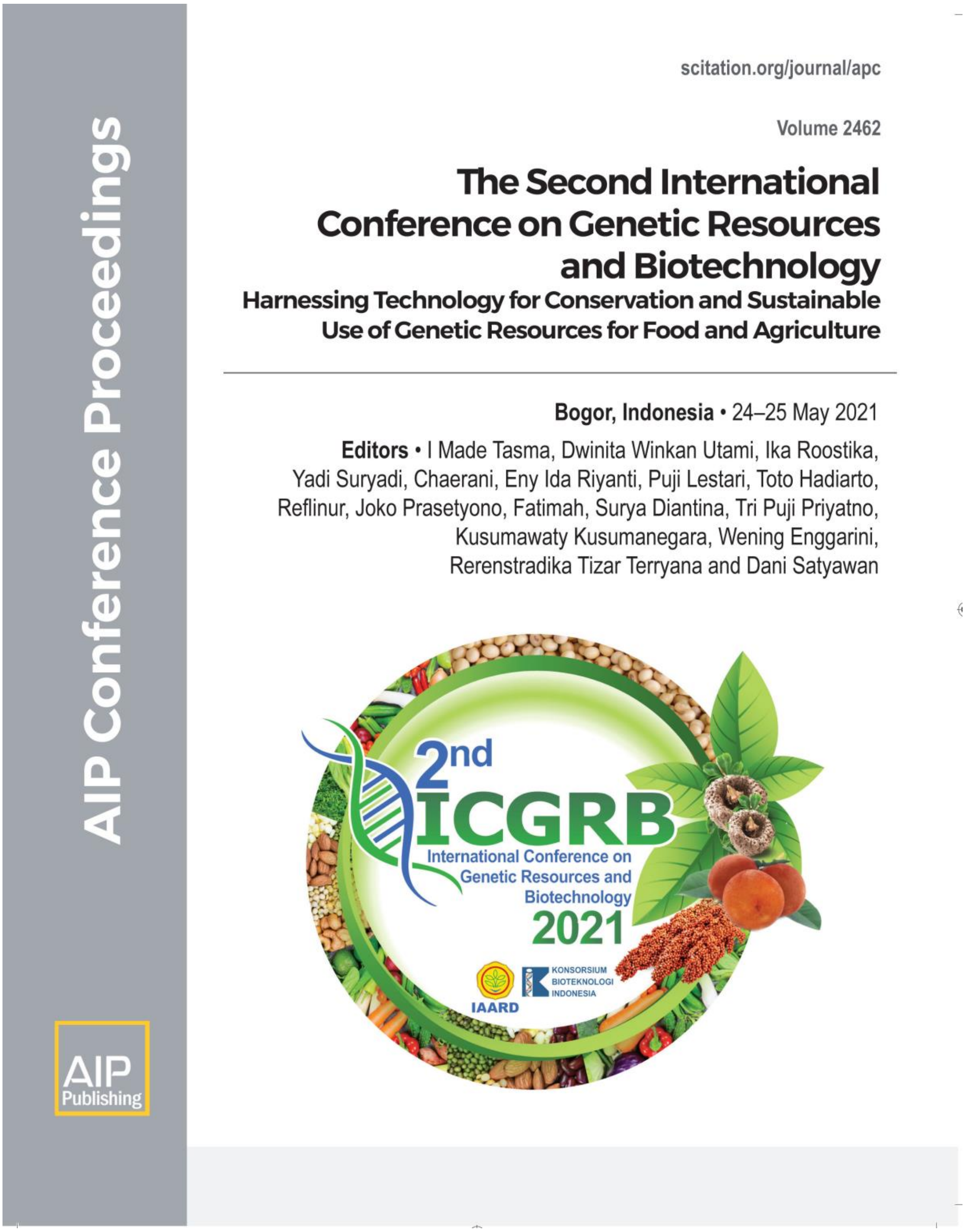

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Committees: The Second International Conference on Genetic Resources and Biotechnology

