AGRIVITA VOLUME 38 No. 1 FEBRUARY-2016 ISSN : 0126 - 0537

INDUCED MUTATION BY GAMMA RAYS IRRADIATION TO INCREASE CHILLI RESISTANCE TO *BEGOMOVIRUS*

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Received: May 20, 2015/ Accepted: December 7, 2015

ABSTRACT

Begomovirus infection has a significant impact of lowering chilli yield in Indonesia. A constraint of narrow genetic variability of chilli in Indonesia has made the mutation breeding program as a solution worth-pursuing in increasing the genetic variability. The objective of this study was to determine the LD_{50} point for each of the five irradiated chilli genotypes and the optimum dose of gamma irradiation in inducing chilli resistance to *Begomovirus* and other improved agronomical traits. The study was conducted in the Indonesian Vegetable Research Institute (IVEGRI) at Cikole-Lembang, elevation 1,200 m above sea level, from March to December 2013. Split plot design was used with genotype as main factor (Kencana, Lembang-1, SSP, Tanjung 2, Seloka) and irradiation dosage as sub-factor (0, 200, 400, 600, 800 Gy). All treatments were replicated three times. The results showed that LD_{50} points of the five irradiated chilli genotypes were in the range of 422.64-629.68 Gy. There were some chilli genotypes in the population of M_2 that had high coefficient variance genetic (CVG) and broad sense heritability (h^2_{bs}) value for disease incubation time. This could be used as resistance parameter to *Begomovirus* and improvement parameter of several agronomical traits.

Keyword: *Begomovirus*; *Capsicum annuum*; gamma rays; induced mutation

INTRODUCTION

Begomovirus has been identified as one of important diseases that influence the yield of chilli in Indonesia. At present, *Begomovirus* infection had been widely spread in all chilli production centers. *Bemisia tabaci* is the insect vector that responsible only for the virus trans-mission, but not for virus propagation (Sulandari *et al*., 2004). Infected plants show bright yellow-ing or yellow-green mosaic, vein clearing, curly, and stunted leaves. Yield loss caused by this disease may reach approximately 20-100% (Setiawati, 2003).

At the farmer's level, some control methods, such as plant eradication or insect vector chemical spraying has been applied to control the *Begomovirus*. However, field observations indicate that these control methods are still not effective yet. Womdim *et al*. (1991) suggests that the most effective and efficient control method to eliminate this disease is to use a resistant variety. Unfortunately, such variety has not been available yet in Indonesia. The germplasm genetic variability, especially for *Begomovirus* resistance is quite low, since Indonesia is not the center of chilli origin. Narrow genetic variability has become a constraint for chilli conventional breeding program because of the difficulty in obtaining the source for resistant genes.

Various strategies to increase chilli genetic variability can be pursued by such as seed introduction, hybridization, and mutation. Mutation seems more preferable than the other two since seed introduction sometimes may function as a media of transferring seed borne diseases, while hybridization will not be effective if there is no resistant gene available. Furthermore, conventional breeding by hybridization usually takes longer, yet sometimes also carries unexpected traits. This may result in an output

Cite this as: Gaswanto, R., M. Syukur, B.S. Purwoko and S.H. Hidayat. 2016. Induced Mutation by Gamma Rays Irradiation to Increase Chilli Resistance to *Begomovirus*. AGRIVITA. 38(1): 24-32. doi: 10.17503/agrivita.v38i1.581
Accredited: SK No. 81/DIKTI/Kep/2011
Permalink/DOI: **http://dx.doi.org/10.17503/agrivita.v38i1.581**

that is not as good as expected as a commercial variety.

Mutation is one of possible alternatives to conventional breeding for crop improvement program (Soeranto, 2011). Mutation is a sudden heritable change in an organism and generally induces structural and composition changes in genome, chromosome, gene, or DNA (Soeranto *et al.*, 2001; Dhanavel *et al*., 2012). Exposing plant genetic material (seed, pollen, rhizome, callus, etc.) to mutagens enhances the chance for isolating unique genetic material. Induced mutation can rapidly create the variability of inherited traits in crops, both quantitatively and qualitatively (Muduli and Misra, 2007). Post induced mutation has been effectively utilized in developing new and valuable alternation in plant characteristics that have contributed to increase yield potential or disease resistance.

