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Characteristics of purple-green stem *Echinacea* in the responses of gamma ray irradiation

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ABSTRACT

The increasing demand of *Echinacea purpurea* as a raw material for herbal medicines and its availability which still relies on imported supplies encourage the development of *Echinacea* on a national scale. This research aims to increase the diversity of *Echinacea* to rise varieties of germplasm diversity, which may be employed to produce good qualities of *Echinacea* in future. The study used a simple experimental design method that compared the control treatment with gamma radiation treatment of 20, 30, and 40 Gray. Each treatment was repeated as many as 20 replicates, so there were 80 experimental units. Data were analysed descriptively and by independent t-test. Limited to this study, it was concluded that gamma ray irradiation enhanced quantitative and qualitative characteristics of purple-green stem *Echinacea* compared to the control treatment.

Key words: Mutation, diversity, variation, morphology, variety, improvement.

INTRODUCTION

The cost of health care that continues to increase every year succeeded in raising public awareness to maintain health by consuming drugs. The emergence of side effects from the use of chemical drugs has resulted in low public interest in consuming these drugs and choosing to consume herbal medicines as an alternative. The results of Riskesdas (2010) showed that 55.3% of the Indonesian population used herbal medicines to maintain their health (Emilda *et al.*,17). One of the medicinal plants that has properties as an immunomodulator is the *Echinacea* plant (*Echinacea purpurea*) (Fu *et al.*, 21)

Echinacea purpurea is a plant that belongs to the Asteraceae family. This medicinal plant is massively developed in the subtropical climate of North America. *Echinacea* has several properties including preventing upper respiratory tract infections and influenza. *Echinacea*'s efficacy as an immunomodulator is evident from its secondary metabolite content. Natural antioxidants in *Echinacea* medicinal plants are flavonoids, phenolic acids, polyphenolic compounds, and phenylpropanoids (Liu *et al.*, 22).

The demand and use of *Echinacea* plants in the pharmaceutical industry continues to increase in various countries. However, the availability of *Echinacea* plants as medicinal raw materials in Indonesia still relies on imported supplies. The Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) has introduced 10 accessions of *Echinacea purpurea* originating from Germany since 2002 as medicinal plants, one of which is purple-green stem (BHU4). This *Echinacea* has advantages of having higher number of leaves, leaf weight, number of flowers, and flower weight compared to other *Echinacea* accessions (Sidhiq *et al.*, 20). Plant breeding is one of the ways to produce good *Echinacea*. Plant breeding needs genetic diversity as its basic which can be obtained by mutation. Mutations can occur spontaneously or by induction. Induction is done by exposing the material to be mutated to mutation agents, resulting in sudden and random changes in genetic makeup.

Mutation with induction techniques can be done with gamma-ray irradiation. Gamma rays as ion radiation treatment can interact with atoms or molecules to produce free radicals in cells. The results of these radicals can affect the growth and development of plants, so that it leads to the changes of morphology and physiology of plant cells and tissues. Gamma rays as a mutation agent can cause genetic changes in plants, so that plants can have different traits or appearances by showing diversity (Beyaz *et al.*, 16).

Gamma radiation is commonly used in plant breeding activities as it does not cause any risks and is relatively fast in the process. Gamma radiation also does not depend on any media and or catalysts, besides that it can also be done with a small number of samples. The application of gamma radiation is proven to induce molecules and encourage gene changes in seeds, so as to produce plants with certain phenotypic criteria that lead to genetic diversity (Prince *et al.*, 20). Research on the development

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of *Echinacea* has not been widely conducted in Indonesia. Research on the behavior of *Echinacea* purple-green stem in the responses of gamma irradiation aims to increase the diversity of *Echinacea* characteristics. This study is expected to provide information on the behavior of purple green stem *Echinacea* resulting from gamma irradiation.

MATERIALS AND METHODS

The research was conducted from August 2022 to February 2023. Seedling was carried out at B2P2TOOT Tawangmangu. Planting and maintenance were carried out at the Faculty of Agriculture, Sebelas Maret University Experimental Field in Jumantono, Karanganyar, while the total flavonoids content test was carried out at the Food Chemistry Laboratory Faculty of Agriculture, Sebelas Maret University.

