BIODIVERSITAS
Volume 20, Number 3, March 2019
Pages: 862-866

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200333

The diversity of morphological characteristics and chemical content of *Celosia cristata* plantlets due to gamma ray irradiation

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Manuscript received: 2 January 2019. Revision accepted: 26 February 2019.

Abstract. *Muhallilin I, Aisyah SI, Dewi Sukma D. 2019. The diversity of morphological characteristics and chemical content of* Celosia cristata *plantlets due to gamma ray irradiation. Biodiversitas 20: 862-866. Celosia cristata* L. or better known as Jengger Ayam is an ornamental plant that has a unique flower shape with attractive colors. Besides, this plant also contains antioxidants and chemical compounds that are used for traditional medicine to treat high blood pressure, and diabetes or as eye ailments. Increased diversity of *C. cristata* through hybridization techniques is constrained by flowers forming small grains that makes it difficult for castration and isolation of reproductive organs. This study aimed to increase *C. cristata* diversity of morphology and chemical content using mutation techniques induced by gamma irradiation. Plantlets were irradiated at 0, 25, 50 and 75 Gy doses. Scoring was conducted for 5 weeks until MV6 generation. Planlets were acclimatized for 3 weeks. The surviving plants were moved onto experimental land until seeded and tested for phytochemicals. Gamma irradiation with 25, 50 and 75 Gy doses produced 4 clones that have morphological changes in color and shape of the stem, leaves, and flowers. Changes in chemical content were observed with the appearance of triterpenoid compounds in 1 clone irradiated with 25 Gy.

Keywords: In vitro, mutant, ornamental plant

INTRODUCTION

Celosia cristata L. or better known as Jengger Ayam is a member of the family Amaranthaceae which grows wide in South America, Africa, and Asia. This plant has a unique flower shape with variety of colors which is widely used for landscape ornamental plants, potted ornamental plants, as cut flowers and for decoration (Porat et al. 1995; Taha and Wafa 2012). The chemical content of *C. cristata* consists of antioxidants and chemical compounds such as saponins, flavonoids, and betalains, and phenol glycosides (Zhang et al. 2016). *C. cristata* has long been used as a traditional medicinal plant in China to treat high blood pressure, paralysis, cataracts, keratitis, diabetes, iridocyclitis, caligo corneae, and sarcoptidosis. The leaves and flowers of this plant are used as vegetables in India, West Africa, and South America. In addition, it can be used as a natural dye and has the potential as a cosmetic ingredient to prevent aging (Pyo et al. 2008; Wang et al. 2010; Sun et al. 2011; Badawi et al. 2015). Ornamental plant industry is faced with the demands of consumers to provide flowers that have added value and unique characters such as flower shape, flower color, and have a long lifespan. As a cut flower, *C. cristata* still encounters various obstacles including the relatively long time of flower production, which is around 4 months (Zuck 2015) and the flower color spectrum tends to be less than Chrysanthemum.

Morphological and chemical diversity of *C. cristata* needs to be developed continuously. Breeding *C. cristata* through hybridization is constrained in the small size of the flower which ranges from 6-8 mm gathered to form a set of inflorescences (Van Steenis et al. 1978), and it is self-pollinated plant (UPOV 2001). This fact makes castration and isolation of reproductive organs more complicated. Induce mutation using physical or chemical mutagens combined with *in vitro* culture technique is an effective method for plant improvement in several vegetative plants since it provides an opportunity to increase the variability of important economic cultivars (Bala and Kanwar 2015).

Induced mutations with gamma-ray irradiation have been widely used in plants. It can induce morphological changes in plant tissues and various biochemical changes at the cellular level to form new mutants that have new productivity potential, produce higher metabolite content and develop varieties with better economic and agronomic characteristics (Wi et al. 2007; Rahimi and Bahrani 2011). In Chrysanthemum flowers, gamma irradiation causes changes in the shape and color of flowers (Kim et al. 2016). Changes in the morphological character of plants due to gamma irradiation also occur in *Polianthes tuberosa*, *Delphinium malabaricum*, and *Rosa hybrida*. (Bala and Kanwar 2015; Kolar et al. 2015; Kayalvizhi et al. 2017). Changes in the chemical content of gamma-ray irradiation occur in *Sesuvium portulacastrum* showing an increase in the content of ecdysteroid 20-hydroxyecdysone which functions to cure muscle atrophy and as protective cosmetic ingredients against UV light, while in *Sesamum indicum*, there is an increase of amino acid content and fatty acids in the seeds (Hussein and Hamideldin 2015; Kapare et al. 2017).

