Agriculture and Natural Resources 52 (2018) 121–124

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Effects of acute gamma irradiation on *in vitro* culture of *Exacum affine* Balf.f. ex Regel

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ARTICLE INFO

Article history:
Received 17 July 2017
Accepted 28 June 2018
Available online 4 July 2018

Keywords:
Exacum affine Balf.f. ex Regel
Gamma irradiation
in vitro culture

ABSTRACT

Exacam affine Balf. f. ex Regel (Persian Violet) is an ornamental plant with small, purple flowers having yellow pollen in their center and it is mostly cultivated as a houseplant. The effect of acute gamma irradiation on *in vitro* cultures of *E. affine* was studied by treatment with gamma radiation using a Mark I Gamma Irradiator at the Nuclear Technology Research Center, Kasetsart University, Thailand with a Cesium-137 source at doses of 0, 10, 20, 30, 40, 50 and 60 Gy and a dose rate of 3.74 Gy/min. After irradiation, subcultures were grown on new Murashige and Skoog (MS) medium; then, after 60 d, the numbers of new branches on surviving plantlets were counted. Samples were subsequently subcultured to new MS medium for morphological study. The median lethal dose was 45 Gy and the 50% decreasing growth rate was 32 Gy. Some variations were observed in irradiated samples in M_1V_2, such as changes in the number of petals in a flower (four and six petals), flower color (light purple, white), smaller leaves, curled-shaped leaves, variegated leaves, lighter-green leaves and shorter internodes than the control.

Introduction

Exacum affine is one of the rare blue-flowering potted plants. In addition to its attractive blue and purple flowers, its popularity is attributable to its fragrance, shiny green foliage, low-mounding habit, ease of shipping and post-harvest shelf life (Riseman, 2007). *Exacum affine* is a species of plant in the family Gentianaceae, native to Socotra, Yemen (Miller and Morris, 2004). It is a biennial plant which grows 20–30 cm tall with green ovate leaves and purple stellate flowers with yellow pollen on the stamens in their center. It is commonly known by its commercial name of Persian violet.

Due to the relatively narrow germplasm base and low level of variation found in *E. affine*, mutation breeding has been a viable method to generate genetic variation (Riseman, 2007). Many researchers have shown interest in induced mutation in *E. affine*. Masae et al. (2014) exposed *E. affine* plants to ultra-violet C radiation (UV–C) at 0–32.40 kJ/m^2 and reported that the median lethal dose (LD_{50}) of shoot apex and axillary explants was 29.7 and 18.9 kJ/m^2, respectively, and the morphological characteristics of irradiated samples were a larger size and darker green color compared to the control. A suggested dose for gamma-ray-induced mutation in tissue cultures of *Exacum* sp. is 4 Gy (Shu et al., 2011)

The current research studied the effect of acute gamma irradiation on tissue culture of *E. affine*. Gamma-ray-induced mutations in tissue culture may be another good technique for increasing the genetic variation in *E. affine*.

Materials and methods

Propagation of Exacam affine (Persian Violet) in tissue culture

Pathogen-free plantlets of *E. affine* were cultured on MS medium (Murashige and Skoog, 1962) until enough plantlets were produced for the experiments. Plantlets were multiplied from single-node cuttings transferred to MS medium 90 d before being subjected to radiation treatments.

Acute gamma irradiation

E. affine plantlets were exposed to acute gamma radiation using a Mark I Gamma Irradiator with a ^{137}Cs source at the Nuclear Technology Research Center, Kasetsart University, Bangkok, Thailand at doses of 0, 10, 20, 30, 40, 50 and 60 Gy (at a dose rate of

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https://doi.org/10.1016/j.anres.2018.06.004

Table 1
Survival percentage and growth rate percentage of *E. affine* at 60 d after irradiation.