The tools used in this research include hoes, shovels, polybags, buckets, sprayers, paranet, stakes, label boards, meters, digital scales, stationery, RHS Color Chart and cameras. The materials used were BHU4 *Echinacea* seeds irradiated with gamma radiation of 20, 30 and 40 Gray (Gy), and control (without radiation).

The implementation of the research included radiation of *Echinacea* seeds, nursery, planting, maintenance, observation, and laboratory tests. Radiation of *Echinacea* seeds was carried out by sending *Echinacea* seeds to the National Nuclear Energy Agency (BATAN) South Jakarta to be irradiated with doses of 20, 30 and 40 Gy using a cobalt-60 gamma ray irradiator for 3 min 37 s and a dose rate of 3.16 kGy/h. The nursery was raised in planting media containing soil, fertilizer and husk charcoal in a ratio of 2:1:1 for 49 days until it could be transferred to small polybags.

Planting was carried out at the Faculty of Agriculture, Sebelas Maret University. Experimental field in Jumantono, Karanganyar after the *Echinacea* was 63 HSS old. Maintenance included weeding, watering, and pest and disease control. Observations were carried out weekly until entering harvest time, namely 3.5 months after planting to observe the growth of *Echinacea*. Laboratory tests were conducted to determine the total flavonoid content using spectrophotometry.

The research design used was a simple experimental design model, which is a research design that compares between experimental treatments and groups without treatment. The research was conducted in a plot measuring 10 m x 8 m which was divided into 4 beds. Each bed has a width of 1 meter with a distance of 1 meter per bed. The research was carried out by comparing *Echinacea* plants without radiation (control) with all *Echinacea* mutants at radiation doses of 20, 30 and 40 Gy. All treatments were repeated 20 times, so there were 80 experimental units.

The observed variables were quantitative and qualitative variables. Quantitative variables included plant height, number of leaves, number of flowers, fresh weight, and total flavonoids, while qualitative variables were flower shape and color. Data were analyzed using descriptive analysis and T test.

RESULTS AND DISSCUSION

The results of the T test analysis showed that all radiation dose treatments were significantly different from the control in plant height (Table 1). Majeed *et al*. (14) stated that low doses of gamma radiation have a stimulative effect, while increasing radiation doses slowed the plant growth. Fadli *et al*. (18) suggested that changes due to physiological damage cause metabolic changes that will result in changes in the chemical content of a plant, morphological characters, and adaptivity of mutant plants.

The results of the T test analysis showed that the radiation dose treatments of 20 and 30 Gy showed significant difference in number of leaves than the control (Table 1). Islamiati *et al.* (22) explained that characteristic of gamma radiation is random so as to create quantitative diversity of plants, one of which is the number of leaves.

The results of the T test analysis showed that all radiation dose treatments were significantly different in number of *Echinacea* flowers than the control (Table 1). Li *et al.* (22) mentioned that the characteristic of gamma radiation is random and can cause DNA damage to plant cells, and can result in the diversity of plant growth in the positive or negative way.

The results of the T test analysis showed that the radiation dose treatment of 20 and 30 Gy was significantly different in fresh weight in comparison to control (Table 1). Asza *et al.* (22) suggested that gamma irradiation treatment at certain levels can have a positive effect in the form of physiological changes, thus increasing plant weight.

Mutants had higher total flavonoids than the control and none of the *Echinacea* mutants had significant difference in the flavonoids content with the control (Table 1). Hwang *et al*. (21) suggested that gamma radiation increases total flavonoids in mugwort extract. The effect of irradiation at higher doses can break the glycone bond of flavonoid aglion. The breaking of bonds makes the molecular weight of flavonoids smaller, making them easily soluble in solvents and increasing total flavonoids.

