



Low Doses of Acute Gamma Radiation Promote Root Formation and Leaf Canopy in Common Cockscomb (*Celosia argentea* var. *cristata*)

Anchalee Jala¹ and Kitti Bodhipadma^{2*}

บทคัดย่อ

ศึกษาผลของรังสีแกมมาเฉียบพลันต่อการงอกของเมล็ดและลักษณะสัณฐานวิทยาของหนอนไก่ (*Celosia argentea* var. *cristata*) เมล็ดซึ่งอยู่บนอาหารพื้นฐานสูตร MS และชิ้นส่วนปลายยอดที่อยู่บนอาหารสูตร MS ซึ่งเติม KNO₃ 100 มิลลิกรัมต่อลิตร ในสภาพปลอดเชื้อ ได้รับรังสีแกมมาเฉียบพลันที่ปริมาณ 0, 5, 10, 20, 40 และ 100 rad พบว่าปริมาณทำให้ตายที่ขนาดมรณะ 30 และ 50 ต่อการงอกของเมล็ดอยู่ที่รังสีแกมมา 15 และ 40 rad ตามลำดับ พีชซึ่งเจริญจากชิ้นส่วนปลายยอดที่ได้รับและไม่ได้รับรังสีมีการแปรผันบางอย่างในสัณฐานวิทยาหลังจากเพาะเลี้ยงเป็นเวลา 12 สัปดาห์ รังสีแกมมาเฉียบพลันปริมาณต่ำ (5 และ 10 rad) ช่วยเพิ่มจำนวนรากและขนาดพุ่มใบให้กับพีชในขวดแก้ว เช่นเดียวกับ การย้ายออกปลูกเป็นเวลา 10 สัปดาห์ พบว่ามีความแตกต่างในสัณฐานวิทยาของขนาดใบที่ส่วนยอดของลำต้นอย่างเห็นได้ชัดเจน นอกจากนี้ปริมาณรังสีแกมมาเฉียบพลันที่ 5 และ 10 rad ยังให้จำนวนของดอกต่อต้นและขนาดดอกใกล้เคียงกับกลุ่มควบคุม

คำสำคัญ: รังสีแกมมาเฉียบพลัน หนอนไก่ การชักนำ การกลาย การแปรผันในสัณฐานวิทยา

Abstract

Effects of acute gamma radiation on seed germination and morphological characters of shoot tip explants of common cockscomb (*Celosia argentea* var. *cristata*) were studied. Aseptic seeds on MS basal medium and shoot tip explants on MS medium supplemented with 100 mg/l KNO₃ were irradiated with acute gamma rays at 0, 5, 10, 20, 40 and 100 rad. It was found that the lethal doses at LD₃₀ and LD₅₀ for seed germination were 15 and 40 rad of gamma rays, respectively. After 12 weeks cultured, irradiated and non-irradiated shoot tip-derived plantlets showed some in vitro morphological variations. Low doses of acute gamma radiation (5 and 10 rad) improved number of root formation and leaf canopy size of in vitro plantlets. As well as 10 weeks of hardening, morphological difference in leaf size was clearly perceived on the top of stem. Furthermore, 5 and 10 rad doses of acute gamma rays made a similar number of flower per plant and flower diameter to the non-irradiated ones.

¹ Associate Professor, Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Rangsit Campus, Prathumthani.

² Associate Professor, Department of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok.

* Corresponding Author, Tel. 0-2913-2500 Ext 4717, E-mail: kbm@kmutnb.ac.th



Keywords: Acute Gamma Radiation, Common Cockscomb, Induced Mutation, Morphological Variation

1. Introduction

Plants in genus *Celosia* originate from the subtropical and temperate regions of Africa, South America, and South East Asia. They are classified as a member of the family Amaranthaceae. Two widespread ornamental varieties of this genus are common cockscomb (*Celosia argentea* var. *cristata*) which has a wide comb-shaped inflorescence, and feathered amaranth (*Celosia argentea* var. *plumosa*) with feather-like flowers. Generally, *Celosia* is put on market as a potted plant, a bedding plant, or a cut flower on decorative purposes [1], [2]. For common cockscomb, it is widely sold as an ornamental plant in all regions of Thailand due to the dazzling and attractive inflorescence color [3].

