	54.25	g	79.03	d	39.95	b
Gamma 150 Gy	67.50	b	84.15	b	50.10	a
SA 2 hours	57.45	e	75.30	e	30.73	c
SA 6 hours	52.48	h	76.83	e	20.22	g
Gamma 100 Gy + SA 2 hours	59.60	d	82.93	c	24.68	e
Gamma 100 Gy + SA 6 hours	61.13	c	73.58	f	22.37	f
Gamma 150 Gy + SA 2 hours	56.90	f	75.20	e	26.94	d
Gamma 150 Gy + SA 6 hours	51.15	i	70.45	g	17.04	h

Remarks: Values followed by the same letter in the same columns and treatments are not significantly different at the 95 % (α = 5 %) of confidence level, according to DMRT, based on arcsine-transformed data

Table 2. Effect of interaction between cultivar and mutagen on percentage of first count on day 5 (%)

Mutagen	Cultivar											
	Inpago Unsoed 1			Rojolele			Inpari 13			Cirata		
Without mutagen	87.40	a	(b)	88.00	a	(b)	92.30	a	(a)	86.20	a	(c)
	B			B			A			C		
Gamma 100 Gy	54.60	f	(mn)	46.10	g	(q)	56.60	f	(l)	59.70	b	(jk)
	C			D			B			A		
Gamma 150 Gy	74.40	b	(e)	61.30	c	(ij)	75.20	c	(e)	59.10	b	(k)
	A			B			A			C		
SA 2 hours	48.50	g	(p)	54.40	d	(mn)	84.30	b	(d)	42.60	f	(r)
	C			B			A			D		
SA 6 hours	47.90	g	(pq)	48.80	f	(p)	75.20	c	(e)	38.00	g	(s)
	B			B			A			C		
Gamma 100 Gy + SA 2 hours	62.50	d	(hi)	47.20	fg	(pq)	75.30	c	(e)	53.40	d	(n)
	B			D			A			C		
Gamma 100 Gy + SA 6 hours	66.70	c	(f)	54.20	d	(mn)	67.80	d	(f)	55.80	c	(lm)
	A			B			A			B		
Gamma 150 Gy + SA 2 hours	60.00	e	(jk)	51.50	e	(o)	63.30	e	(gh)	52.80	d	(no)
	B			C			A			C		
Gamma 150 Gy + SA 6 hours	37.30	h	(s)	64.40	b	(g)	56.70	f	(l)	46.20	e	(q)
	D			A			B			C		

Remarks: Values followed by the same lowercase letter in the same columns, the same capital letter in the same rows and the same lowercase letter within parenthesis in the same table are not significantly different at 95 % (α = 5%) of confidence level, according to DMRT. DMRT is based on arcsine-transformed data

Table 3. Effect of interaction between cultivar and mutagen on percentage of final count on day 14 (%)

Mutagen	**Cultivar**											
	Inpago Unsoed 1			**Rojolele**			**Inpari 13**			**Cirata**		
Without mutagen	87.80	a	(ef)	88.90	a	(de)	94.10	b	(b)	86.20	bc	(gh)
	B			B			A			C		
Gamma 100 Gy	73.20	d	(mnop)	75.40	b	(m)	82.60	ef	(jk)	84.90	c	(hi)
	C			C			B			A		
Gamma 150 Gy	84.80	b	(hi)	72.50	c	(op)	83.10	ef	(ijk)	96.20	a	(a)
	B			C			B			A		
SA 2 hours	59.40	e	(rs)	74.70	bc	(mno)	84.40	e	(ij)	82.70	d	(jk)
	C			B			A			A		
SA 6 hours	78.30	c	(l)	74.40	bc	(mno)	81.60	f	(k)	73.00	e	(nop)
	B			C			A			C		
Gamma 100 Gy + SA 2 hours	83.20	b	(ijk)	71.20	c	(p)	90.40	c	(c)	86.90	b	(fg)
	C			D			A			B		
Gamma 100 Gy + SA 6 hours	75.20	d	(mn)	68.40	d	(q)	90.20	cd	(cd)	60.50	g	(r)
	B			C			A			D		
Gamma 150 Gy + SA 2 hours	88.60	a	(e)	57.80	e	(s)	88.00	d	(ef)	66.40	f	(q)
	A			C			A			B		
Gamma 150 Gy + SA 6 hours	41.20	f	(t)	73.70	bc	(mno)	95.60	a	(a)	71.30	e	(p)
	D			B			A			C		