Echinacea as part of the Astraceae family has a flower head that is actually a collection of seeds

Table 1. Quantitative character of *Echinacea.*

Treatment (Gy)	Plant height (cm)	No. of leaves (strands)	No. of flower (florets)	Fresh weight (g)	Total flavonoids (%)
Control	85.96 ± 10.897	74.95 ± 20.114	11.95 ± 3.268	307.25 ± 150.992	0.0360 ± 0.006
20	99.77 ± 11.704*	89.65 ± 20.137*	16.80 ± 6.693*	417.25 ± 151.340*	0.0360 ± 0.002
30	94.81 ± 12.578*	90.55 ± 21.583*	18.30 ± 7.019*	421.50 ± 156.374*	0.0363 ± 0.002
40	93.81 ± 11.668*	80.50 ± 20.135	14.55 ± 4.559*	381.25 ± 153.029	0.0453 ± 0.003

(*):significantly different from the control based on T-test. The data is represented as mean±SE.

of the flower itself. This plant is characterized by a spiny flower head with a cone-like flower base. The colour of the petals of *Echinacea* flowers varies, however, according Burlou-Nagy *et al*. (22) *Echinacea* purpurea has a purplish-pink petal colour. The results showed that radiation dose treatments of 20, 30 and 40 Gy caused changes in flower colour. The control *Echinacea* with strong purplish pink colour (63C) after gamma irradiation gives a diversity of flower colours including pale purplish pink (62D), strong purplish red (63B), light purplish pink (63D), moderate purplish red (64A), dark purplish pink (64D), and moderate purplish pink (65A). A dose of 20 Gy gave a higher diversity of flowers. Pallavi *et al*. (17) explained that floral pigments consist of flavonoids including anthocyanins, flavones, and flavonols. Mutations that occur in structural genes in the initiation of anthocyanin pigment formation result in colour changes in flowers.

The flower color of the control *Echinacea* was strong purplish pink (63C). This colour was also observed in the 20 Gy (3 plants), 30 Gy (5 plants), 40 Gy (2 plants) *Echinacea* mutants (Fig. 1a). Gamma radiation causes a change in flower color from strong purplish pink (63C) to pale purplish pink (62D). The pale purplish pink color was observed in 20 Gy (2 plants) and 40 Gy (1 plant) *Echinacea* mutants (Fig. 1b).

Gamma radiation causes a change in flower colour from purplish pink (63C) to strong purplish red (63B). The strong purplish red colour (63B) was observed in the 20 Gy (2 plants), 30 Gy (6 plants), and 40 Gy (7 plants) *Echinacea* mutants (Fig. 1c). Gamma radiation causes a change in flower color from strong purplish-pink (63C) to light purplish-pink (63D). The light purplish pink colour (63D) was observed in the 20 Gy (9 plants), 30 Gy (4 plants), and 40 Gy (3 plants) *Echinacea* mutants (Fig. 1d).

Gamma radiation causes a change in flower colour from strong purplish pink (63C) to moderate purplish-red (64A). The moderate purplish red colour (64A) was observed in the 20 Gy *Echinacea* mutant (1 plant) (Fig. 1e). Gamma radiation causes a change in flower colour from strong purplish pink (63C) to dark purplish pink (64D). The dark purplish pink colour (64D) was observed in 30 Gy (5 plants) and 40 Gy (7 plants) *Echinacea* mutants (Fig. 1f). Gamma radiation causes a purplish-pink (63C) to moderate purplish pink (65A) color change. The moderate purplish pink (65A) was observed in the 20 Gy *Echinacea* mutant (3 plants) (Fig. 1g).

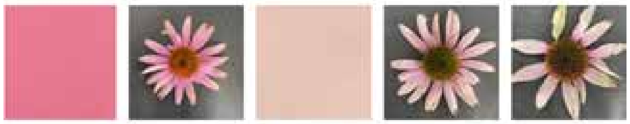
The results showed that radiation dose treatments of 20, 30 and 40 Gy resulted in changes in flower shape. The control *Echinacea* that have a triangular flower receptacle shape, dense-sparse petal density, and 45^{o} flower angle gave rise to a new diversity after gamma-ray irradiation in the form of round and flat round receptacle shapes, dense and sparse petal density, and 90^{o} flower angle and 0^{o} flat. Maharani *et al*. (15) suggested that gamma radiation causes mutation that occur randomly. Gamma radiation is a type of electromagnetic radiation without electric charge consisting of photons that move at high speed. The interaction of gamma radiation with atoms in the material causes absorption, scattering, or formation of new particles, so that mutant plants, have different characters per individual. The difference in flower shape due to radiation is also stated in the research of Monikasari *et al*. (18) on sunflower plants, which states that gamma radiation changes the shape of tube flowers to be not fully round and has a sparse density of ribbon flowers.

