Ionizing Irradiation Using Electron Beam to Control Importation of Biothreat Agents in Geranium Cuttings Inhibits Rooting

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Abstract. *Pelargonium × hortorum* ‘Tango Dark Red’, ‘Tango Pink’, ‘Americana Red’, and ‘Rocky Mountain Lavender’ unrooted cuttings were subjected to Electron beam (E-beam) irradiation at 0 (control, no treatment), 0.61 ± 0.04 , 0.83 ± 0.07 , or 1.02 ± 0.01 kGy (mean $\pm$ SE, $n = 4$) in Expt. 1 and 0 (control, no treatment), 0.08 ± 0.00 , 0.16 ± 0.00 , 0.31 ± 0.00 , or 0.57 ± 0.02 kGy (mean $\pm$ SE, $n = 4$) in Expt. 2. Cuttings exposed to E-beam irradiation other than the control treatment did not root or form callus and exhibited a change in leaf color from green to red and eventually yellow. Our results suggest that the use of ionizing irradiation for preventing the accidental importation of biothreat agents through unrooted *Pelargonium* cuttings is not feasible.

Ionizing irradiation such as Electron beam (E-beam) irradiation is used to destroy microbial pathogens in food (Smith and Pillai, 2004). In addition to food, E-beam irradiation can be used for sterilizing pharmaceutical products, curing polymers, and for phytosanitary applications to prevent the importation of dangerous insects and pests. We hypothesized that E-beam irradiation could be used for the disinfestation of *Pelargonium* cuttings to allow importation of cuttings produced offshore without the risk of introducing disease agents that currently are not present in the United States. Experiments were conducted to assess the feasibility of using E-beam irradiation doses of 1 kGy or less as a disinfestation tool for geranium cuttings following current USDA-APHIS regulations, which restrict the use of ionizing radiation to 1 kGy or less for phytosanitary applications (APHIS, 2002).

Materials and Methods

Pelargonium × hortorum ‘Tango Dark Red’, ‘Tango Pink’, ‘Americana Red’, and ‘Rocky Mountain Lavender’ unrooted cuttings were obtained from Fischer USA (Boulder, CO) and transported in the original shipping container to the National Center for Electron Beam Research facilities at Texas A & M University in College Station, TX, for E-Beam irradiation. Cuttings of each cultivar were unpacked and divided into lots of 25 and repackaged in resealable polyethylene bags. The bags from each cultivar were randomly assigned to one of four groups with each group containing one bag of each cultivar. In the first experiment, the groups of cuttings were treated with 0 (control no treatment), 0.61 ± 0.04 , 0.83 ± 0.07 , or 1.02 ± 0.01 kGy (mean $\pm$ SE, $n = 4$). The E-Beam doses were quantified using alanine dosimeters. Immediately after treatment, the cuttings were transported to the Texas A & M University, TAES–Dallas Center and stored overnight at 3 ± 1 °C in the box in an unlit incubator. The next morning, the cutting ends were dipped in Hormodin #1 (0.1% indolebutyric acid; OHP, Mainland, PA) powder. The cuttings from each irradiation treatment were randomly divided into four lots for each cultivar (six cuttings in lots one to three and seven cuttings in lot four). The cuttings were stuck in four randomized complete blocks in

a mist bench containing 50% perlite/50% vermiculite rooting media with bottom heat (24 ± 2 °C). Mist frequency was controlled by an electronic leaf (Phytronics, Earth City, MO). In Expt. 2, the same procedure was followed except that the treatment dosages were 0 (control no treatment), 0.08 ± 0.00 , 0.16 ± 0.00 , 0.31 ± 0.00 , or 0.57 ± 0.02 kGy (mean $\pm$ SE, $n = 4$). The cuttings were checked for rooting at 14 d for both experiments. In Expt. 1, the cuttings were pulled on day 25 after sticking, and rooting data were collected. In Expt. 2, the cuttings were pulled on day 17 to collect final rooting data. Data consisted of number of roots and length of roots.

Results and Discussion

In both experiments, 100% of the cuttings of the four cultivars in the control treatment rooted within 14 d. However, none of the cuttings treated with E-beam irradiation at any dosage exhibited any sign of callusing or rooting at day 25 for Expt. 1 or day 17 for Expt. 2. Additionally, the leaves turned a crimson red after treatment with E-beam irradiation and there was no growth of the shoot apex (Fig. 1). The rate and intensity at which the leaves turned red was directly proportional to the dosage received by the cuttings with the cuttings treated with the highest dosage (1.02 kGy) turning red within 1 week of the treatment (data not shown). The loss of chlorophyll and exhibition of red pigments did not occur in any of the control cuttings and indicates that the E-beam irradiation was detrimental to the health of the cuttings.

Studies have reported that ionizing radiation can affect chlorophyll content and photosynthesis. Conter et al. (1986) showed that in the cyanobacterium *Synechococcus lividus*, very low ionizing radiation doses caused an earlier onset of photosynthesis as evidenced by an increase in pigment content and enhancement of glyceraldehyde-3-phosphate dehydrogenase. The authors suggested that radiostimulation was related to activation of enzymes protecting against peroxides induced under oxidative circumstances and to the activation of a glucose catabolism through the oxidative pentose phosphate pathway. Jo et al. (2001) reported that chlorophyll b is susceptible to ionizing irradiation. Jovanić and Dramićanin (2003) showed that at doses as low as 0.003 kGy (3 Gy), ionizing radiation was capable of altering the fluorescence spectra [fluorescence intensity ratio (FIR) at 695 and 735 nm] of pumpkin leaves. They believed that the spectral difference was manifested by a decrease of FIR as a result of a change in chlorophyll luminescence possibly resulting from an increase of chlorophyll concentration during the recovery process of the plant. However, Fan and Thayer (2001) reported that ionizing irradiation had no effect on chlorophyll content in alfalfa sprouts, whereas Mukhin and Sal’kova (1961) reported that potato tubers exposed to ionizing radiation lost their ability to form chlorophyll. In our

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Fig. 1. Pelargonium cuttings treated with electron beam irradiation; 0.0 kGy (control) on left and 0.61 ± 0.04 kGy on right.

experiments, the loss of chlorophyll and the lack of rooting or callus formation in treated cuttings indicate that pelargonium is very sensitive to ionizing irradiation.

In conclusion, because the pelargonium cuttings did not root at doses that were significantly below the current USDA-APHIS regulations for phytosanitary applications,

this indicates that the use of ionizing irradiation for preventing the accidental importation of biothreat agents through unrooted *Pelargonium* cuttings is not feasible.

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