Previous research showed the radiosensitivity of *C. cristata* planlets LD_{50}, LD_{30} and LD_{20} were 68.73, 46.68 and 35.65 Gy respectively. The Mutant response of plantlets produced in MV3 generation is abnormal growth such as shortening of internode and curly leaves (Hayati et al. 2016). The aim of this research was to obtain morphologically and chemically variants of *C. cristata* plants as a result of gamma irradiation.

MATERIALS AND METHODS

The research was conducted from August 2017 to August 2018 at the Network Culture Laboratory 1 Department of Agronomy and Horticulture, Bogor Agricultural Institute, Bogor, Indonesia and Sabisa Farm, Bogor, Indonesia. Plantlets of *C. cristata* consisted of buds containing 1-2 nodes with 3-5 leaves, and were planted under sterile conditions on MS media without plant growth regulators (MS0). Plantlets were irradiated at doses of 0, 25, 50 and 75 Gy of gamma-rays at the National Nuclear Energy Agency of Indonesia, South Jakarta, Indonesia. Plant maintenance was carried out for 5 weeks for each generation then they were cultured until MV6 generation. MV6 plants were then transferred to pre-acclimatization media to strengthen the plants for 1 month before acclimatization. Prepared acclimatized *C. cristata* plantlets were planted in a closed plastic cup with some holes using sterile media in the combination of 1: 1 cocopeat and husk for 3 weeks. Weekly readings were conducted on plantlets including the number of shoots, number of leaves, number of nodes, and number of roots measured. In plants that were successfully acclimatized the reading was taken on plant height (cm), stem diameter (cm), number of branches, and number of flowers. To describe qualitative characters, the colors and shapes of leaves, stems, and flowers were observed following the UPOV standard for Celosia (2001) as well as the special phenotypic characters that appeared in irradiated plants. Flower colors were compared using the RHS mini color chart guide. Data is processed using Microsoft Excel and PB STAT.

Phytochemical content was analyzed based on the Harborne (1984) method to determine the presence of secondary metabolites of alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids. The color of white sediment indicates the presence of alkaloids with Meyer reagents, brown deposits with Wagner reagent, and red deposits by Dragendroff reagents. The orange color with Mg^{2+} powder, HCl solution in ethanol (1: 1) and amyl alcohol solution showed the presence of flavonoids. The addition of 1% $FeCl_3$ to the extract gives a dark blue color indicating the presence of tannin compounds. Dissolution of samples with distilled water and heating and shaking will show the presence of saponin compounds. The sample extract with Lieberman Burchard reagent showing red or blue color indicates the presence of terpenoids, while the blue or green color indicates the presence of steroids.

RESULTS AND DISCUSSION

Planlets growth until acclimatization

Mutations induced by gamma irradiation occur randomly so that changes in an individual plant can be seen by the existence of extreme values on the graph. In the character of the number of plantlet buds, there was an extreme value at 50 Gy with a total of 15 shots. The character of the number of leaves in plantlets irradiated at 25 Gy has an extreme value of 72 leaves. The character of the number of nodes has 2 extreme values at different radiation doses. At a dose of 25 Gy, there were 17 nodes while at a dose of 50 Gy there were only 14 nodes (Figure 1).