The shape of the flower receptacle in control is triangular. This flower receptacle shape was also observed in the 20 Gy (8 plants), 30 Gy (10 plants), and 40 Gy (15 plants) *Echinacea* mutants (Figure 2a). Gamma radiation causes a change in the shape of the triangular flower receptacle to round. The rounded flower receptacle shape was observed in 20 Gy (8 plants), 30 Gy (6 plants), and 40 Gy (5 plants) *Echinacea* mutants (Fig. 2b).

Gamma radiation causes changes in the shape of the triangular flower receptacle to become flat-round. Flat-rounded flower receptacle shape was observed in 20 Gy (4 plants) and 30 Gy (4 plants) *Echinacea* mutants (Fig. 2c). The petal density in the control *Echinacea* was dense-sparse. Dense-sparse petal density was also observed in the 20 Gy (8 plants), 30 Gy (15 plants), and 40 Gy (18 plants) *Echinacea* mutants (Fig. 2d).

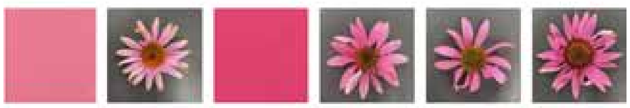
Colour 63C Control 20 Gy 30 Gy 40 Gy

(a) Colour and appearance of strong purplish pink *Echinacea* (63C).



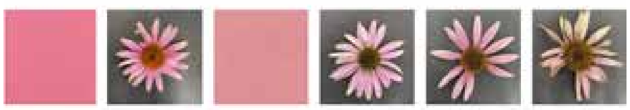
Colour 63C Control Colour 62D 20 Gy 40 Gy

(b) Colour and appearance of pale purplish pink *Echinacea* (62D).



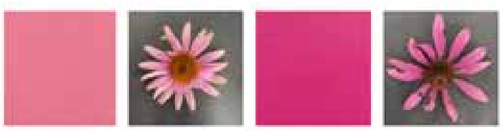
Colour 63C Control Colour 63B 20 Gy 30 Gy 40 Gy

(c) Colour and appearance of strong purplish red *Echinacea* (63B).



Colour 63C Control Colour 63D 20 Gy 30 Gy 40 Gy

(d) Colour and appearance of light purplish pink *Echinacea* (63D).

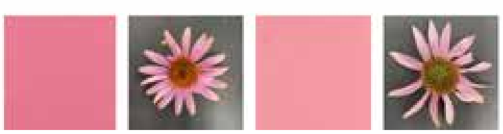


Colour 63C Control Colour 64A 20 Gy

(e) Colour and appearance of moderate purplish red *Echinacea* (64A).

Colour 63C Control Colour 64D 20 Gy 40 Gy

(f) Colour and appearance of dark purplish pink *Echinacea* (64D).



Warna 63C Kontrol Warna 65A 20 Gy

(g) Colour and appearance of moderate purplish pink (65A).

Fig. 1. Variability in flower colour in *Echinacea* mutants.

Control 20 Gy 30 Gy 40 Gy

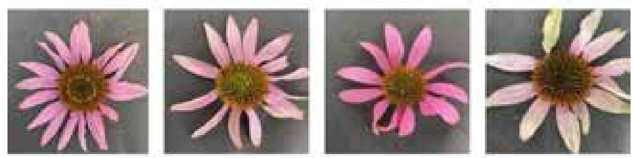
(a) Triangular shape of *Echinacea* receptacle.

Control 20 Gy 30 Gy 40 Gy

(b) Round shape of *Echinacea* receptacle.