In plant species, a wide range of genetic variations could be noticeably obtained via tissue culture. Typically, this in vitro modification offered a shorter time comparing to the conventional breeding programs. Thus, the genetic variations of useful traits are currently becoming a valuable outcome for crop improvement. On this matter, radiation, a mutagenic agent, has proved to be able to induce mutations and create genetic alterations. Using tissue culture in combination with the radiation, therefore, will certainly make a considerable contribution to plant breeding [4] - [6].

So far, there were a few publications on common cockscomb tissue culture. The first report was flowering in vitro in common cockscomb [7] and the latest research was comparison of in vitro and in vivo inflorescence of this plant [3]. However, the

mutation induction of common cockscomb had never been investigated hitherto. Hence, the objectives of this study were to evaluate the effects of acute gamma radiation on seed germination and morphological characters of common cockscomb and find out any beneficial features from this induced mutation.

2. Materials and Methods

Plant materials and surface sterilization

Seeds of common cockscomb, *Celosia argentea* var. *cristata*, purchased from Thai Seed and Agriculture Co., Ltd., Bangkok, Thailand were surface sterilized twice with Clorox (a commercial bleach solution comprised 5.25% (w/w) sodium hypochlorite as available chlorine), first with 10% (v/v) for 15 min and then 5% (v/v) for 10 min. The seeds were subsequently, rinsed 3 times with sterile distilled water (1 min each time).

Effects of acute gamma radiation on seed germination

Surface-sterilized common cockscomb seeds were transferred to MS basal medium [8] as a control. For the experimental groups, these seeds on the MS basal medium were irradiated with acute gamma radiation at 5, 10, 20, 40 and 100 rad to investigate the lethal dose of the radiation at LD₃₀ and LD₅₀ on seed germination in the growth room under 16 h illumination with white fluorescent lamps and 8 h of darkness at 25 ± 2 °C. Percentage of germination in each group was recorded after 4 weeks.

Influences of acute gamma radiation on morphological characters of shoot tip explants

After common cockscomb seeds on MS basal medium germinated and developed into seedlings for 2 weeks, shoot tips were cut each 0.8-1 cm long

and placed in MS medium supplemented with 100 mg/l KNO_3 as a control. For the treatments, shoot tip explants on MS medium consisted of 100 mg/l KNO_3 were exposed to acute gamma radiation at the same quantity and conditions as mentioned above. These explants were subcultured every 4 weeks. Root number, root length, plantlet height, leaf number and leaf canopy were examined after 12 weeks.

Impacts of acute gamma radiation on morphological characters of ex vitro plants

Irradiated and non-irradiated plantlets grown on MS medium fortified with 100 mg/l KNO_3 for 12 weeks were transferred into a soil mixture (soil: sand:coconut husk in a 1:1:1 ratio) on the plastic pot under in vivo conditions. Leaf size in each part of stem was measured after 10 weeks of acclimatization while number of flower per plant and flower diameter were observed when the last plant in each treatment flowering.

Statistical analysis

A completely randomized design (CRD) was employed for all experiments. Analysis of variance (ANOVA) was first carried out at the significance level of $P < 0.05$ and then Duncan's multiple range test (DMRT) comparison of means were performed at $P < 0.05$.

3. Results and Discussion

Lethality of common cockscomb seeds

Following 4 weeks germination of common cockscomb seeds, the lethal doses of gamma radiation at LD_{30} and LD_{50} were calculated. It was found that when gamma radiation dose increased, the survival

percentage of common cockscomb seeds grown in vitro decreased. The levels of gamma radiation that caused 30 and 50% lethal seeds were 15 and 40 rad, respectively. The 100 rad irradiation dose had only 4% of endurance seeds. These results were dissimilar on pea (*Pisum sativum* L.) since gamma irradiation had an insignificant effect on seed germination [9]. Moreover, seeds of durum wheat (*Triticum durum* Desf.) treated with gamma radiation had an increase in germination rate and capacity when compared with non-irradiated ones [10]. Thus, seeds from different plant species might have diverse responses on different doses of gamma irradiations and common cockscomb seeds were more susceptible to gamma radiation than pea and durum wheat.