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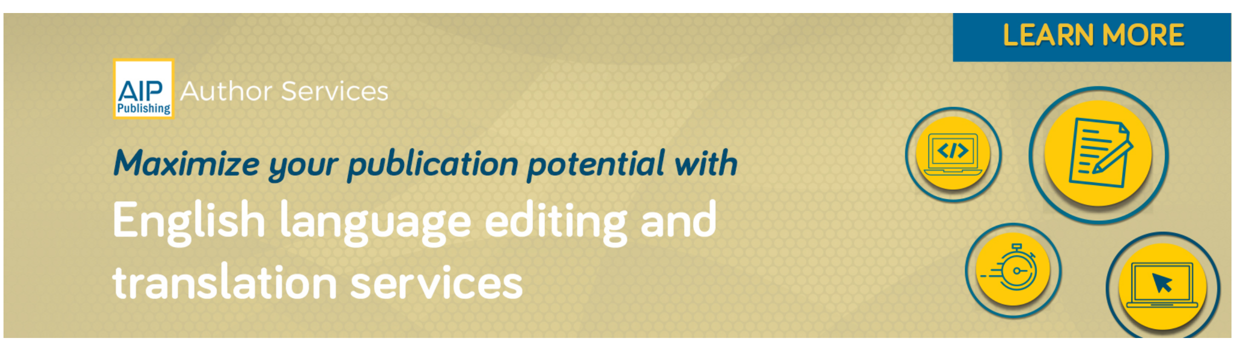

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Andri Fadillah Martin, Dyah Retno Wulandari, Tri Muji Ermayanti, et al.

Radiosensitivity and Phenotypic Characterization of Gamma Ray-Induced Mutant Population of Four *Capsicum annuum* L. Cultivars Grown in Screen House

Andri Fadillah Martin[a)], Dyah Retno Wulandari, Tri Muji Ermayanti, Betalini Widhi Hapsari, Erwin Al Hafiizh and Laela Sari

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Abstract. Efforts to increase *Capsicum annuum* L. production can be done by inducing mutations with gamma ray irradiation. The purpose of this study was to investigate the growth response of seedlings for survival rate after gamma ray irradiation and to characterize the phenotypes of four chili cultivars grown in a screen house. The phenotypic characterization was conducted at 28 weeks after planting in the screen house. Gamma ray irradiation affected all cultivar growth as reflected by increasing irradiation dose that reduced the survival rate. The LD_{50} values obtained were 372.63, 373.78, 373.78, and 499.03 Gy for Tanjung-2, Kencana, Kopay, and Anies cultivars, respectively. Plant habitus and leaf morphology was carried out according to the *Capsicum* descriptor from IPGRI using ten wild-type plants and 3 of Tanjung-2 and Kencana, 13 of Kopay, and 20 mutant candidates of Anies cultivars. The results showed that as compared to their wild-type plants, according to the *Capsicum* descriptor, the mutant candidates differed in stem color, nodal anthocyanin, stem shape, plant height, plant canopy average, branching habit, leaf density, and leaf shape. In contrast, the diameter of the stems, plant growth habit, presence of tillers, color, edge, length, and width of the leaves of wild type and mutant candidates were similar.

INTRODUCTION

Chili (*Capsicum annuum* L.) is one of the most important and vastly cultivated horticultural crops globally [1, 2]. Chilies are often used in the pharmaceutical, cosmetic, and food industries since they are rich in medicinal substances such as carotenoids, capsaicinoids, and vitamins A and C [3]. Chili cultivars that are widely cultivated in Indonesia are big and curly chilies. The grouping of those two cultivars was based on the difference mainly in their fruit surface appearance. The big chili has a smooth surface, while the curly chili has a wrinkled surface on its fruit [4]. There were 133 cultivars of big chili and 135 cultivars of curly chili that registered in the database of the Ministry of Agriculture [5].

In modern breeding for improving essential crop traits, e.g. yield, nutritional value, and secondary metabolite content, it is vital to have a wide genetic variety created by mutagenesis technique [6, 7]. Mutagenesis techniques can generate genomic diversity and have vast potential for crop improvement [8]. In addition, mutagenesis could produce new plants that are safe and relatively fast because the regulation of their post utilization is easier compared to genetically modified organism (GMO) plants, especially when combined with precise and comprehensive selection [8].

The mutagenesis technique with gamma ray irradiation is categorized as a physical mutation. Physical mutagens are preferably used to induce gross lesions, such as chromosomal abbreviation or rearrangements. Gamma radiation from cobalt (^{60}Co) radioactive is widely used since it has a high penetrating potential to penetrate deeper into plant

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tissue and is hazardous. However, gamma ray can irradiate whole plants and delicate materials, such as pollen grains. Accordingly, various mutants have been developed through gamma irradiation [9].

Several physical mutagenesis applications were reported on *Capsicum*. For example, first, to increase the number and weight of fruit by fast neutron irradiation on Nigerian pepper [10]. Second, to induce chromosome aberration to produce a new breeding parent [11]. Third, to increase tolerance to *Begomovirus* attack [12, 13], and finally to enhance capsaicin content in ornamental chilies [14].