One of physical mutagens in mutation breeding is gamma rays. Gamma rays belong to ionizing radiation and radiation-induced ionizations may act directly on the cellular component molecules or indirectly on water molecules, causing water-derived radicals. Since water is the major constituent of cells, the absorption of energetic radiations by water results in both excitations and ionizations leading to production of free radicals that in turn can attack other critical molecules (indirect effect). Free radicals react with nearby molecules in a very short time, re-sulting in breakage of chemical bonds or oxidation (addition of oxygen atoms) of the affected molecules. These radicals can damage or modify important components of plant cells and they have been reported to affect the morphology, anatomy, biochemistry and physiology of plants differently depending on the irradiation level (Kim *et al.*, 2004).

Gamma rays irradiation is an efficient tool to produce mutants in crop breeding and more than 1,800 cultivars either obtained as direct mutants or derived from their crosses have been released worldwide in 50 countries (Ahloowalia and Maluszynski, 2001). In Indonesia, gamma rays irradiation has been used in agriculture research and development since several decades ago, especially in fields of mutation breeding, pest control, plant nutrition, and animal health. In breeding program, this approach has not only contributed several crop varieties to national agriculture, but also generated hundreds of promising mutant lines that are ready for further multi-location trials. However, it is noted that no work on chilli resistance to *Begomovirus* has been carried out yet.

The aim of the present study is to determine the LD_{50} pointsfor each of the five irradiated chilli genotypes and the optimum dose of gamma irradiation in inducing chilli resistance to *Begomovirus* and other improved agronomical traits.

MATERIALS AND METHODS

The study was conducted in the Indonesian Vegetable Research Institute (IVEGRI) at Cikole-Lembang, elevation 1,200 m above sea above level, from March to December 2013. The genetic materials used were five open pollinated chilly varieties (M_0): Kencana, Lembang-1, SSP, Tanjung 2, Seloka that were all susceptible to *Begomovirus*. M_0 seeds used had the water content of 10-12% and the viability of 90%. Each seed variety was packed in 3 g (± 750 seeds) small plastic bag for each irradiation doses.

Gamma rays irradiation was carried out at The Center for Isotopes and Radiation Application-PATIR, Pasar Jumat, Jakarta. The seeds were subjected to gamma radiation at an irradiation dose of: 0 (control); 200 Gy; 400 Gy; 600 Gy; 800 Gy. After irradiation, the status of M_0 seeds changed to M_1 seeds automatically because the cell condition of each irradiated M_0 seed was changed and different. Then M_1 seeds were sown in the small polybag filled with media consisted of soil: humus = 1 : 1. After six weeks, seedlings were ready for transplanting in the open field.

The experimental design used was split plot with genotype as main factor, consisted of five treatments (Kencana, Lembang-1, SSP, Tanjung 2, Seloka) and gamma rays irradiation dose as sub factor, consisted of five treatments (0, 200, 400, 600, 800 Gy). All treatments were replicated three times. Land preparation was carried out thoroughly. Basal fertilizer was organic manure (30 t ha^{-1}) mixed with 100 kg ha^{-1} of P_2O_5 applied a week before transplanting. Split fertilization was provided three times for a total of 180 kg ha^{-1} of N and 120 kg ha^{-1} of K_2O applied at 3, 6 and 9 weeks after transplanting (Nurtika and Hilman, 1991; Hilman and Suwandi, 1992). Beds were covered by silver plastic mulch with 40 x 60 cm planting distance. Standard crop maintenance that includes plant protection (pesticide spraying) and irrigation (watering) was also applied. Self pollination was done on flowers of the irradiated M_1 plant within the range of LD_{50}point to produce the M_2 seeds.

