Journal of Tropical Crop Science Vol. 3 No. 2, June 2016
www.j-tropical-crops.com

Radiosensitivity Levels of *In Vitro* Cultured *Celosia cristata* Planlets by γ - Ray Irradiation

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Abstract

Plantlets of the ornamental plant *Celosia cristata* were irradiated with gamma rays to increase their genetic diversity. This study was aimed to establish the lethal levels of gamma radiation (LD_{20}, LD_{30} and LD_{50}) for *C. cristata* plantlets. The irradiation doses used were 0, 25, 50 and 75 Gy. The growth of irradiated plantlets was evaluated to the third generation. Irradiated *C. cristata* MV1 plantlets showed a decrease in growth, with plantlets irradiated at 75 Gy showing only 30% survival. Abnormal growth characteristics observed in the third generation plantlets included the shortening of internodes, and curling of leaves. LD_{50}, LD_{30} and LD_{20} of *C. cristata* were 68.73 Gy, 46.68 Gy and 35.65 Gy, respectively.

Keyword: ornamental plant, genetic diversity, gamma irradiation, lethal dose.

Introduction

C. cristata (2n = 36) is one of the tropical ornamental plants that have the potential to be developed as plants for landscaping, potted plants, and flowers (Porat et al., 1995). *C. cristata* also has medicinal properties (Ranjan and Deokule, 2013; Gholizadeh et al., 2004). The potential of the flowers of *C. cristata* relates to their striking and bright colors (Ahmad and Dole, 2014). *C. cristata* was initially found growing in areas with a dry, tropical climate in Africa and Asia. However, it is now cultivated throughout the world. *C. cristata* grows well in fertile moist soils, but can also l be grown in most soil categories, including acid soils, sands, loamy and clay soils. The optimum soil temperature for growing *C. cristata* is 16° C (Gilman and Howe, 1999). In addition to their unique shape, the flowers of *C. cristata* are long lasting, particularly when supplemented with plant growth hormones (Shanan et al., 2014).

Genetic variations might occur in undifferentiated cells, callus, tissues and morphological traits of *in vitro* cultured plants. Genetic variability raised from tissue culture propagation has provided a new tool to the breeders, particularly for crops which are either difficult to breed or have narrow genetic base (Krishna et al., 2016). In addition *in vitro* culture does not require an extensive area for planting. *In vitro* methods for the development of *Celosia* have been reported (Bodhipadma et al., 2010; Taha and Wafa, 2012; Bakar et al., 2014). Taha and Wafa (2012) reported that bud explants grown in a basic medium MS can provide the basis for *in vitro* regeneration of *Celosia* flowering plants.

Another method than can potentially be used to increase plant genetic diversity is by mutation breeding based on gamma ray irradiation. Gamma rays are high-frequency electromagnetic waves that carry energy protons that can penetrate plant cells and cause the ionization process (Aisyah, 2006). Ionization in the plant cells leads to the disruption of metabolic processes. Generally low doses of gamma ray irradiation have fewer side effects than higher doses, the latter sometimes causing cell damage that can directly affect plant phenotype, including damage various cell organelles and biochemical components (Ali et al., 2016).

Among the physical mutagens, gamma rays are the most common mutagen used in mutation breeding. Mutations of this type have been used in the fields of medicine, food preservation, and for the mutation breeding of plants (IAEA, 2011). Mutations based on gamma-ray irradiation can potentially produce mutant plants with improved morphological and physiological characteristics. The combination of *in vitro* culture and mutations might potentially result in greater genetic diversity, which is the main objective of ornamental plant breeders.

In vitro mutagenesis methods to generate genetic diversity has been reported. Jala and Bodhipadma

(2011) reported that the acute effects of mutation-induced gamma rays in *C. cristata* have been able to generate mutant plants with different numbers of roots, and differences in leaf width and leaf size, relative to control plants. Gamma ray irradiation had also resulted in color changes of the petals of *Spathoglottis plicata* Blume (Romeida, 2012). Aisyah (2006) reported that the use of gamma ray has resulted in the production of 87 mutants in carnation (*Dianthus caryophyllus*)

Growth inhibition is the most common indicator of the adverse effects of irradiation. The LD_{50} (Lethal Dose) is the dose required to kill 50% of a tested population after a specified test duration. The LD_{50} value of the *in vitro* culture of mini roses is 3 Krad, and irradiated plantlets changed their petal color from pink or red to white (Handayati et al., 2001). Other indicators of changes following irradiation are growth inhibition or lethality, somatic mutations, chromosome fracture and changes in the number and size of chromosomes (Datta, 2001). The changes that occur after irradiation can be include morphological changes in in relation to size, shape, and color of the leaves and flowers.