Radiation dose (Gy)	Plantlet survival (as % of control)	Growth rate (as % of control)
0	100.00[a1]	100.00[a]
10	84.76[ab]	68.32[b]
20	93.38[ab]	68.85[b]
30	70.37[bc]	53.36[bc]
40	56.08[cd]	40.45[c]
50	44.45[de]	37.08[cd]
60	28.84[e]	21.15[d]
F-test	[a]	[a]
Coefficient of Variation (%)	20.33	19.21

[a] Significant at 1% level.
[1] means within columns followed by a common superscript letters are not significantly different ($p < 0.05$).

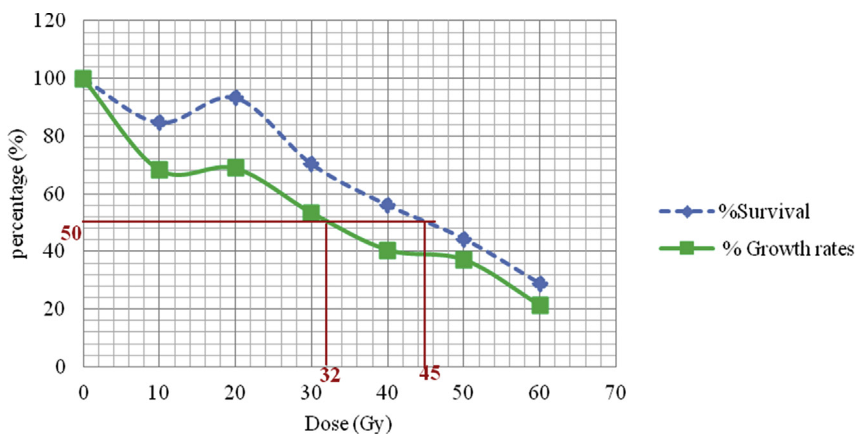

Fig. 1. Survival percentage and growth rate percentage at 60 d after irradiation.

3.74 Gy/min). A completely randomized design was used with three replications for each treatment (dose), with 20 plantlets per replication. Following irradiation, the plantlets were then multiplied from two-node cuttings by subculturing on the same medium. After 60 d, for M_1V_1 generation the number of surviving plantlets and the number of new branches were recorded to calculate the median lethal dose ($LD_{50(60)}$) (50% lethal dose at 60 d after irradiation) and the median decreasing growth rate ($GR_{50(60)}$). Desirable variations were recorded and selected in the M_1V_2 generation.

Statistical analysis

The data were analyzed using analysis of variance, after which means were compared using the least significant difference (LSD). The analyses were facilitated by the STAR program (International Rice Research Institute, 2013).

Results and discussion

Effect of acute gamma irradiation on the survival and growth rate of E. affine plantlets

Exposing *E. affine* plantlets raised in aseptic conditions to acute gamma radiation at doses of 0, 10, 20, 30, 40, 50 and 60 Gy showed that 60 d after irradiation, the survival percentages of plantlets (M_1V_1) that had received 10 and 20 Gy of radiation were not different from the control. The percentage of surviving plantlets decreased as the dosage of radiation increased (Table 1). The LD_{50} at 60 d was 45 Gy (Fig. 1) and the effect of irradiation dose on survival percentage was highly significant ($p < 0.01$). The results of the current study differed from Shu et al. (2011) who reported the suggested dose for gamma-ray-induced mutation in tissue cultures of *Exacum* sp. is 4 Gy, but the dose rate in that study was not reported. It is suspected that the difference was due to different dose rates and different ages of plantlets.

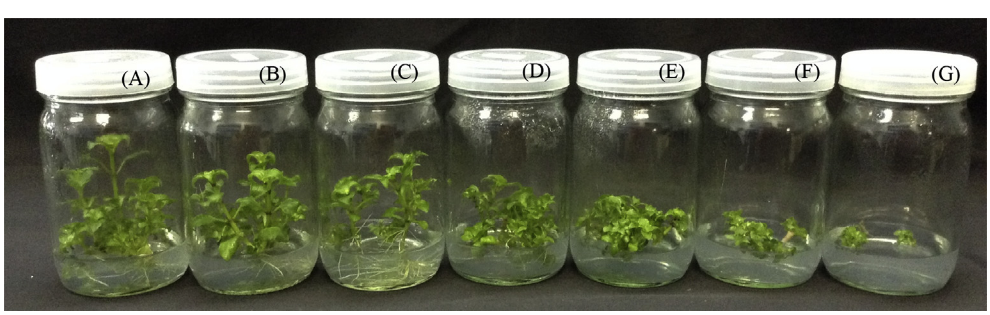

Fig. 2. Control and irradiated samples 60 d after irradiation: (A) Control; (B) 10 Gy; (C) 20 Gy; (D) 30 Gy; (E) 40 Gy; (F) 50 Gy; (G) 60 Gy.