Morphological characters of shoot tip explants after irradiation

When irradiated and non-irradiated shoot tip explants developed on MS medium containing 100 mg/l KNO_3 for 12 weeks, some morphological differences of these explants had been found (Table 1). Generally, 20 and 40 rad doses made the reduction in all characters except leaf canopy comparing to the control. At 100 rad, explants turned brown and died at last. However, it was quite interesting that low doses of acute gamma rays (5 and 10 rad) enhanced a number of root formation and increased leaf canopy size. These findings were similar to hard wheat (*Triticum durum* Desf.) as low doses of the radioactive gamma rays gave root number per plant better than control as well [11]. Besides, root lengths of some grapevine varieties at low doses of gamma-rays exposure were significantly higher than those of the non-irradiated ones [12].



Table 1 Morphology of irradiated and non-irradiated plantlets after cultured on MS medium supplemented with 100 mg/l KNO₃ for 12 weeks

Gamma radiation (rad)	Root number	Root length (cm)	Plantlet height (cm)	Leaf number	Leaf canopy (cm)
0	2.83b	8.66a	13.88a	22a	6.47c
5	3.67a	7.65a	13.67a	21a	7.67b
10	2.83b	7.55a	11.92a	19a	9.12a
20	2.17c	5.55b	7.76b	15b	7.28bc
40	1.5d	4.47b	5.65b	14b	6.81c
100	0	0	0	0	0

Data are means of 10 replications and those sharing the same letter in a column are not significantly different (ANOVA, P < 0.05)

Morphological characters of ex vitro plants after irradiation

With the successful hardening, irradiated and non-irradiated plants could survive and grow well under ex vitro conditions. After 10 weeks of acclimatization, morphological variation in leaf size (width x length) was obviously seen on the top of stem (Table 2). These outcomes were the same as leaf canopy in the previous experiment. Plants irradiated with 5 and 10 rad had bigger leaf size on the top of stem than other treatments. Exposure of acute gamma rays had no influence on leaves at the base and in the middle of stems. It is possible that young leaves of common cockscomb react to low doses of acute gamma rays (5 and 10 rad) better than the old leaves. Nevertheless, in niger (*Guizotia abyssinica* Cass.) and makhana (*Euryale ferox* Salisbury), reduction in leaf length and width with increasing levels of gamma irradiation was obtained [13], [14].

Table 2 Average size of leaf in each part of stem after growing in vivo for 10 weeks

Gamma radiation (rad)	At the base of stem WxL (cm)	In the middle of stem WxL (cm)	On the top of stem WxL (cm)
0	2.38x3.45a	2.03x3.43a	2.82ax3.17c
5	2.15x2.72a	2.15x3.25a	2.92ax4.83a
10	2.23x3.08a	2.08x3.93a	2.32abx4.55ab
20	2.18x3.02a	2.15x2.77a	2.10bx3.68bc
40	1.71x2.78a	1.48x2.82a	1.78bx3.23c
100	0	0	0

Data are means of 10 replications and those sharing the same letter in a column are not significantly different (ANOVA, P < 0.05), W = leaf width and L = leaf length

For the flowering appearance, though acute gamma rays at doses of 5 and 10 rad delayed the time of flower formation, number of flower per plant and flower diameter were comparable to non-irradiated ones (Table 3). When irradiation doses over 20 rad, none of flowering had been noticed. These results agree with the works on African marigold because lessening in flower number and size were recorded in higher doses of gamma ray [15]. In addition, floral abnormalities in shape and size of makhana flowers were initiated from gamma radiation [14].