Many results indicated that mutagenic effectiveness and efficiency decreased with an increase in mutagenic treatments [2, 13, 15–19]. Correspondingly, the lower or intermediate treatments of gamma rays and the alkylating agents were found more effective and efficient in creating maximum mutations with more minor biological damage [15]. The LD_{50} value can show intermediate treatment, thus, this value is essential to determine the optimal irradiation dosage. The LD_{50} value for each species or cultivar of the plant can also differ due to the genotype-dependent mechanism and the kind of propagule treated [20].

The LD_{50} value of cultivars Kencana, Lembang-1, SSP, Tanjung-2, and Seloka were 538.41, 448.84, 614.79, 422.64, and 629.68 Gy, respectively, using M_0 seeds propagule [13]. The water content of M_0 seeds used was 10–12%, with a viability of 90%. Increasing dosage of gamma ray irradiation caused alteration in physiological and agronomical traits of M_1 plant population [13]. The application of gamma ray irradiation for five chili seeds genotypes with ANS, GLR, HOT, SSR, and YUN codes was recommended ranging from 400 to 600 Gy, implying the LD_{50} value [12]. Innate response of grown seed to radiation treatment which is indicated at the LD_{50} value refers to radiosensitivity. Gamma irradiation treatment will produce phenotypic changes. Hence, the plants which undergo phenotype changes are called mutants candidate.

Mostly, mutant candidate plants have different characters from the wild type. Characterization of mutant candidates in the M_1 generation is necessary for determining mutation efficiency and identifying dominant character changes in plants [1]. The characterization of M_1 mutant generation of five Indonesian chili genotypes showed that nine out of the 14 characters that change from the wild type, i.e. plant growth type, stem color, branching type, leaf shape, leaf color, number of flowers per axil, fruit surface, fruit tip shape, and fruit base shape. In addition, some mutant plants have chimera leaves and become dwarf [12].

Hence, the efforts to increase chili production can be done by inducing mutations with gamma ray irradiation. Therefore, this study aimed to investigate the growth response of survival seeds rate after gamma ray irradiation and characterize the phenotypes of four chili cultivars grown in a screen house.

MATERIALS AND METHODS

Plant Materials

Two big chili cultivars, namely Tanjung-2 and Anies, and two curly chili cultivars, Kencana and Kopay (Table 1) were used in this study. All cultivars were obtained from seed providers and recommended farmers.

TABLE 1. Source of chili cultivars and dose of gamma ray irradiation used in this study.

No.	Cultivar name	Chili type	Plant origin and year released	Gamma ray irradiation treatments (Gy)
1	Tanjung-2	Big	Indonesian Vegetable Research Institute (IVEGRI), 2001	0 (control), 400, 500, 600, 700
2	Anies	Big	IPB University, 2015	0 (control), 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000
3	Kencana	Curly	IVEGRI, 2011	0 (control), 400, 500, 600, 700
4	Kopay	Curly	Payakumbuh local variety, 2009	0 (control), 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000

Radiosensitivity Determination

One hundred seeds of Tanjung-2 big chili and Kencana curly chili cultivars were irradiated by gamma rays at 0 (control), 400, 500, 600, 700 Gy (according to LD_{50} value of Gaswanto *et al*. [13]). Whereas for Kopay curly chili and Anies big chili cultivars were irradiated at 0 (control), 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 Gy. Irradiation was carried out at the National Nuclear Energy Agency of Indonesia.

Irradiated seeds were sown in a tray with the planting medium consisting of a mixture of sand, soil, husks charcoal, and compost in a ratio of 1:3:1:1. The planting tray has a 5 × 5 cm hole size. The seeds were sown in the screen house and became seedlings. At the 4th week after planting (WAP), the seedling's survival rate was calculated to determine the effect of gamma ray irradiation on chili seed (radiosensitivity test) and determined LD_{50} values.

Phenotypic Characterization

Eight WAP, the seedlings were moved from trays, then all seedlings were transferred to 10 cm × 15 cm polybags containing a mixture of soil, manure goat, and raw husk in a ratio of 3:1:1. All polybags were kept in the screen house. After 16 WAP, the plants were transplanted to bigger polybags (15 × 20 cm) containing the same planting medium. The plants were maintained in the screen house until the generative stage, in which all plants formed fruits and ready for characterization observation.

After 28 WAP the phenotypic characteristics of four chili cultivars grown in a screen house were recorded. Phenotypic characterization of plant habitus and leaf morphology was carried out according to the *Capsicum* descriptor [21] using 10 wild-type plants for each cultivar, 3 candidate mutant plants of Tanjung-2, 3 of Kencana, 13 of Kopay, and 20 of Anies cultivars, respectively. Seventeen characters were observed. Color-related characters were determined with color chart from the Royal Horticultural Society [22].