The M_2 individual seedling of each genotype was screened by using mass transfer method. *B. tabaci* was prepared by collecting ± 250 imago non viruliferous using an aspirator tool, then the imago was released on *Begomovirus* infected chilli plants for 48 hours. After that, 20-30 imago units of *B. tabaci* viruliferous was used in one cage which covered by screen net for 50 individuals seedlings (2-4 true leaves). In this study, an inoculum strain collected from Kersana-Brebes was used. Genotype IPBC-12 was used as a control resistant genotype. The number of screened seedlings for each genotype was 200 individuals without replication.

Some parameters were observed from M_1 and M_2 population. In M_1 population, parameters observed were seeds germination, seedling height, percentage of abnormal seedlings, chimera and male sterility phenomena, LD_{50} point, plant height at flowering stage, fruit length, fruit diameter, weight per one fruit, and fruit numbers per plant. Meanwhile, parameters observed in M_2 population were the coefficient variance genetic (CVG), heritability (h^2_{bs}), disease symptoms (scoring) and incubation time as resistance parameter to *Begomovirus* and also some other agronomical traits. Scoring of disease symptoms was shown Figure 1. All data were initially analyzed by using F test and any significant result was further tested by using the DMRT (Duncan Multiple Range Test) at 5% significance level.

Figure 1. Scoring of disease symptom: 0 = no symptom (healthy); 1 = mild mosaic; 2 = mosaic, yellowing contrast; 3 = mosaic, yellowing contrast, leaf malformation, 4 = acute mosaic, yellowing contrast, leaf malformation

RESULTS AND DISCUSSION

Seeds Germination Percentage and Seedling Height

Results showed that there were significant interactions between the five chilli genotypes and gamma rays irradiation dosages for seed germination percentage (F_{count} = 35.99** > F_{tab01} = 2.49) and seedling height (F_{count} = 11.08** > F_{tab01} = 2.49). The highest percentage of seed germination was showed by control dose (0 Gy) on five chilli genotypes (85.67-89.33%), while the combination of genotype SSP and 200 Gy dosages (85.00%) showed the highest germination percentage among the irradiated genotypes (Table 1). Reduction in seed germination percentage might have been due to the effect of gamma rays irradiation on meristematic tissues of the seed (Sheppard *et al*., 1986). Moreover, Dhakshanamoorthy *et al*. (2010) indicated that the decrease in seed germination at higher dosage of mutagens may be attributed to disturbances at cellular level caused either at physiological or physical level. Ussuf and Nair (1974) said that gamma rays irradiation interfered with the synthesis of enzymes and at the same time accelerated the degradation of existing enzymes involved in the formation of auxins, and thus reduced the seed germination.

Table 2 showed that the treatment of Genotype Lembang-1 irradiated by 200 Gy dosage had the highest seedling height (12.00 cm) even compared to control (0 Gy). This indicated that 200 Gy dosage of gamma rays treatment had a stimulatory effect on the growth of seedling. This result was in line with the study of Dhakshanamoorthy *et al*. (2010) which suggested that the stimulatory effect on the seedling height was usually observed at a lower dosage of gamma rays. Hypothetically, the origin of these stimulations by irradiation was due to cell division rates, as well as an activation of growth hormone, e.g. auxin (Zaka *et al*., 2004). Results also showed that the dosage of 800 Gy would drastically reduce chilli seedling height in all genotypes. This indicated that 800 Gy was considered as the dosage of gamma rays irradiation that was too extremely high for chilli seed.

Seedlings physiological damaged that caused by gamma rays irradiation usually correlated with mutation frequency. Irradiation could have direct effect on chromosomes. They might directly break chromosomes or alter one of the DNA bases or indirectly might initiate a chain of physical and chemical reactions. The biological effect also depended on the type of cell and stage of nuclear cycle. For instance, chromosomes were extremely sensitive to breakage in mitotic prophase. The frequency of mutants per viable organism often increased linearly with the dosage (Dhanavel *et al*., 2012).