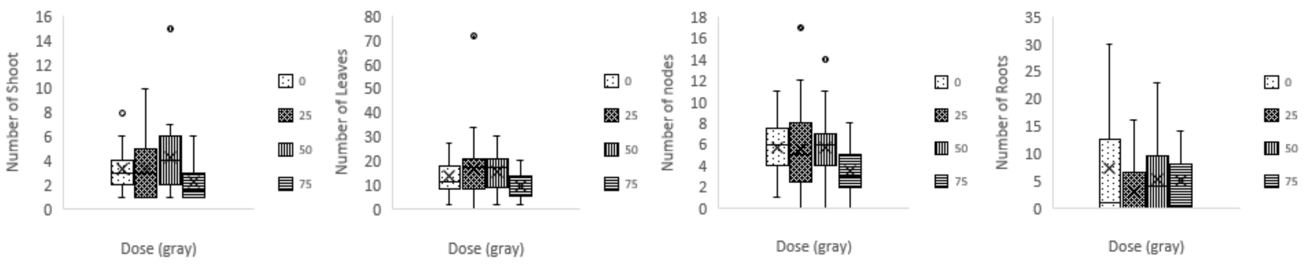

Figure 1. Boxplot of various characters on planlets in MV6 generation

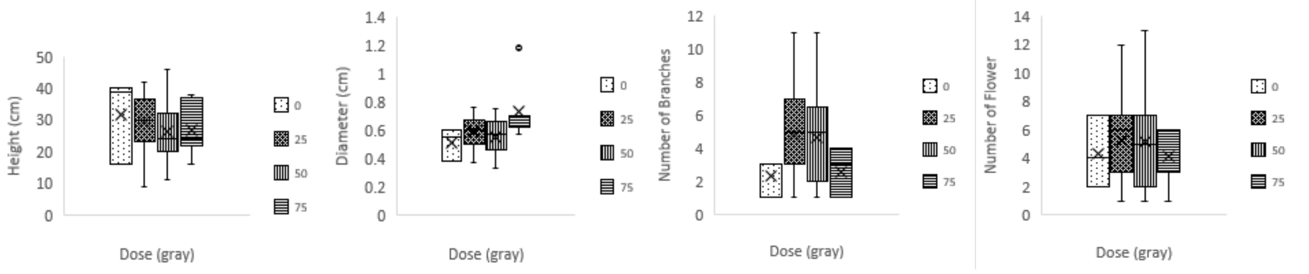

Figure 2. Boxplot of various characters on acclimatized plants

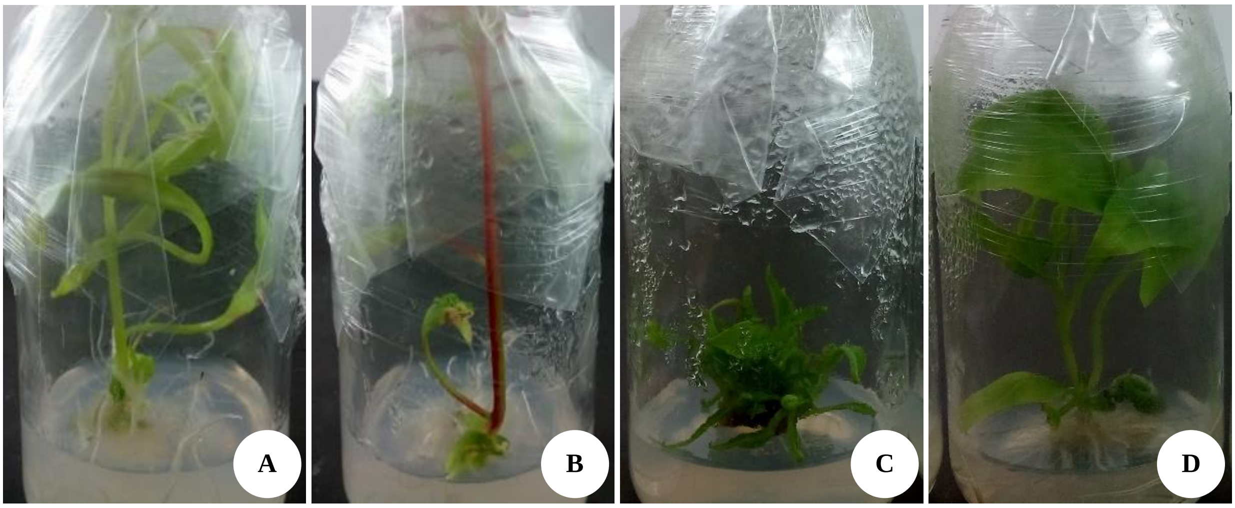

Figure 3. Morphology of plantlets in MV4 generation. A. Control planlets, B. 50 Gy mutant plantlets which had chimera, C. irradiated plants with internode shortening and crowded leaves, D. Irradiated plant leaves larger than control plants