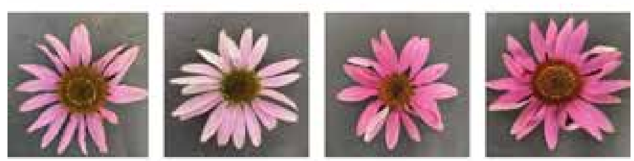
Control 20 Gy 30 Gy

(c) Flat-round shape of *Echinacea* receptacle.



Control 20 Gy 30 Gy 40 Gy

(d) Dense-sparse petal density of *Echinacea.*



Control 20 Gy 30 Gy 40 Gy

(e) Dense petal density of *Echinacea.*

Control 20 Gy 30 Gy

(f) Sparse petal density of *Echinacea.*

Control 20 Gy 30 Gy 40 Gy

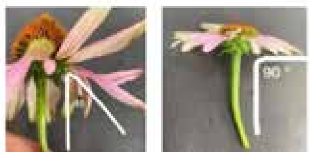
(g) Flower angle 45° of *Echinacea.*

Contd...

Fig. 2 contd...

Control 20 Gy 40 Gy

(h) Flower angle 0° of *Echinacea.*



Control 20 Gy

(i) Flower angle 90° of *Echinacea.*

Fig. 2. Variability in shapes of *Echinacea.*

Gamma radiation causes changes in petal density from dense-sparse to dense. Dense petal density was observed in the equine mutants at 20 Gy (5 plants), 30 Gy (3 plants), and 40 Gy (2 plants) *Echinacea* mutants (Fig. 2e). Gamma radiation causes changes in petal density from dense-sparse to sparse. Sparse petal density was observed in 20 Gy (7 plants) and 30 Gy (2 plants) *Echinacea* mutants (Fig. 2f).

The flower angle in the control was 45°. The 45° flower angle was also observed in the 20 Gy (12 plants), 30 Gy (20 plants), and 40 Gy *Ecinachea* mutants (16 plants) (Fig. 2g). Gamma radiation caused the 45° flower angle to change to a flat 0°. A flat flower angle of 0° was observed in the 20 Gy (6 plants) and 40 Gy (4 plants) *Echinacea* mutants (Fig. 2h).

Gamma radiation also caused the flower angle to change from 45° to 90°. The 90° flower angle was observed in the 20 Gy *Echinacea* mutant (2 plants) (Fig. 2i).

AUTHORS' CONTRIBUTION

Designed (EP) and experiment conduct (AY, DP, AS), writing manuscript (AY, EP, AS, FR).

DECLARATION

The authors declare that there is no conflict of interest.

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REFERENCES

1. Asza, A.A., Moeljani, I.R. and Koentjoro, Y. 2022. Induksi radiasi sinar gamma 60Co dosis 3 Gy terhadap keragaman genetik populasi Mutan (M4) tanaman bawang merah varietas Bauji. *Agrohita* **7**: 332-36.

2. Beyaz, R., Kahramanogullari, C.T., Yildiz, C., Darcin, E.S. and Yildiz, M. 2016. The effect of gamma radiation on seed germination and seedling growth of *Lathyrus chrysanthus* Boiss. under *in vitro* conditions. *J. Environ. Radioact.* **162**: 129–133. https://doi.org/10.1016/j.jenvrad.2016.05.006

3. Burlou-Nagy, C., Bănica, F., Jurca, T., Vicas, L.G., Marian, E., Muresan, M.E., Bácskay, I., Kiss, R., Fehér, P. and Pallag, A. 2022. *Echinacea purpurea* (L.) Moench: Biological and pharmacological properties–A review. *Plants* **11**. https://doi.org/10.3390/plants11091244

4. Emilda, E., Hidayah, M. and Heriyati, H. 2017. Analisis pengetahuan masyarakat tentang pemanfaatan tanaman obat keluarga (studi kasus kelurahan situgede, kecamatan bogor barat). *Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam* **14**: 11-20.

