Plant Breeding and Evaluation

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Section Editor

Mutagenesis of *Illicium parviflorum* for Novel Phenotypes

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Significance to Industry *Illicium parviflorum* Michx. is a prevalent landscape shrub for a myriad of reasons: its adaptability, fast growth, ability to form a dense mass, evergreen nature, and relatively pest-free landscape performance [1, 2]. Although it is a popular Florida and Georgia native for hedges and beds, very few cultivars exist. Much of the nursery stock *Illicium parviflorum* is clonal from very few wild sources [3, 4]. This inherent survival risk attributed to mass reproduction of the same genotype, plus the phenotypic uniformity among plantings, begs for the introduction of distinct cultivars for homeowners and landscapers. Gamma radiation was used to induce phenotypic variation in material for potential cultivar development.

Nature of Work: Small anise tree (*Illicium parviflorum*) performs well as a reliable landscape shrub providing a green to olive green vegetative mass in USDA hardiness zones 6-9. Due to its uniform appearance and the shortage of cultivars, there is much room for improvement in market options. Mutation induction was chosen over traditional breeding because of deterring factors like low fruit and seed set, small flowers, and reported self-incompatibility [5, 6]. Mutation breeding has led to many ornamental cultivars, and gamma irradiation is relatively quick, with minimal waste, and capable of changing one to few traits in plants [7, 8]. The objectives of this project were to observe and select for phenotypic variations induced by gamma irradiation of stem cuttings, determine the optimal dose for rooting cuttings, as well as evaluate the interaction of the effects of the stage of tissue growth with irradiation.

The cultivar ‘Forest Green’ which is described as having lustrous, dark green foliage was utilized for this study and cutting material was obtained from the State Botanical Garden of Georgia. Shoot tip cuttings were collected 2016 and 2017 in Feb., May and July/Aug. for hard-, soft- and semi-hardwood tissue types, respectively. Cuttings had 4-6 nodes and following irradiation treatment were dipped in potassium salt of Indole-3-butyric acid (K-IBA) at 3,000 ppm for approx. 5 seconds. After allowing to air-dry for a few minutes, cuttings were stuck into propagation mix (2:1 Jolly Gardener® Pro-Line Growing Mix: Aero-soil® perlite), randomized by irradiation treatment level, and placed under mist (8 secs. every 5 mins. from 7am-7pm) for 9-12 weeks (Table 1). Irradiation was administered with a ^{60}Co source and dose levels varied for tissue type and year (see Table 1) but always included an untreated control. The experimental unit was a single stem cutting and there were 10 replicates per irradiation level for each tissue type.

Stem cuttings were taken out of mist and evaluated on rooting. A successfully rooted cutting was scored as 1 and an unrooted cuttings was scored as a 0. Data for 2017 was analyzed as a randomized complete block design (RCBD) where each tissue type, or collection date, was treated as a block and irradiation as the main effect. For 2016, each tissue type was treated as a completely randomized design (CRD) and irradiation was the main effect. Data for 2017 was analyzed as a two-way ANOVA and for 2016 as a one-way ANOVA with transformation of the binomial data in R [9].

Results and Discussion Stem cuttings of *Illicium parviflorum* were affected by exposure to gamma irradiation and type of tissue when evaluated for rooting success. Data from each year was analyzed separately due to differences in irradiation levels. For 2017, there was a 60 Gy treatment for hardwood and softwood but not semi-hardwood. Additionally, neither tissue type had any successful rooting at that level, therefore it was omitted in a two-way ANOVA for sake of balancing data (Figure 1). The analysis of rooting found irradiation to have an effect ($p<0.001$), as well as tissue type ($p<0.01$) with a non-significant interaction ($p=0.30$). The highest number of rooted cuttings was for the untreated control using hardwood tissue. Within each of the three tissue types, no irradiation treatment had higher rooting than the non-irradiated control. Each of the three tissues had zero rooting when irradiation was greater than 10 Gy, with the exception of semi-hardwood with one rooted cutting at 20 Gy. The level of irradiation for which cuttings had peak rooting at 5 Gy for hardwood, 3 Gy for softwood, and 10 Gy for semi-hardwood tissue.