Determining of LD_{50} Point

The success of gamma rays irradiation was determined by the genotype radio sensitivity. They could be measured by lethal dose 50 (LD_{50}) point. Usually, the parameter used for determining LD_{50} point is the percentage of seed germination. LD_{50} point is lethal dosage for 50% of seed germination. Within the range of LD_{50} point the existence of maximum cell mutant number can be predicted (Dwiatmini *et al*., 2009). LD_{50} point was determined by using Finney method that was standardized to control (0 Gy).

Table 1. Seed germination of five irradiated chilli genotypes by several dosage levels of gamma rays at 30 days after sowing (DAS)

Genotypes	Gamma rays dosages (Gy)				
	0	200	400	600	800
Kencana	88.67 aA	79.67 bB	55.33 cC	41.00 dB	4.00 eAB
Lembang-1	85.67 aB	80.33 bB	55.00 cC	15.17 dC	3.33 eAB
SSP	88.67 aA	85.00 bA	75.50 cA	48.00 dA	5.00 eA
Tanjung-2	88.33 aA	76.67 bC	51.00 cD	14.00 dC	2.33 eB
Seloka	89.33 aA	79.33 bB	68.33 cB	50.17 dA	5.00 eA

Remarks: Numbers followed by different lowercase in a row and uppercase letters in a column indicated significant differences at 0.05 level according to DMRT

Table 2. Seedling height of five irradiated chilli genotypes by several dosage levels of gamma rays at 30 days after sowing (DAS)

Genotypes	**Gamma rays doses (Gy)**				
	0	**200**	**400**	**600**	**800**
Kencana	10.50 aB	10.33 aC	8.33 bD	3.17 cB	1.00 dA
Lembang-1	9.83 bC	12.00 aA	9.00 bBC	2.67 cB	1.17 dA
SSP	10.33 aB	9.17 bD	9.00 bBC	5.17 cA	1.00 dA
Tanjung-2	11.50 aA	11.17 aB	8.83 bCD	2.50 cB	1.00 dA
Seloka	11.17 aA	10.83 aBC	10.50 bA	5.83 cA	1.17 dA

Remarks: Numbers followed by different lowercase in a row and uppercase letters in a column indicated significant differences at 0.05 level according to DMRT

Table 3. Lethal doses 50 (LD_{50}) point of five irradiated chilli genotypes by gamma rays

Genotype	**Curve formula**	**LD_{50} (Gy)**
Kencana M_1	$Y=100.108-0.043x-9.2625e005x^2$	538.41
Lembang-1 M_1	$Y=99.38+0.11x-0.00076x^2+5.8364e-007x^3$	448.84
SSP M_1	$Y=98.67+0.04x-0.0002x^2$	614.79
Tanjung-2 M_1	$Y=99.45+0.045x-0.0006x^2+4.64e007x^3$	422.64
Seloka M_1	$Y=100.22-0.091x+0.00021x^2-3.031e.007x^3$	629.68
Average		530.87

The analysis showed that LD_{50} point of five irradiated chilli genotypes ranges between 422.64 Gy and 629.68 Gy with Genotype Tanjung-2 was the lowest and Seloka was the highest (Table 3). The average LD_{50} point (530.87 Gy) was different from previous study conducted by Omar *et al.* (2008) that indicated the LD_{50} point for chilly was 445 Gy. This might be explained by the fact that radio sensitivity among chilli genotypes was varied and each chilli genotype had specific LD_{50} point. Therefore, it was necessary to determine LD_{50} point for assessing an optimum dosage for each chilli genotype and obtaining the positive mutant.

Effect of Irradiation on Physiological Abnormality

The results showed that at 600 Gy dosages, there were three M_1 plants of Genotype Kencana (0.50%) were failed to produce fruits. There was a possibility that the three plants were male sterile plants. In general, the shape of sterile pollen was clear and wrinkle because it did not contain amylum. The amount and diameter of sterile pollen were lower than that of fertile one. Moreover, the existence of male sterility phenomenon could be used for improving hybrid variety cytoplasmic male sterile (CMS). In M_1 population with 600 Gy, there were also five plants of Genotype Kencana (0.83%) and two plants of Genotype Tanjung (0.33%) that showed chimera leaf phenomenon. This phenomenon indicated that there was a competition between mutant and normal leaf cells inside the plants. At the growing stage, if normal cells were more dominant than mutant cells, the developing young leaves would be normal again. On the contrary, if mutant cells were more dominant, the chimera phenomena would be going to their progenies. Seedlings condition at 800 Gy dosages had seed germination lower than 20% and a lot of abnormal seedlings, therefore all the irradiated seeds could not be planted in the field.