Data Analysis

The obtained seedling survival rate data were analyzed using CurveExpert 1.4 [23], and the LD_{50} fit equation and values were determined. The bar graph and linear fit regression were plotted using R statistical software version 4.04 [24] with *ggplot2* package [25].

RESULTS AND DISCUSSIONS

Radiosensitivity Determination

The survival rate of untreated chili seeds for Tanjung-2, Anies, Kencana, and Kopay cultivars was 41, 33, 49, and 47%, respectively, as shown in Fig. 1. The germination ability decreased along with the increase of gamma ray irradiation dose. The survival rate on all chili cultivars control treatments was lower than 50%; apparently, this was caused by the dormancy of its chili seed. Several methods have been applied to alleviate seed dormancy, such as thermal, mechanical, chemical, and hormonal treatment [26]. However, in our experiment, we did not pretreat the chili seeds with dormancy breaking treatment; therefore, we observed a low germination rate on all chili seed's cultivar control treatments. Consequently, these will also affect the basis of LD_{50} calculation because the 50% lethal dose (LD_{50}) will be based on the default germination rate of chili seeds without gamma ray irradiation.

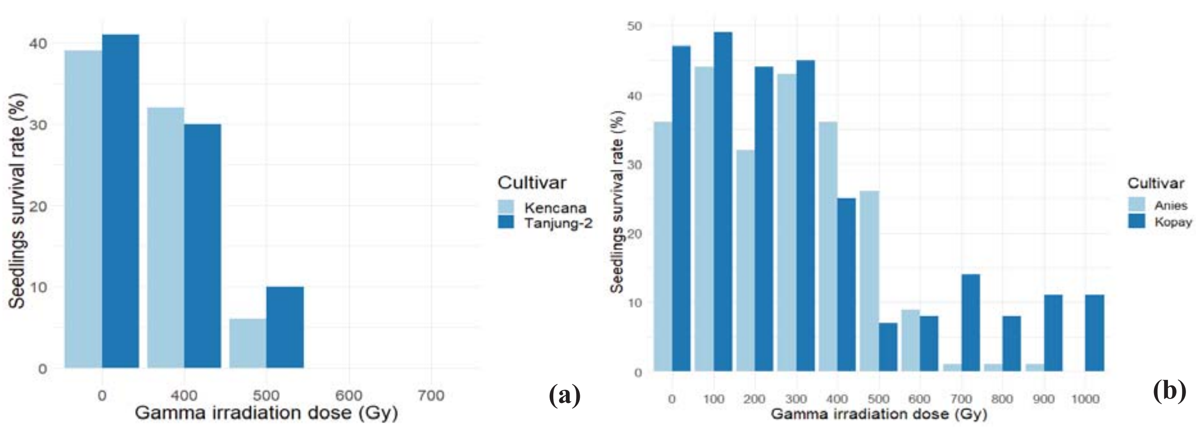

FIGURE 1. Seedling survival rate (%) of chili cultivars irradiated by various doses of gamma rays at 4 WAP. (a) Seedling survival rate of Kencana curly chili and Tanjung-2 big chili cultivars at 0–700 Gy doses. (b) Kopay curly chili and Anies big chili cultivars at 0–1,000 Gy doses.