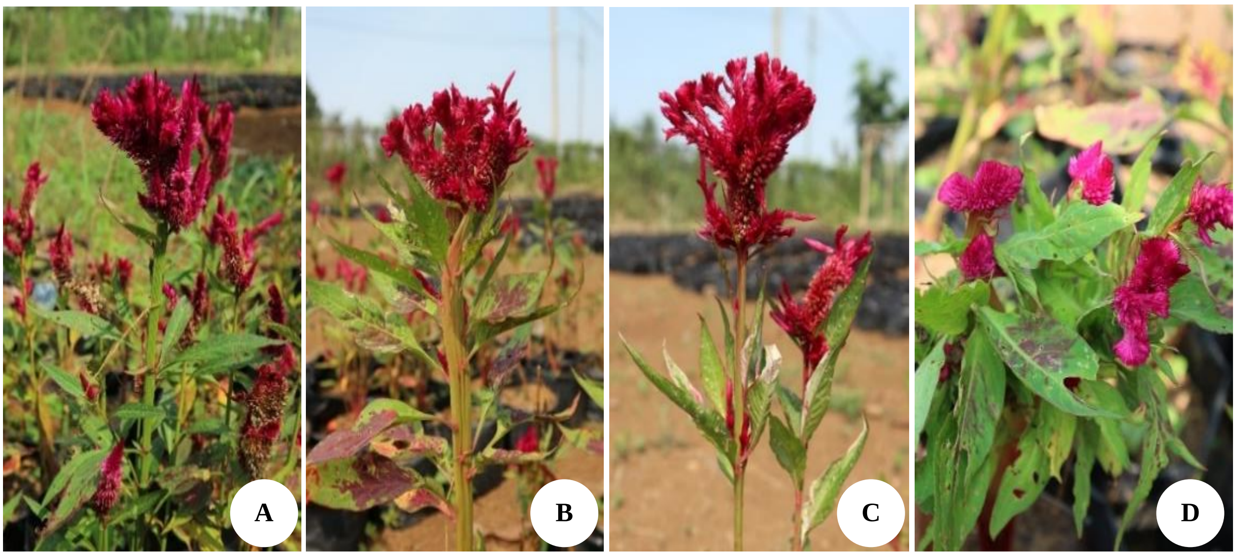

Figure 4. Successfully acclimatized control plant (A) and irradiated plants C3U2 1.3.2 (B), C1U2 5.1.1 (C), C2U1 6.1.5 (D)

After 3 weeks of acclimatization, the plants were transferred to the experimental field. Extreme values of plant clones that were successfully acclimatized were found in trunk diameter characters with a value of 1.18 cm in irradiated clones at 75 Gy (Figure 2). In the character of the number of roots, height, the number of branches and the number of flowers, no extreme value was found. The extreme value of the observed character has greater value than the control.

In *Dianthus,* there was a diplontic selection phenomenon where new mutants were formed in the MV3 generation. This mutant did not appear in next generation (Aisyah et al. 2009). Kayalvizhi (2017) reports that in the *Polianthes tuberosa* L. which were irradiated with gamma rays, there were leaves which were variegated in the M1V2 generation and changed in the color and number of flowers.

After 3 weeks of acclimatization, the plants were then transferred to the experimental field. The morphological characteristics of the plant were observed until the plants produced flowers and seeds. Clone C3U2 1.3.2 which was irradiated at 75 Gy showed changes in the shape of the broad stems with twisted ends and reddish colors (Figure 4.B) whereas, in control plants, there were also plants with slightly wider stems but not twisted in green and reddish in the lower part (Figure 4.A). Discoloration of stems and leaves (Figure 4.C) was indicated by the presence of white streaks on the stems and leaves (variegated). This change only occurred in C1U2 5.1.1 which was irradiated at 25 Gy. The flower of the mutant plant in Figure 4.D only formed sterile flowers. Those plants did not produce seeds (sterile). This change occurred in plants irradiated at 50 Gy, C2U1 6.1.5. The color and shape of the flower and leaf color did not indicate the change. Both the control plants and the irradiated plants were reddish purple (Purple Group 71 A) with cristate shape of flower. The color of leaves was green with purple in the main vessels of the leaves. The diversity of qualitative characteristics in plants was summarized in Table 1.

Gamma rays are one type of electromagnetic wave radiation that produces energy by releasing electrons from target cells which can cause direct damage to molecules due to the energy of radiation is absorbed by DNA molecules. Indirectly, that energy is not absorbed by DNA but with other molecules in the cell to produce free radicals which ultimately cause changes in DNA molecules. It includes damage to double strand, loss of nitrogen base and chemical changes in base structures that can change pairs of bases causing gene mutations (Chahal and Gosal 2003). This has a direct impact on living tissue because it can cause damage or changes to important components in the cell, causing changes in morphology, anatomy, biochemistry and plant physiology (Mba et al. 2011). Morphological changes in Rose's internode explants which were irradiated by gamma rays at doses of 25, 40 and 55 Gy were indicated by the presence of albino leaves, variegated leaves, fused leaves and flowers with different colors were also found (Bala and Kanwar 2015).