Plant breeders suggested that the use of irradiation dosage should not be higher than LD_{50} point because the mutagens will cause bad physiological effects, such as low seed germination percentage, male sterility, chimera phenomenon, seedling abnormality, dwarf, late flowering, etc. On the other hand, if the dosage was lower than LD_{50} point, there was a possibility for being unable the obtain mutants (Soeranto *et al.*, 2001). In general, high gamma rays dosages, particularly 600 and 800 Gy had negative effects physiologically on chilli seedlings derived from irradiated seeds. This indicated that gamma rays irradiation had been used higher than the recommended dosage for chilli seed.

Table 4. Several agronomical traits of five irradiated chilli M_1 genotype affected by some gamma rays dosages

Treatments	Plant height at flowering stage (cm)	Fruit Length (cm)	Fruit diameter (mm)	Weight per fruit (g)	Fruit number per plant (number)
Main plot:					
Kencana	20.42 b	11.18 b	7.79 c	2.75 c	165.33 a
Lembang-1	26.17 a	9.06 c	7.15 c	2.34 c	108.67 b
SSP	20.17 bc	13.92 a	11.05 b	5.54 b	97.00 b
Tanjung-2	19.00 bc	10.05 c	15.14 a	6.28 b	24.00 d
Seloka	16.42 d	13.29 a	15.86 a	7.58 a	65.58 c
Sub plot:					
0	23.53 a	12.20 a	11.86 a	5.38 a	110.13 a
200	21.87 b	11.74 ab	11.39 b	5.04 b	98.67 b
400	19.53 c	11.38 b	11.16 b	4.63 c	88.00 c
600	16.80 d	10.69 b	11.19 b	4.54 c	71.67 d
ANOVA					
Genotype (G)	**	**	**	**	**
Irradiation doses (D)	**	**	ns	**	**
G x D	ns	Ns	ns	ns	ns

Remarks: Mean followed by the same letter are not significantly at 0.05 level according to DMRT; ** = highly significant; ns = non-significant

Effect of Gamma Rays Irradiation to Several Agronomical traits on M_1 Plant Population

Results of variance analysis (Table 4) showed that there was no interaction between irradiated chilli genotypes and gamma rays irradiation dosages for some parameters, such as plant height at flowering, fruit length, fruit diameter, weight per fruit, and fruit number per plant. GenotypeM_1 Seloka showed the longest fruit length (13.29 cm), longest fruit diameter (15.86 mm), and higher weight per fruit (7.58 g) as compared to the other four genotypes. Genotype M_1 Lembang-1 had the highest plant height (26.17 cm), while Genotype M_1 Kencana had the highest fruit number per plant (165.33 fruits). In general, increasing gamma rays irradiation dosage might have a consequence of increasing negative effects. All parameters were recorded maximum at the control treatment (0 Gy), and recorded minimum at 600 Gy dosage. Previous study suggested that lower dosage could become a stimulatory effect to some agronomical traits (Dhakshanamoorthy *et al*., 2010), however in this study, both lower and higher dosage of gamma rays treatments had shown an inhibitory effect as compared to control.

Genetic Variance of Some Characters of M_2 Plant Population

Table 5 showed that in M_2 plant population, both the coefficient of variance genetic (CVG) and broad sense heritability (h^2_{bs}) of disease symptom scoring parameter for *Begomovirus* were low. However, the disease incubation parameter was rather high. This indicated an escalation of resistance to *Begomovirus*for each individual in M_2 as the incubation time varied (Figure 2). Genotype M_2 Kencana performed the best in terms of disease incident (29.17%) and disease intensity percentage (10.00%). But, out of the five irradiated chilli genotypes, IPBC-12 as a resistant genotype control, showed a high resistance level. It was indicated by the low disease incident percentage that was only 21.35% and disease intensity that was 8.33%. Unfortunately, the fruit performance of Genotype IPBC-12 could not satisfy the criteria of an ideal chilli fruit idiotype. Nevertheless, this genotype could still be collected as germplasm for the source of *Begomovirus* resistant gene.