Table 3 Flower characters of irradiated and non-irradiated plants after acclimatization

Gamma radiation (rad)	Day after transplanted until the last plant flowering (days)	Number of flower per plant	Flower diameter (cm)
0	84.00b	1.4a	1.56a
5	87.75b	1.25a	1.08ab
10	113.67a	1a	0.8b
20	-	-	-
40	-	-	-
100	-	-	-

Data are means of 10 replications and those sharing the same letter in a column are not significantly different (ANOVA, P < 0.05)



In summary, mutation in vitro could be occurred naturally or artificially. The present research showed that some advantageous characteristics of common cockscomb were able to induce via acute gamma irradiation like other plant species. Great number of root, leaf canopy and leaf size on the top of stem reflected the alteration in genes for the desired trait of common cockscomb under low doses of acute gamma rays exposure. This variability may be respected as an alternative way to control plant growth, such as slow flowering could delay the time to sell and possibly increase more value of this ornamental plant on the market.

References

- [1] R. Porat, E. Shlomo, and A.H. Halevy, "Horticultural techniques to improve *Celosia plumosa* growth for cut flowers," *Sci. Hortic.*, vol. 63, pp. 209–214, 1995.
- [2] Y. Cai, M. Sun, W. Schliemann, and H. Corke, "Chemical stability and colorant properties of betaxanthin pigments from *Celosia argentea*," *J. Agric. Food Chem.*, vol. 49, pp. 4429–4435, 2001.
- [3] K. Bodhipadma, S. Noichinda, I. Yadbuntung, W. Buaeiam, and D.W.M. Leung, "Comparison of in vitro and in vivo inflorescence of common cockscomb (*Celosia argentea* var *cristata*)," *ScienceAsia*, vol. 36, pp. 68–71, 2010.
- [4] F.J. Novak and H. Brunner, "Plant breeding: Induced mutation technology for crop improvement," *IAEA Bull.*, vol. 4, pp. 25–33, 1992.
- [5] D.C.W. Brown and T.A. Thorpe, "Crop improvement through tissue culture," *World J. Micro. Biotech.*, vol. 11, pp. 409–415, 1995.
- [6] S.M. Jain, "Tissue culture-derived variation in crop improvement," *Euphytica*, vol. 118, pp. 153–166, 2001.
- [7] J. Yamada, K. Nakata, W. Amaki, and H. Higuchi, "In vitro flowering in *Celosia argentea* var. *cristata*," *Bull. NODAI Res. Inst.*, vol. 8, pp.22–24, 1997.
- [8] T. Murashige and F. Skoog, "A revised medium for rapid growth and bioassays with tobacco tissue culture," *Physiol. Plant.*, vol. 15, pp. 473–497, 1962.
- [9] C.Y. Çiftçi, A.D. Türkan, K.M. Khawar, M. Atak, and S. Özcan, "Use of gamma rays to induce mutations in four pea (*Pisum sativum* L.) cultivars," *Turk. J. Biol.*, vol. 30, pp. 29–37, 2006.
- [10] M. Melki and T. Dahmani, "Gamma irradiation effects on durum wheat (*Triticum durum* Desf.) under various conditions," *Pak. J. Biol. Sci.*, vol. 12, pp. 1531–1534, 2009.
- [11] M. Melki and A. Marouani, "Effects of gamma rays irradiation on seed germination and growth of hard wheat," *Environ. Chem. Lett.*, vol. 8, pp. 307–310, 2010.
- [12] T. Charbaji and I. Nabulsi, "Effect of low doses of gamma irradiation on in vitro growth of grapevine," *Plant Cell Tiss. Org. Cult.*, vol. 57, pp. 129–132, 1999.
- [13] P.M. Naik and H.N. Murthy, "The effects of gamma and ethylmethanesulphonate treatments on agronomical traits of niger (*Guizotia abyssinica* Cass.)," *Afr. J. Biotechnol.*, vol. 8, pp. 4459–4464, 2009.
- [14] A.K. Verma, B.K. Banerji, D. Chakrabarty, and S.K. Datta, "Studies on makhana (*Euryale ferox* Salisbury)," *Curr. Sci.*, vol. 99, pp. 795–800, 2010.
- [15] V. Singh, M. Saxena, B.K. Banerji, A.K. Dwivedi, and A.K. Verma, "Studies on effects of gamma irradiation on 'Pusa Narangi Gainda' (African Marigold cultivar)," *B.V.A.A.P.*, vol. 18, pp. 55–61, 2010.