5. Fadli, N., Syarif, Z., Satria, B. and Akhir, N. 2018. The Effect of gamma cobalt-60 ray irradiation on cultivar growth in Taro White (*Xanthosoma sagittifolium* L.). *Inter. J. Environ. Agric. Biotechnol.* **3**: 2020-25. https://doi.org/10.22161/ijeab/3.6.9

6. Fu, R., Zhang, P., Deng, Z., Jin, G., Guo, Y. and Zhang, Y. 2021. Diversity of antioxidant ingredients among *Echinacea* species. *Ind. Crops Prod*. **170**: 113699. https://doi.org/10.1016/j.indcrop.2021.113699

7. Hwang, K.E., Ham, Y.K., Song, D.H., Kim, H.W., Lee, M.A., Jeong, J.Y. and Choi, Y.S. 2021. Effect of gamma-ray, electron- beam, and X-ray irradiation on antioxidant activity of mugwort extracts. *Radiat. Phys. Chem.* **186**: 109476. https://doi.org/10.1016/j.radphyschem.2021.109476

8. Islamiati, K, Lestari, A, Samullah, H.Y. and Sanjaya, L.L. 2022. Analisis karakter kuantitatif klon-klon krisan (*Chrysanthemum morifolium* Ramat.) Generasi MV 7/8 hasil mutasi sinar Gamma. *J. Ilmiah Wahana Pendidikan*, **8**: 304-13.

9. Li Y, Chen L, Zhan X, Liu L, Feng F, Guo Z, Wang D, Chen H. 2022. Biological effects of gamma-ray radiation on tulip (*Tulipa gesneriana* L.). *Peer J*, **10**: 1-22. https://doi.org/10.7717/peerj.12792

10. Liu, D.F., Bai, M., Zhang, X., Sun, X., Song, S.J. and Huang, X.X. 2022. Phytochemical investigation of *Echinacea purpurea* (Linn.) Moench and their chemotaxonomic significance. *Biochem. System. Ecol*. **100**: 104378. https://doi.org/10.1016/j.bse.2021.104378

11. Maharani, S., Khumaida, N., Syukur, M. and Ardie, S.W. 2015. Radiosensitivitas dan keragaman ubi kayu (*Manihot esculenta* Crantz) hasil iradiasi sinar Gamma. *J. Agro Indonesia* **43**: 111. https://doi.org/10.24831/jai.v43i2.10412

12. Majeed, A., Muhammad, Z., Majid, A., Shah, A.H. and Hussain, M. 2014. Impact of low doses of gamma irradiation on shelf life and chemical quality of strawberry (*Fragaria* × *ananassa)* cv. ‘Corona.’ *J. Anim. Plant. Sci.* **24**: 1531-36.

13. Monikasari, I.N.S., Anwar, S. and Kristanto, B.A. 2018. Keragaman M_1 Tanaman hias bunga matahari (*Helianthus annuus* L.) akibat iradiasi sinar Gamma. *J. Agro. Complex* **2**: 1. https://doi.org/10.14710/joac.2.1.1-11

14. Pallavi, B., Nivas, S.K., D’Souza, L., Ganapathi, T.R. and Hegde, S. 2017. Gamma rays induced variations in seed germination, growth and phenotypic characteristics of *Zinnia elegans* var. Dreamland. *Adv. Hortic. Sci*. **31**: 267-73. https://doi.org/10.13128/ahs-20289

15. Prince, S., Dhull, S.B., Punia, S. and Rohilla, S. 2020. Effect of γ- radiation on physico-chemical, morphological and thermal characteristics of lotus seed (*Nelumbo nucifera*) starch. *Int. J Biological Macromol.* **157**: 584-90. https://doi.org/10.1016/j.ijbiomac.2020.04.181

16. Sidhiq, D.F., Widiyastuti, Y., Subositi, D., Pujiasmanto, B. and Yunus, A. 2020. Morphological diversity, total phenolic and flavonoid content of *Echinacea purpurea* cultivated in Karangpandan, Central Java, Indonesia. *Biodiversitas* **21**: 1265-71. https://doi.org/10.13057/biodiv/d210355

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