This study aimed to define the lethal dose (LD) of radiation with gamma rays for *C. cristata* plantlets *in vitro.*

Material and Method

Plant Material

The study was conducted in the Tissue Culture Laboratory 1, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB, Bogor. Eight-week-old C. *cristata* plantlets were used in this study. Plantlets were cultured on media MS + TDZ 0.5 mg. L^{-1} for four weeks for shoot induction, and then subcultured to medium to MS0 medium for another four weeks. Scoring was conducted on the growth of the first (MV1) to the third generation (MV3) of the irradiated plantlets. MV1 and MV2 irradiated plantlets were planted on MS0 media, whereas MV3 on MS + NAA (0.5 mg. L^{-1}) for root induction.

Gamma Ray Irradiation

Five jars containing five uniform plantlets each were irradiated using Gammacell 220, a Cobalt 60 irradiator (Atomic Energy of Canada) at 0 (control), 25, 50 and 75 Gy. Gamma-ray irradiation was performed in PAIR BATAN Jakarta in July 2016. Irradiated *C. cristata* plantlets were maintained and monitored for its growth between July to December 2016.

Statistical Analysis

The values of radiosensitivity were determined using the curve-fit analysis, a statistical analysis program to determine the best equation of the death rates of a population (Finney 2005). Curve-fit model adjusts the numerical values of the MV2 plantlets at 10 weeks after irradiation. A model that has the highest correlation coefficient (R) was selected to determine the lethal doses of *C. cristata*.

Results

The increasing doses of irradiation resulted in increasing percentage of the dead plantlets (Figure 1). Plantlets irradiated at 75 Gy showing only 30% survival at 10 weeks after irradiation (WAI) (Figure 1). Celosia plantlets survived up to four weeks after irradiation, but plantlets irradiated with the high doses (50-75 Gy) died at 8 WAI. The symptoms prior to plants' death were browning of old leaves, which were gradually spread to the upper shoots of the plantlets. These symptoms demonstrated damaged cells and tissues caused by gamma irradiation and had resulted in plant death. Plantlets treated with 25 Gy had 100% survival and were still alive at 10 weeks after irradiation. Exposure to gamma irradiation at this level was causing damage but not resulted in death of the plantlets. Plantlets that survived had the potential to be mutants.

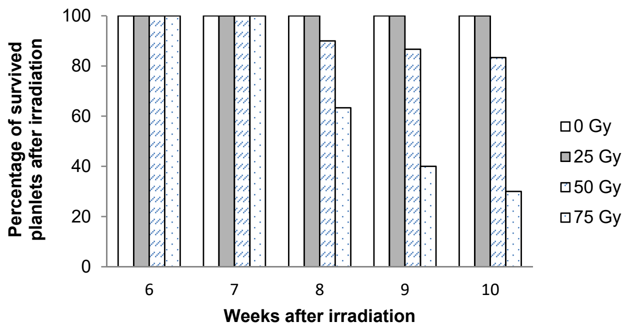

Figure 1. Percentage of survived *C. cristata* plantlets (n = 30 per treatment) after gamma irradiation treatment

LD_{50} is an indicator of irradiation level that can cause genetic changes. Lethal dose values of *C. cristata* plantlets were determined using curve-fit analysis to find the linear fit (Figure 2).This model demonstrated the radio-sensitivity of the MV2 *C. cristata* plantlets at 10 WAI. The sensitivity screening of the MV2 generation plantlets was reported by Abdullah et al. (2009) in ornamental pot plant *Curcuma alismatifolia.* The curve-fit method was used to extrapolate the percentage of mortality from the plantlets data into a formula of: y = a + bx. The calculated R value in *C.*

cristata was 0.8824, giving the value of LD_{50} of 68.73 Gy, LD_{30} of 46.68 Gy and LD_{20} of 35.65 Gy (Table 1, Figure 2).

Table 1. Radiosensitivity level of *C. cristata* plantlets based on *best curve-fit analysis*.

Doses lethal	*Model*	*Equation*	*Score (Gy)*
LD_{50}	Linear fit y=a+bx	y = 112.33-0.9068x	68.73

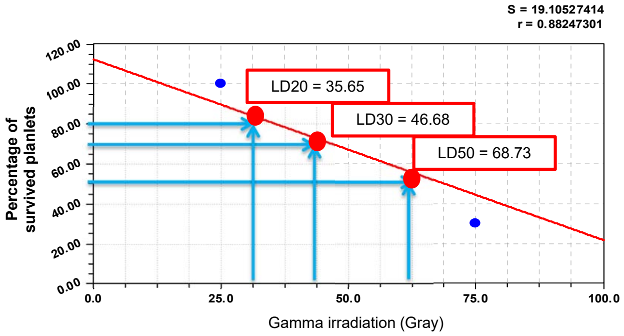

Figure 2. Lethal doses of *C. cristata* plantlets at 10 week after irradiation

Genetic variability is the most important prerequisite for a successful plant improvement program, particularly for ornamental crops, as it provides a broader spectrum of phenotypic variants for selection. Screening for the improved or unique phenotypes is important to develop new flower or ornamental cultivars. From this study, changes in phenotypes were observed in the third generation of irradiated *C. cristata* plantlets. Twelve out of 20 plantlets irradiated at 50 Gy had shortened internodes (Figure 3 b), and 4 out of 20 plantlets had curly leaves (Figure 3 c). Plantlets irradiated at 75 Gy also demonstrated similar but with a smaller frequency. One of the plantlets irradiated at 25 Gy had split leaves (Figure 3 a).