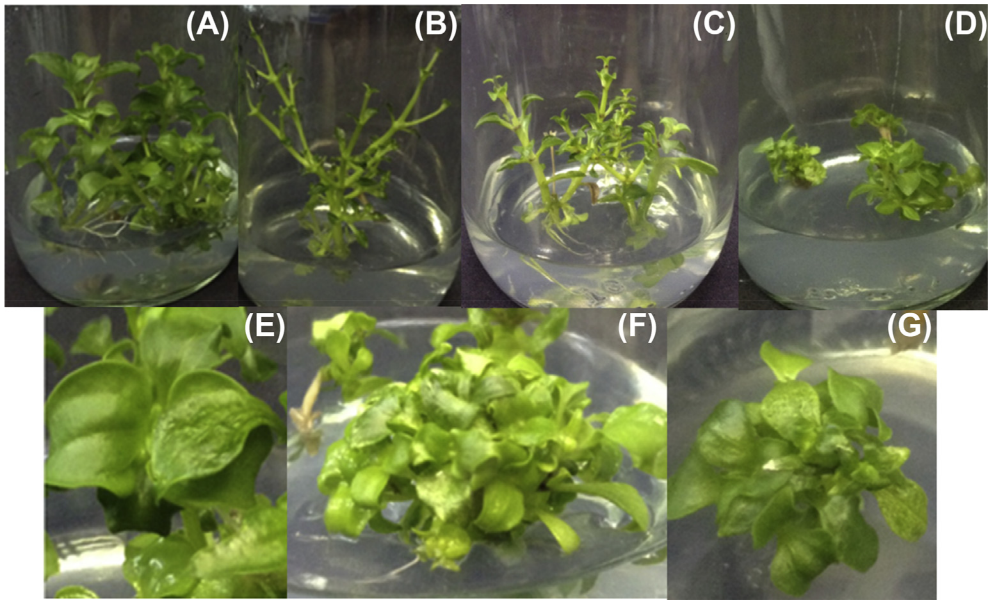

Fig. 3. Variations observed in M_1V_2 generation: (A) control (0 Gy); (B) small leaves at doses of 10 and 30 Gy; (C) small leaves and short internodes at doses of 20 and 40 Gy; (D) dwarfism at doses of 10, 30, 40, 50 and 60 Gy; (E) curl shaped leaf at 40 Gy; (F) light green leaves at doses of 30 and 40 Gy; (G) variegated leaves at doses of 40, 50 and 60 Gy.

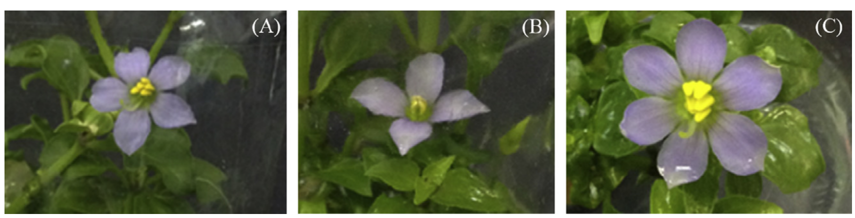

Fig. 4. Variations in flower petal number observed in M_1V_2 generation: (A) control (0 Gy) with 5 petals; (B) 30 Gy with 4 petals; (C) 6 petals in a flower found at doses of 30, 40, 50 and 60 Gy.