Remarks: Values followed by the same lowercase letter in the same columns, the same capital letter in the same rows and the same lowercase letter within parenthesis in the same table are not significantly different at 95 % (α = 5%) of confidence level, according to DMRT. DMRT is based on arcsine-transformed data

Table 4. Effect of interaction between cultivar and mutagen on percentage of germination rate (KCt) for 5 days (%/et mal)

Mutagen	Cultivar											
	Inpago Unsoed 1			Rojolele			Inpari 13			Cirata		
Without mutagen	50.70 B	b	(c)	30.50 D	d	(i)	77.20 A	a	(a)	38.70 C	ab	(fg)
Gamma 100 Gy	40.90 B	c	(e)	34.70 D	c	(h)	46.40 A	c	(d)	37.80 C	B	(g)
Gamma 150 Gy	62.40 A	a	(b)	46.80 C	a	(d)	51.10 B	b	(c)	40.20 D	A	(ef)
SA 2 hours	20.50 A	f	(o)	28.90 B	de	(ij)	49.40 A	b	(c)	24.10 C	C	(klm)
SA 6 hours	20.60 B	f	(o)	23.70 A	fg	(klm)	25.40 A	f	(k)	11.20 C	F	(r)
Gamma 100 Gy + SA 2 hours	25.30 B	d	(k)	24.90 B	f	(kl)	30.50 A	d	(i)	18.00 C	D	(p)
Gamma 100 Gy + SA 6 hours	23.80 B	de	(klm)	27.30 A	e	(j)	21.90 C	g	(no)	16.50 D	E	(q)
Gamma 150 Gy + SA 2 hours	23.30 C	e	(lmn)	39.10 A	b	(efg)	27.70 B	e	(j)	17.70 D	de	(pq)
Gamma 150 Gy + SA 6 hours	11.60 C	g	(r)	23.00 A	g	(mn)	17.20 B	h	(pq)	16.30 B	E	(q)

Remarks: Values followed by the same lowercase letters in the same columns, the same capital letters in the same rows and the same lowercase letters within parenthesis in the same table are not significantly different at 95 % (α = 5 %) of confidence level, according to DMRT. DMRT is based on arcsine-transformed data

A B

Remarks : The arrows point to the rice albino seedlings. A = The population of rice seedling of Inpago Unsoed 1 was treated with sodium azide 10^{-3} M 2 hours; B = The population of rice seedling of Inpago Unsoed 1 was treated with gamma 100 Gy + sodium azide 10^{-3} M 2 hours

Figure 1. The rice albino seedlings

In addition to quantitative deviation, qualitative deviation was also found. Deviation was indicated by the presence of the albino seedlings. It occurred in the Inpago Unsoed 1 treated with a sodium azide 10^{-3} M 2 hours, and gamma 100 Gy + sodium azide 10^{-3} M 2 hours (Figure 1). Ando and Montalvan (2001) reported that gamma irradiation and sodium azide effectively made the rice plant become albino. The albino condition is caused by the errors in chloroplast genome replication, resulting in abnormalities of chlorophyll (Lin *et al.*, 2008)

According to Warghat *et al.* (2011), albino is a chlorophyll mutation appearing as white leaf

indicating that it does not contain any pigment, and the plants will die 1-2 weeks after germination. However, the albino seedlings found in this research could last up to 8 weeks after germination. It shows that the acquired chlorophyll abnormalities were not a perfect albino.

CONCLUSION

The best cultivar response for germination traits was Inpari 13, the best mutagen to build cultivar for germination traits was Gamma 150 Gy, and the best combination between cultivar and mutagen for germination traits was Inpago Unsoed 1 that was treated with Gamma 150 Gy. The result was expected to continue on the next rice breeding programs.

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