Data from 2016 could not be analyzed as a two-way ANOVA due to substantial unbalanced data from differences in irradiation treatment levels. Therefore, data for each collection date, or tissue type, was analyzed separately and irradiation had a significant effect for each tissue type ($p<0.001$). Cuttings taken of hardwood and softwood were influenced by irradiation and even the control had a relatively moderate rooting percentage (Figure 2). Semi-hardwood had the highest rooting for 2016 with all cuttings rooting for the control and just below that were cuttings treated at 5 Gy with 90% rooting and 3 Gy with 70% rooting. This observation of such a high rooting % in the summer for *Illicium parviflorum* was expected based on propagation suggestions found in Dirr and Heuser [10].

Rooting similarity was high for 3 and 5 Gy treated cuttings, which would seem to be the optimal level for irradiating *Illicium parviflorum* to obtain successful rooted cuttings. Cuttings readily root at <3 Gy, however at such a low rate of irradiation, it seems unlikely that any mutation would occur. From phenotypic observations thus far, a few plants have started to display differences compared to controls. Two plants treated in the summer of 2016 at 5 Gy have a unique foliage morphology (Figures 3 & 4). The shape appears curved, rather than the uniformly linear shape typical of the species, and the surface has a warped, wrinkly texture which is very different from the smooth untreated plants. These findings suggest that a dose of 5 Gy for semi-hardwood cuttings are optimal for obtaining phenotypic mutations in rooted cuttings of *Illicium parviflorum*.

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Table 1. Details of individual treatment levels by tissue type over 2016 and 2017.

Stem cutting type	Initiation Date	Weeks Under Mist	Rates Applied (Gy*)
Hardwood	2/16/16	9	0, 20, 40, 60
Softwood	5/20/16	12	0, 5, 10, 20, 40, 60
Semi-hardwood	8/4/16	12	0, 3, 5, 10, 20, 40
Hardwood	2/17/17	10	0, 5, 10, 20, 40, 60
Softwood	5/16/17	10	0, 3, 5, 10, 20, 40, 60
Semi-hardwood	7/27/17	10	0, 3, 5, 10, 20, 40