The effect of radiation on growth was assessed by counting the number of new branches at 60 d after irradiation and calculating the growth rate (Table 1, Fig. 2). Non-irradiated plantlets (control) produced a higher number of new branches compared to irradiated plantlets. The result from ANOVA indicated that there was highly significant difference in the percentage of growth rate between non-irradiated control and irradiated with gamma-rays at any dose ($p < 0.01$; Table 1). This was in agreement with Jompuk et al. (2009) who studied the effects of acute gamma radiation on *Cryptocoryne wendtii* “brown” and Tangpong et al. (2009) who worked *in vitro* on *Anubias congensis* N.E. Brown and found that the growth rate decreased with increasing radiation dose. Lamseejan et al. (2002) reported similar results for *Curcuma* spp. In the current study, $GR_{50(60)}$ was 32 Gy (Fig. 1). Theoretically, exposure to gamma rays produces negative effects on plant growth and development. The extent of growth reduction varies with species and dosimetry and the radio sensitivity of plant tissue must be determined by exposing the seeds, plants or explants to different doses of radiation and by studying the ‘dose-response’ effect on several growth traits (Wongpiyasatid et al., 2007; Marcu et al., 2013; Yadav, 2016). From this research the appropriate dose of acute gamma radiation to be used for inducing mutation in tissue cultures of *Exacum affine* Balf.f. ex Regel ranged from 20 to 40 Gy.

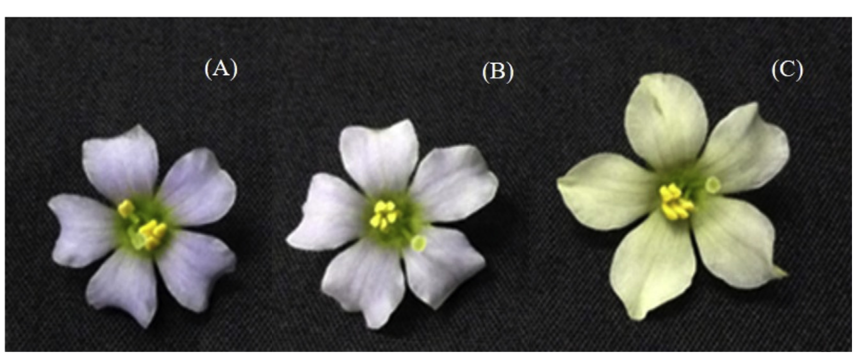

Fig. 5. Flower color and flower size variability of *Exacum affine* Balf.f. ex Regel after treatment of *in vitro* culture with different doses of gamma-rays: (A) control (0 Gy); (B) 20 Gy; (C) 30 Gy.

Variation in M_1V_2 generation

Morphological variation was observed in the M_1V_2 generation. In the 10 Gy treatment group, some plants with small leaves and dwarfism were observed; in the 20 Gy treatment group there were some plants with small leaves and short internodes; in the 30 Gy treatment group there were some plants with small leaves, light green leaves and dwarfism; in the 40 Gy treatment group there were some with small leaves and short internodes, curl-shaped leaves, light green leaves, variegated leaves and dwarfism; and in the 50 Gy and 60 Gy treatment groups there were some with variegated leaves and dwarfism (Fig. 3). In this study, the leaf variation changes observed in irradiated samples included smaller leaf size and lighter green leaves than in the control and variegated leaves in the M_1V_2 generation, whereas Masae et al. (2014) reported their irradiated samples as having a larger leaf size and darker green leaves compared to their control. Perhaps the differences between the two studies could be attributed to the different types of radiation used in each study, as the current study used irradiation with gamma rays (ionizing radiation) but Masae et al. (2014) used ultraviolet irradiation (non-ionizing radiation).

In the M_1V_2 generation, some plants in the 30 Gy treatment group exhibited flowers with four petals and six petals in a flower; in the 40 Gy, 50 Gy and 60 Gy treatment groups there were some flowers with six petals (Fig. 4) and flower color changes were also observed at doses of 20 and 30 Gy (Fig. 5). Schum and Preil (1998) concluded that for many species, mutation induction led to changes in flower shape and flower color. In a number of species, either an increase or decrease of petal number, or both, were recorded.

Conflict of interest

None.

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