Table 5. Coefficient of variance genetic (CVG) and broad sense heritability (h^2_{bs}) on M$_2$ plant population

Traits	**Population of gamma rays irradiation (400-600 Gy)**				
	M$_2$ Kencana	**M$_2$ Lembang1**	**M$_2$ SSP**	**M$_2$ Tanjung 2**	**M$_2$ Seloka**
Symptom Scoring (0-4)					
Mean	1.39	2.47	1.83	2.06	2.06
σ^2_g	(-1.07)	0.15	(-0.70)	(-0.08)	(-0.46)
h^2_{bs}	(-2.06)	0.08	(-0.63)	(-0.05)	(-0.30)
CVG	(-74.42)	15.68	(-45.72)	(-13.73)	(-32.92)
Criteria	Low	Rather low	Low	Low	Low
Incubation time (DAI)					
Mean	16.32	14.95	16.19	15.77	15.86
σ^2_g	13.56	12.25	12.91	13.89	13.20
h^2_{bs}	0.77	0.42	0.72	0.81	0.71
CVG	22.56	23.41	22.2	23.64	22.91
Criteria	Rather high	Rather high	Rather high	Rather high	Rather high
Plant Height (cm)					
Mean	72.17	53.29	59.75	37.92	55.09
σ^2_g	604.59	355.09	215.83	89.28	165.8
h^2_{bs}	0.97	0.95	0.91	0.92	0.94
CVG	34.07	35.36	24.59	24.92	23.37
Criteria	High	High	Rather high	Rather high	Rather high
Fruit Number (fruits)					
Mean	180.05	119.36	120.45	49.57	87.67
σ^2_g	809.28	683.64	232.44	268.81	369.88
h^2_{bs}	0.87	0.91	0.8	0.98	0.88
CVG	15.8	21.91	12.66	33.08	21.94
Criteria	Rather low	Rather high	Rather low	High	Rather high
Fruit Length(cm)					
Mean	12.84	10.49	14.02	11.17	13,60
σ^2_g	8.14	1.37	1.43	1.50	2.64
h^2_{bs}	0.85	0.59	0.62	0.59	0.70
CVG	22.23	11.16	8.53	10.96	11.94
Criteria	Rather high	Rather low	Low	Rather low	Rather low
Fruit Diameter (mm)					
Mean	7.44	7.45	11.44	15.20	15.83
σ^2_g	0.18	0.14	2.46	0.45	12,30
h^2_{bs}	0.46	0.38	0.34	0.39	0.94
CVG	5.70	5.02	13.71	4.41	22.16
Criteria	Low	Low	Rather low	Low	Rather high
Weight of One Fruit (g)					
Mean	3.02	2.57	6.86	7.73	7.86
σ^2_g	0.56	0.04	2.43	3.55	3.48
h^2_{bs}	0.95	0.33	0.84	0.92	0.95
CVG	24.75	7.77	22.71	24.36	23.73
Criteria	Rather high	Low	Rather high	Rather high	Rather high

Remarks: Criteria of CVG: (0<x≤10.94 = low; 10.94<x≤21.88 = rather low; 21.88<x≤ 32.83 = rather high; 32.83<x≤43.77 high; 43.77<x≤ ≤~= very high. Criteria of h^2_{bs}: h^2_{bs}< 0.20 = low; 0.20 ≤ h^2_{bs} ≤ 0.50 = moderate; h^2_{bs}> 0.50 = high