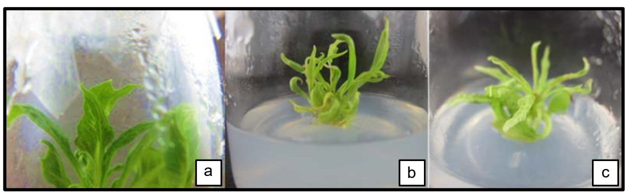

Figure 3. Changes of *C. cristata* after gamma irradiation treatment: (a) a split leaf; (b) shortened internodes; and (c) curly leaves.

Changes in the plants morphology and growth are indicators of the plant responses to different doses of irradiation.

Discussion

Variable responses of plants growth to the gamma irradiation has been reported. Broertjes and Van Harten (1988) showed that low doses of irradiation generated a low frequency of mutants but the plants had a high survival rate. On the other hand, the chance of mutation was higher when only a small percentage of plants survived after irradiation. Similar results were reported in other ornamental plants treated with gamma irradiation including *Gladiolus sp.* (Cantor and Korosfoy, 2002), *Torenia fournieri* and *Torenia baillonii* (Jala, 2011; Sawangmae et al., 2011), *Gerbera jamesonii* (Hasbullah et al., 2012) and *Philodendrons scandens* (Khateeb et al., 2016). Different plant species has different radiosensitivity levels and the response was species specific.

The effective doses of irradiation is usually around the LD_{50}, or slightly below. The LD_{50} irradiation level allows the plant tissues to recover despite lacking of the mutation frequency. Therefore, the LD_{30} and LD_{20} values can also be used as the optimal mutagen doses (IAEA, 2011). Suhesti (2015) used LD_{20} radiosensitivity level as an indicator to determine the mutation rate on sugarcane callus following treatment with gamma-ray irradiation. The LD_{20} and LD_{50} of sugarcane was 12.57 Gy and 28.88 Gy, respectively. Irradiated callus at LD_{20} to LD_{50} had resulted in a high frequency of mutation (Suhesti, 2015). The inhibition of plantlet development at specific doses of irradiation is considered normal in the mutation studies. Cells which underwent irradiation suffered some type of physiologic or chromosome damage and had lower mitotic capacity.

Other study reported an LD_{50} of 63.65 Gy for *Torenia fournieri* Lind leaf explants (Chancula et al., 2015), whereas Jala and Bodhipadma (2011) reported that the LD_{30} value and LD_{50} *C. cristata* seeds was 0.15 Gy and 0.4 Gy, respectively. Seeds are very susceptible to death when treated with high doses or irradiation (Aisyah, 2006). The levels of LD_{50} in this study was similar those reported in *Torenia* (Chancula et al., 2015) and green-leaf Coleus (Aisyah et al., 2015).

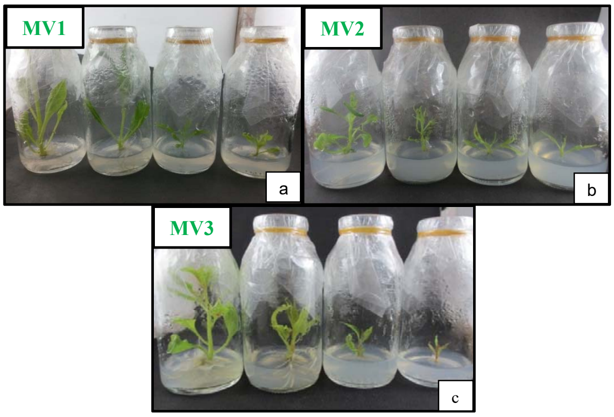

Figure 4. *C. cristata* plantlets of MV1 (a), MV2 (b) and MV3 (c) ten weeks after irradiation treatment; from left to right: control plantlets, plantlets irradiated at 25, 50, and 75 Gy.

Low dose of irradiation increases oxygen absorption and production of free radicals that might overcome seed dormancy, as reported in an ornamental plant *Mollucela laevis* L. (Minisi et al., 2013). However, the final results of the irradiation treatment might vary depending on the metabolic state of the irradiated seed, temperature and oxygen pressure during irradiation (Minisi et al., 2013).

Abnormality of growth in the third generation (MV3) plantlets was reported by Tangpong et al. (2009) in *Anubias congensis* N.E. Brown. The leaf of the irradiated plantlets became smaller, wavy on the edge, or stunted, but had bright green color. Yadav (2016) reported abnormal plant morphology including stem thickening, shorter internodes, increased branching and and larger leaves in the irradiated flowering shrub *Canscorra decurrens* Dalz.

Conclusion

Gamma irradiation has resulted in morphological changes in the third generation of *C. cristata* plantlets, i.e. the shortening of internodes, and curling of leaves. LD_{50}, LD_{30} and LD_{20} of *C. cristata* were 68.73 Gy, 46.68 Gy and 35.65 Gy, respectively.

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