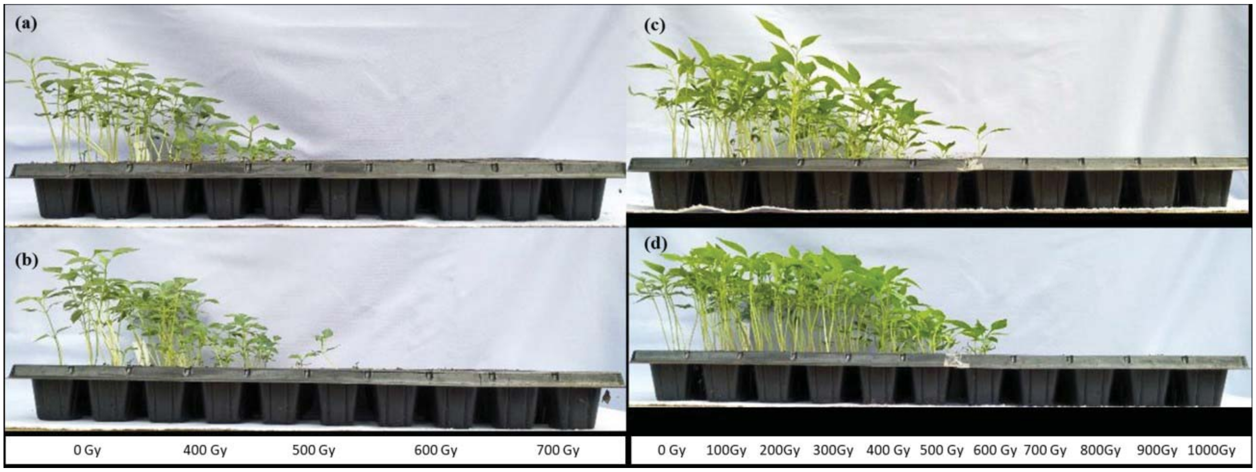

FIGURE 2. Survived seedlings of Kencana curly chili (a), Tanjung-2 big chili (b), Kopay curly chili (c), and Anies big chili (d) irradiated by various doses of gamma rays at 4 WAP.

The mutant clones developed in this study were based on LD_{50} value of Kencana and Tanjung-2 cultivars in the previous study [13]. The radiosensitivity data of the Kencana and Tanjung-2 cultivars were presented in Fig. 1(a) and Figs. 2(a) and 2(b). The seedling's survival rate of Kencana cultivar for 0, 400, 500, 600, 700 Gy were 39, 32, 6, 0, 0%, respectively. Likewise, the seedling's survival rate of Tanjung-2 cultivar for the same doses were 41, 30, 10, 0, 0%, respectively. The LD_{50} value for Kencana and Tanjung-2 cultivars were 373.78 and 372.63 Gy, respectively (Table 2), and the linear regression visualization was presented in Fig. 3.

Since no previous study reported mutant induction using gamma ray irradiation on Kopay and Anies chili cultivar, a broad range of gamma ray irradiation doses were used, i.e. 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 Gy. The radiosensitivity data of Kopay and Anies cultivar were presented in Fig. 1(b) and Figs. 2(c) and 2(d). The seedling's survival rate of Kopay cultivar for mentioned gamma ray doses was 47, 49, 44, 45, 25, 7, 8, 14, 8, 11, 11%, respectively. Moreover, the seedlings survival rate of Anies cultivar for mentioned gamma ray dose were 36, 44, 32, 43, 36, 26, 9, 1, 1, 1, 0%, respectively. The LD_{50} value for Kopay and Anies cultivars were 499.03 and 585.43 Gy, respectively (Table 2), and the linear regression visualization was presented in Fig. 3.

Gamma ray irradiation affected all cultivar growth as demonstrated in this study. Their LD_{50} values ranging from 400–600 Gy were in accordance with the previous studies on several chili cultivars [12, 13]. Determination of radiosensitivity is a prerequisite for large-scale irradiation for induction of mutation. Radiosensitivity can be assayed by chromosome breakage, somatic mutation, growth inhibition, or lethality. Various biological, physical, and chemical factors such as water content of seeds, age of seeds, genotype, state of cellular development, chromosome number, storage, oxygen, chemicals, temperature, ionization density, and combined treatment can modify the effects of radiation in plants and genotype-dependent [20]. Notably, various gamma ray doses treated on the chili decreased the seed survival rate (%) by increasing gamma ray doses. Similar responses also observed in the previous study using chili pepper [16], banana [17], *Tacca* [18], and maize [19].

TABLE 2. LD_{50} value of four chili cultivars (CurveExpert 1.4).

No.	Chili cultivar	Equation	LD_{50} value (Gy)
1	Kencana	$y = 51.2x - 7.69e^{-2}$	373.78
2	Tanjung-2	$y = 44.2x - 6.38e^{-2}$	372.63
3	Kopay	$y = 49.5x - 5.39e^{-2}$	499.03
4	Anies	$y = 46.0x - 5.05e^{-2}$	585.43