*Gy : joule of radiation energy per kilogram of matter

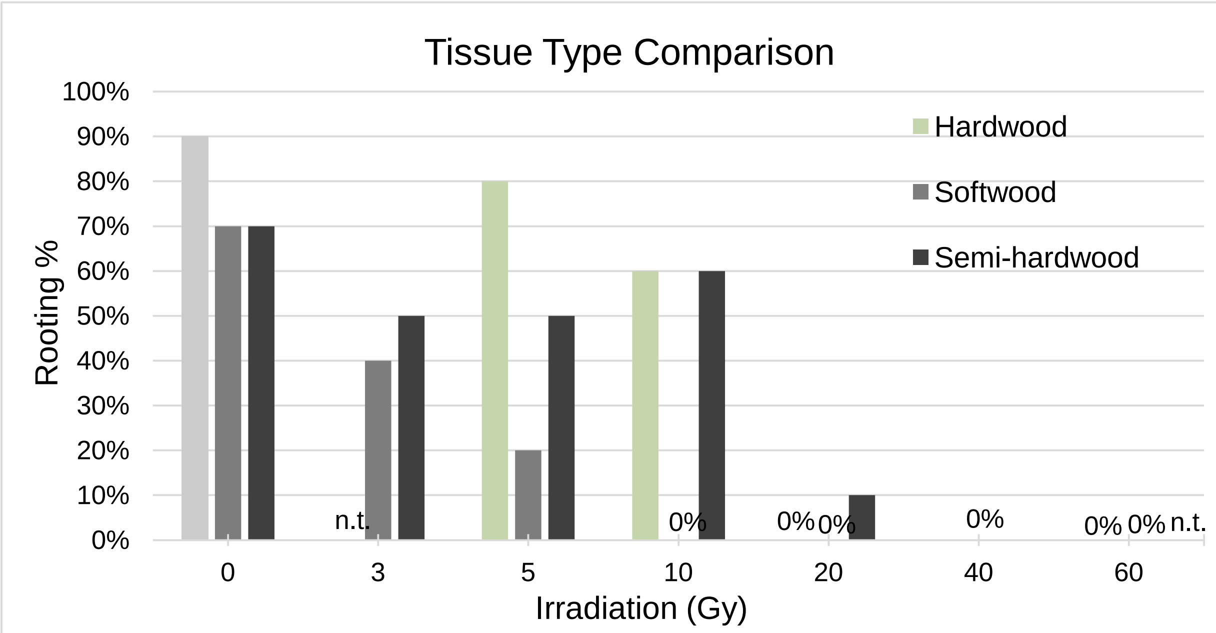

Figure 1. Rooting percentages for 2017 irradiation treatments of *Illicium parviflorum* by stem cutting type. 'n.t.' indicates there was no treatment for tissue type at that irradiation level.

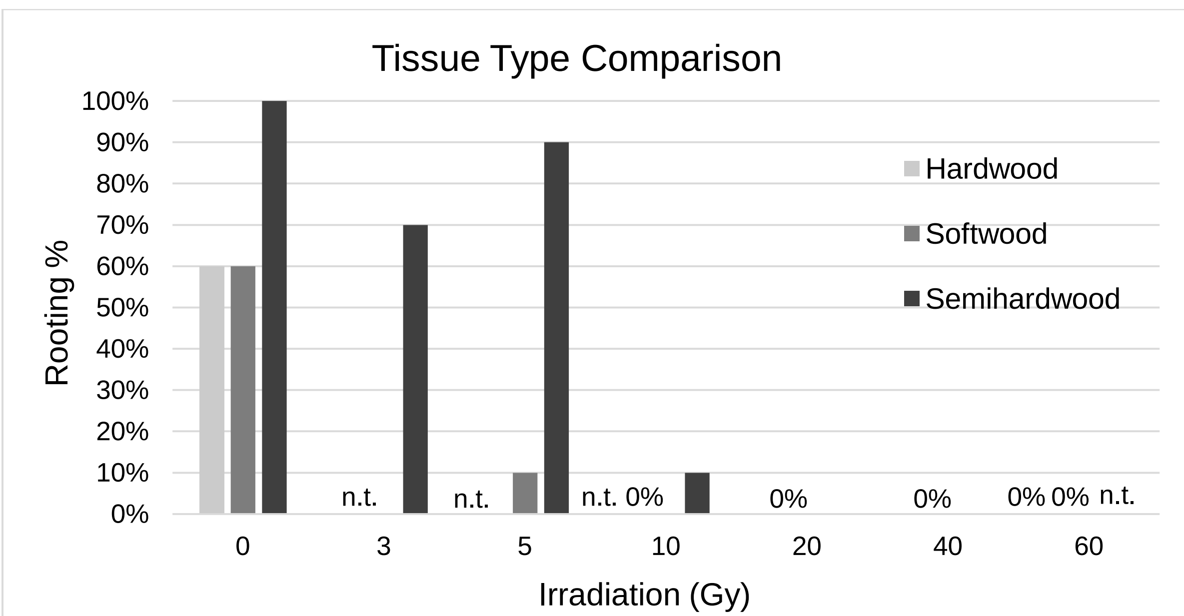

Figure 2. Rooting percentages for 2016 irradiation treatments of *Illicium parviflorum* by stem cutting type. 'n.t.' indicates there was no treatment for tissue type at that irradiation level.

Figure 3. Shoots (top) and leaves (below) from plants of *Illicium parviflorum* of control (L) and irradiated at 5 Gy (R) on July 28, 2016.

Figure 4. Shoots (top) and leaves (below) from plants of *Illicium parviflorum* of control (L) and irradiated at 5Gy (R) on Aug. 4, 2016.