Phytochemical analysis

Successfully acclimatized plantlets were analyzed by phytochemical tests to determine the presence of flavonoids, alkaloids, tannins, saponins, quinones, steroids and triterpenoids. In this study, phytochemical tests were carried out on 2 control plants and 3 plants at each irradiation dose (table 2). Phytochemical test resulted from control plants (K1 and K2) showed the presence of flavonoid, saponin and steroid compounds. Plant C1U3 2.3.1 shows triterpenoid content which controls did not have. That plant had been irradiated at a dose of 25 Gy. Triterpenoids are secondary metabolites with carbon frames derived from six isoprene units and derived from C 30 acyclic hydrocarbons, namely squalene. These compounds are cyclic or acyclic and often have alcohol, aldehyde, or carboxylic acids (Harborne 1984). Most Triterpenoid compounds have prominent physiological activities so that in daily life, many of them are used as medicine for diabetes, menstrual disorders, snake failure, skin disorders, liver damage, and malaria. Meanwhile, for plants containing Triterpenoid compounds, there are ecological values because these compounds work as anti-fungus, insecticides, anti-predator, anti-bacterial and anti-viral (Robinson 1995).

Table 1. Diversity of morphological characters in acclimatized *C. cristata*

Clone	Intensity of anthocyanin coloration at base	Shape in cross section	Leaf shape	Shape of leaf apex	Fertility of inflorescence
Control	Strong, medium, weak	Circular, flattened	Ovate, elliptic	Long acuminate	Fertile
C1U2 5	Strong, medium	Circular, flattened	Ovate, elliptic	Long acuminate	Fertile, sterile
C1U3 4	Strong	Circular, flattened	Ovate, elliptic	Long acuminate	Fertile
C1U3 2	Strong, medium	Circular	Ovate, elliptic	Long acuminate	Fertile
C1U5 5	Medium, weak	Circular	Ovate, elliptic	Long acuminate	Fertile
C1U2 3	Strong	Flattened	Ovate	Long Acuminate	Fertile
C2U1 2	Strong, medium	Circular	Ovate, elliptic	Long acuminate	Fertile, sterile
C2U1 5	Strong, medium, weak	Circular, flattened	Ovate, elliptic	Long acuminate	Fertile
C2U1 6	Strong, medium, weak	Circular	Ovate, elliptic	Long acuminate	Fertile
C2U2 4	Strong, medium	Circular, flattened	Ovate, elliptic	Long acuminate	Fertile
C3U1 3	Strong, medium	Circular, flattened	Ovate, elliptic	Long acuminate, short acuminate	Fertile
C3U2 1	Strong, medium	Circular, flattened	Ovate, elliptic	Long acuminate, short acuminate	Fertile

Table 2. Test results of phytochemical compounds on *C. Cristata*

Clone	Dose (Gy)	Flavonoid	Alkaloid	Tanin	Saponin	Steroid	Triterpenoid
K1	0	+	-	-	+	+	-
K2	0	+	-	-	+	+	-
C1U2 5.1.1	25	+	-	-	+	+	-
C1U5 5.4.1	25	+	-	-	+	+	-
C1U3 2.3.1	25	+	-	-	+	+	+
C2U2 4.1.1	50	+	-	-	+	+	-
C2U1 6.9.1	50	+	-	-	+	+	-
C2U1 2.1.1	50	+	-	-	+	+	-
C3U2 1.3.2	75	+	-	-	+	+	-
C3U2 1.3.1	75	+	-	-	+	+	-
C3U1 3.1.1	75	+	-	-	+	+	-

Note:-= not identified, + = identified

Gamma irradiation has energy that is able to change the atomic structure to ionize so that it directly affects the tissues (Mba et al. 2011). The response of ionizing is production of reactive oxygen species (ROS) as radiolysis of cellular water. These ROS are reported as free radicals that play an important role during plant defense responses and induce changes in plant (Esnault et al. 2010; Moghaddam et al. 2011). These changes include cellular structure and metabolic processes such as enlargement of the thylakoid membrane, photosynthetic changes, modulation antioxidant systems and accumulation of phenol compounds (Kovács and Keresztes 2002; Wi et al. 2007). The study of Ramabulana et al. (2015) reported the presence of the chalcone, phloretin-3',5'-di-C-β-glycopyranosyl in *Phaseolus vulgaris* which were irradiated by gamma rays so that gamma radiation has a function against oxidative stress.

Irradiation with doses of 0, 25, 50 and 75 Gy on *C. cristata* plantlets on MV6 generation showed changes in morphological characters in the stem, leaves, and flowers. These changes occurred in 4 plants. Changes in chemical content occurred in clone C1U3 2.3.1 that irradiated at 25 Gy. Change in chemical content was indicated by the triterpenoid content.

ACKNOWLEDGEMENTS

The author would like to thank Bogor Agricultural University, Indonesia and Indonesia Endowment Fund for Education, Ministry of Finance, the Republic of Indonesia for the financial support and facilities provided in this study.

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