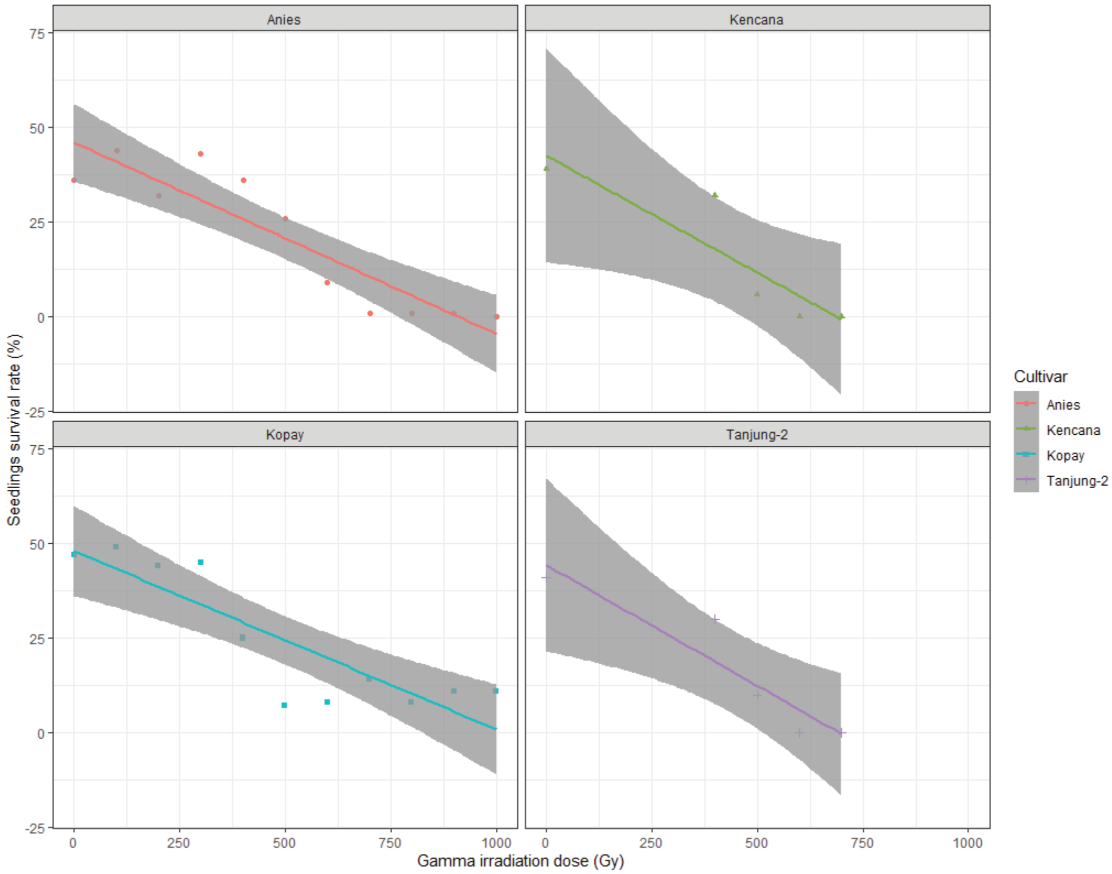

FIGURE 3. LD_{50} curve of Anies, Kencana, Kopay, and Tanjung-2 irradiated-chili cultivars at 4 WAP.

Phenotypic Characterization

Phenotypic traits at M_1 generation were characterized in order to analyze any possible change due to irradiation treatment. It is possible to detect dominant mutation on M_1 generation [1, 2], although stable phenotype usually observable at M_2 due to segregation of the alleles to create stable homozygous recessive of dominant alleles [27]. Furthermore, this background is used as the basis of our phenotypic characterization of M_1 generation and screens it visually to observe possible phenotypic changes, i.e. disease resistance, plant habitus, color changes, or growth [28].

The mutant candidate from Kencana curly chili has three unique morphological characters in terms of stem shape, branching habit, and leaf shape compared to the wild type (Table 3). Additionally, the mutant candidate of Tanjung-2 big chili has five unique characters in terms of branching habit, plant height, plant canopy width, leaf density, and leaf color (Table 4). Meanwhile, the mutant candidate of Kopay curly chili has four unique characters in terms of plant canopy width, stem length, branching habit, and leaf density (Table 3). Similarly, the mutant candidate of Anies big chili has seven unique characters in stem color, nodal anthocyanin, stem shape, plant height, branching habit, leaf density, and leaf color (Table 4).

It was found that Anies big chili cultivar has the most significant number of different mutant characters, indicating that Anies big chili cultivar has the highest mutation efficiency. Accordingly, the order mutation efficiency could be determined based on the number of dominant mutant characteristics formed, i.e. Anies > Tanjung-2 > Kopay > Kencana. The phenotypic appearance of each chili cultivar with the comparison between the wild type and the mutant candidate is presented in Fig. 4.