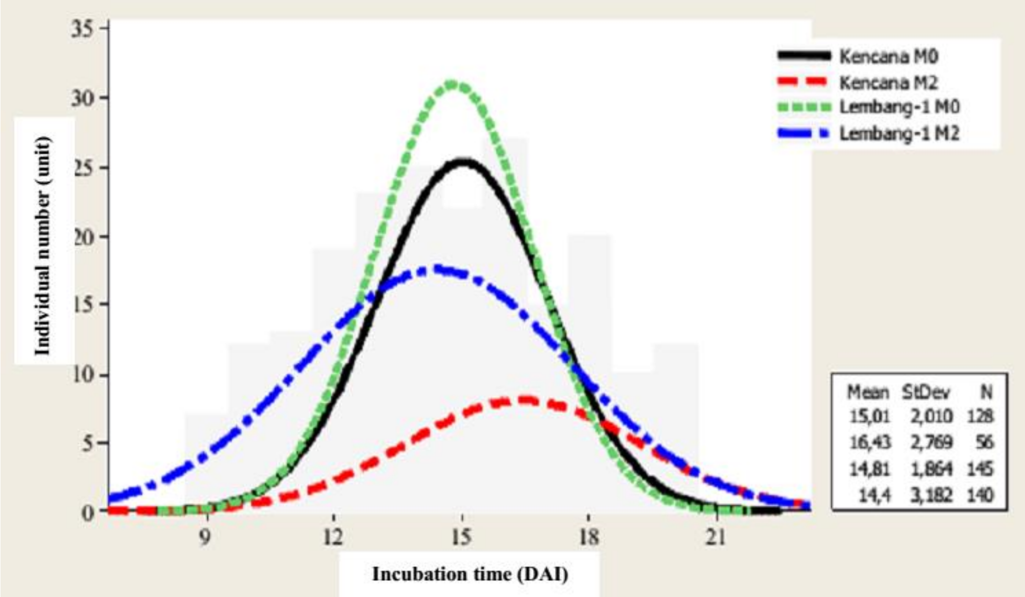

Figure 2. Incubation time variability of *Begomovirus* in the generation of M_0 and M_2 Kencana genotype (resistant category) and Lembang-1 genotype (susceptible category)

Mutations were phenotypically classified into two groups: macro and micro mutations. Macro mutation was easily detectable in individual plants, phenotypically visible, morphologically distinct, qualitatively inherited genetic changes, and it occurred in major genes or oligogenes. Meanwhile, micro mutation resulted in a small effect that usually could only be detected by the help of statistical methods, quantitatively inherited genetic changes and it occurred in minor genes or polygenes (Dhanavel *et al*., 2012). Quantitative traits of M_2 plant population derived from irradiated seed by gamma rays could be seen in the Table 5 also. Results in Table 5 showed that the CVG and h^2_{bs} for plant height of the five irradiated chilli genotypes were rather high to high. Chilli genotypes of M_2 Lembang-1, M_2 Tanjung-2, and M_2 Seloka had rather high to high CVG and h^2_{bs} value for fruit number. Only Genotype M_2 Kencana and M_2 Selokahad rather high CVG and h^2_{bs} value for fruit length and fruit diameter, respectively. Except Genotype M_2 Lembang-1, the other genotypes had rather high CVG and h^2_{bs} value for weight per fruit. This proved that gamma rays irradiation could also increase chilli genetic variability in morpho-agronomical traits.

CONCLUSION AND SUGGESTION

Increasing dosage of gamma rays irradiation caused alternation in physiological and agronomical traits of M_1 plant population. LD_{50} points of Genotype Kencana, Lembang-1, SSP, Tanjung-2, Seloka were 538.41 Gy, 448.84 Gy, 614.79 Gy, 422.64 Gy, and 629.68 Gy respectively.

There were some chilli genotypes in the population of M_2 that had high coefficient variance genetic (CVG) and broad sense heritability (h^2_{bs}) value for disease incubation time. This could be used as resistance parameter to *Begomovirus* and improvement parameter of several agronomical traits.

ACKNOWLEDGEMENTS

Authors are thankful to the Indonesian Agency for Agricultural Research and Development (IAARD) for funding the present study through the KKP3N 2013 Project, contract No. 710/LB.620/I.1/2/2013.

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