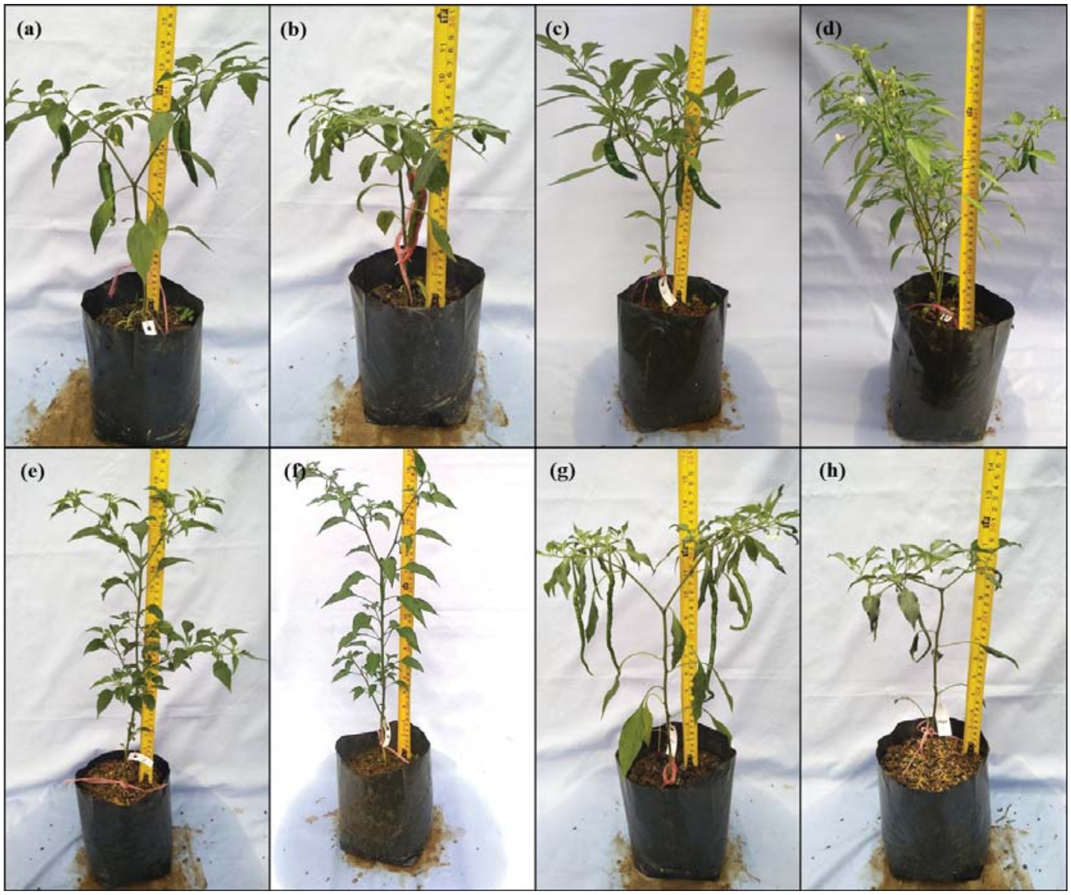

FIGURE 4. Phenotypic performance of Tanjung-2 wild type (a), Tanjung-2 mutant candidate 400 Gy-gamma-irradiated (b), Anies wild type (c), Anies mutant candidate 600 Gy-gamma-irradiated (d), Kencana wild type (e), Kencana mutant candidate 400 Gy-gamma-irradiated (f), Kopay wild type (g), and Kopay mutant candidate 500 Gy-gamma-irradiated (h) at 28 WAP.

In this study, the changing of vegetative characters of mutant candidates from our chili cultivars was similar to other chili cultivar mutagenesis studies. Morphology changes on five chilies mutant cultivars, studied in Indonesian Vegetable Research Institute, Ministry of Agriculture are plant growth habit, stem color, branching habit, leaf shapes, and leaf color [12]. The irradiation treatment provides diversity in plant height, number of branches, number of leaves, and leaf shapes produced on ornamental chili "Garda Firework" [14]. Several morphological variations were observed on the mutant of 'Hot pepper smart' from Bali, i.e. plant height, leaf size, the pattern of the branch, and the number of the main stems [29].

A mutation is a random process; therefore, the genome is damaged randomly in each cell of the treated seed in M_1 generation [29]. A mutation is defined as any alteration in the DNA sequence. Gamma rays produce energy, and this can cause molecular damage through a spontaneous reaction in which the DNA molecule absorbs radiation energy. In an indirect reaction, energy is not absorbed by the DNA but by other molecules in cells that produce free radicals, resulting in changes in DNA molecules. Changes in the DNA base sequence will cause changes in the protein encoded by genes [30]. Genotypic variability and correlations between the environmental parameters will be expressed as different phenotypic of mutant [31].

TABLE 3. Characteristic comparison among wild type and mutant candidates of Kencana and Kopay curly chili.

No.	Character		Kencana		Kopay	
			Wild type	Mutant	Wild type	Mutant
1	Annual life cycle		100	100	100	100
2	Stem color	Green	60	66.67	0	0
		Green with purple stripes	40	33.33	100	15.38
		Purple	0	0	0	84.61
3	Nodal anthocyanin	Green (YGG147A)	10	0	0	0
		Dark purple (PGN77A)	80	33.33	100	100
		Dark purple (PGN77B)	10	0	0	0
		Dark purple (PG79A)	0	66.67	0	0
4	Stem shape	Cylindrical	100	33.33	100	71.42
		Angled	0	66.67	0	28.58
5	Plant height	<25 cm	0	0	0	0
		25–45 cm	20	33.33	40	14.29
		46–65 cm	80	66.67	60	85.71
6	Plant growth habit	Intermediate	0	0	0	0
		Erect	100	100	100	100
7	Average of plant canopy width (cm)		26.05	24	38.2	29
8	Average of stem length (cm)		31.3	35.16	24.35	16.71
9	Average of stem diameter (cm)		3.36	2.64	4.3	3.82
10	Branching habit	Sparse	0	33.33	0	100
		Intermediate	70	66.67	80	0
		Dense	30	0	20	0
11	Sparse tillering		100	100	100	100
12	Leaf density	Sparse	50	33.33	0	14.28
		Intermediate	30	33.33	100	85.71
		Dense	10	33.33	0	0
13	Leaf color	Green (GGNN137A)	80	100	100	100
		Yellow (YGP147A)	20	0	0	0
14	Leaf shape	Ovate	50	0	0	14.28
		Lanceolate	50	100	100	85.71
15	Undulate lamina margin		100	100	100	100
16	Average of mature leaf length (cm)		5.51	6.35	7.17	6.24
17	Average of mature leaf width (cm)		2.49	2.62	2.39	2.08

Values are in percentage (%), except when specified as in cm in character column. Color classification (in parentheses) follows RHS color chart code [22]

TABLE 4. Characteristic comparison among wild type and mutant candidates of Tanjung-2 and Anies big chili.

No.	Character		Tanjung-2		Anies	
			Wild type	Mutant	Wild type	Mutant
1	Annual life cycle		100	100	100	100
2	Stem color	Green	0	0	0	40
		Green with purple stripes	100	100	100	60
3	Nodal anthocyanin	Green (YGG147A)	0	0	0	10
		Dark purple (PGN77A)	100	100	0	70
		Dark purple (PGN79A)	0	0	100	20
4	Stem shape	Cylindrical	100	100	0	60
		Angled	0	0	100	40
5	Plant height	<25 cm	0	0	0	5
		25–45 cm	100	100	50	95
		46–65 cm	0	0	50	0
6	Plant growth habit	Intermediate	0	0	0	5
		Erect	100	100	100	95
7	Average of plant canopy width (cm)		38	25.16	34.3	31.1
8	Average of stem length (cm)		21.1	12.67	20.5	16.7
9	Average of stem diameter (cm)		4.68	3.52	4.61	4.18
10	Branching habit	Sparse	90	33.33	0	0
		Intermediate	10	33.33	100	55
		Dense	0	33.33	0	45
11	Sparse tillering		100	100	100	100
12	Leaf density	Sparse	80	0	100	0
		Intermediate	20	100	0	65
		Dense	0	0	0	35
13	Leaf color	Green (GG137A)	30	0	0	0
		Green (GGNN137A)	70	100	40	80
		Green (GGNN137B)	0	0	60	15
		Green (YGG147A)	0	0	0	5
14	Leaf shape	Ovate	0	0	20	50
		Lanceolate	100	100	80	50
15	Undulate lamina margin		100	100	100	100
16	Average of mature leaf length (cm)		5.51	6.35	7.48	6.85
17	Average of mature leaf width (cm)		2.49	2.62	2.84	2.57

Values are in percentage (%), except when specified as in cm in character column. Color classification (in parentheses) follows RHS color chart code [22]

CONCLUSION

Gamma ray irradiation at 400–600 Gy LD_{50} was able to produce mutant candidates from the four chili cultivars, as can be seen from the seedling's survival rate from various doses of gamma ray. The seedling survival rate of mutant was reduced to half at irradiation doses close to the LD_{50} values. Cultivar Anies demonstrated the highest efficiency of mutation in contrast to cultivar Kencana based on the number of dominant character changes resulting from phenotypic characterization based on *Capsicum* descriptors. Overall, the phenotypic characterization proved to be used for early detection of mutant chili candidates.

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