Ilex Embryo Germination Saving Your Time©

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INTRODUCTION

Holly (*Ilex*) is a large genus in *Aquifoliaceae*, which is comprises of 400 to 600 deciduous and evergreen species. This genus is cultivated as important medicinal and ornamental plants in the temperate and subtropical regions (Galle, 1997; Hu, 1989). The great diversity and adaptability of hollies make them as the king in gardens and landscapes. They can be used as shade trees, dividing lines, hedges, and groundcovers. They have beautiful effects of fruits in autumn, masses of evergreen foliage, and bright glistening color of variegated cultivars (Robinson, 1984). *Ilex crenata* Thunb. is native to eastern China, Japan, Korea, Kuril, Sakhalin, Philippines, and the Himalayas. Now it is widely planted as an ornamental plant in the southeastern US for its dense evergreen foliage and various forms (Dirr, 2009). Many cultivars have been released for commercial production such as *I. crenata* (Fastigiata Group) 'Sky Pencil', which is popular in the landscape for its strongly upright habit and lustrous, dark evergreen foliage (Dirr, 2011).

Similar to the majority of *Ilex* species, seed germination of 'Sky Pencil' is inefficient as a result of low germination rate and long germination time. It usually takes 2 to 3 years to overcome the double dormancy from hard, impermeable seed coat and immature embryos (Dirr and Heuser, 2006). Normally, 'Sky Pencil' is propagated by rooting of stem cuttings with 1000-3000 ppm IBA (The United States National Arboretum). But for plant breeders, it is hard to select new cultivar from cuttings. New cultivars are from open pollinated and artificial cross. Therefore, seed germination is the key point to select new cultivars from I. crenata 'Sky Pencil'. To shorten the germination time and select new cultivars efficiently, we investigated the embryo germination of *I. crenata* 'Sky Pencil'.

MATERIALS AND METHODS

Plant Material and Culture Establishment

On 13 Jan. 2014, mature fruits from an 8-year-old plant of *I. crenata* 'Sky Pencil' seedling at the Horticulture Farm University of Georgia were collected and washed with running tap water for 20 min, then rinsed with distilled water. Surface disinfection was carried out with 75% ethanol for 5 min followed by immersion for 15 min in Clorox (included 8.3% sodium hypochlorite, Clorox Company, Oakland, California). Subsequently, they were washed five times with double distilled water and kept in sterile water until excision of embryos under a stereomicroscope in a laminar-flow hood. The immature embryos at heart-shaped stage (Fig. 1A) were placed into 6-cm petri-dishes containing various basic culture media plus 3% sucrose (Sigma-Aldrich, Co., Louis, Missouri) and 0.65% agar for germination. The pH of the media were adjusted to 5.8 with NaOH or HCl before adding agar (Fisher Science Education, Nazareth, Pennsylvania). A total of 12 ml of the media was transferred by pipette into autoclaved dishes. All dishes with embryos were cultured in a growth chamber at room temperature in darkness. After 2 weeks, on 28 Jan. 2014, germinated embryos were moved to a growth chamber with 14-h photoperiod under cool-white fluorescent lamps (115 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Embryo germination rate and the height of seedling were recorded. Four weeks later, on 1 Mar. 2014, seedlings with at least two true leaves were transferred to a tray with 32 cells ($6.5 \times 6.5 \times 9$ cm^3) with a mixture of Aero-Soil perlite (Dicalite, Dicaperl Minerals, Inc., Bala Cynwyd, Pennsylvania) and a commercial substrate (Fafard 3L Mix, Sun Gro Horticulture Canada Ltd., Agawam, Massachusetts) in a ratio of 1:1 (v/v) and kept in greenhouse. Flats were placed under

intermittent mist. Misting frequency was controlled by a misting controller (Phytotronics, Inc., Earth City, Missouri) and set at 15 s every 10 min for the first 2 weeks. Mist system was on in the morning and off in the evening. No additional light was provided. Germination rate, seedling height, the number of leaves and number of roots were recorded before transplanting into a tray. After 2 months, plant survival rate was also recorded.

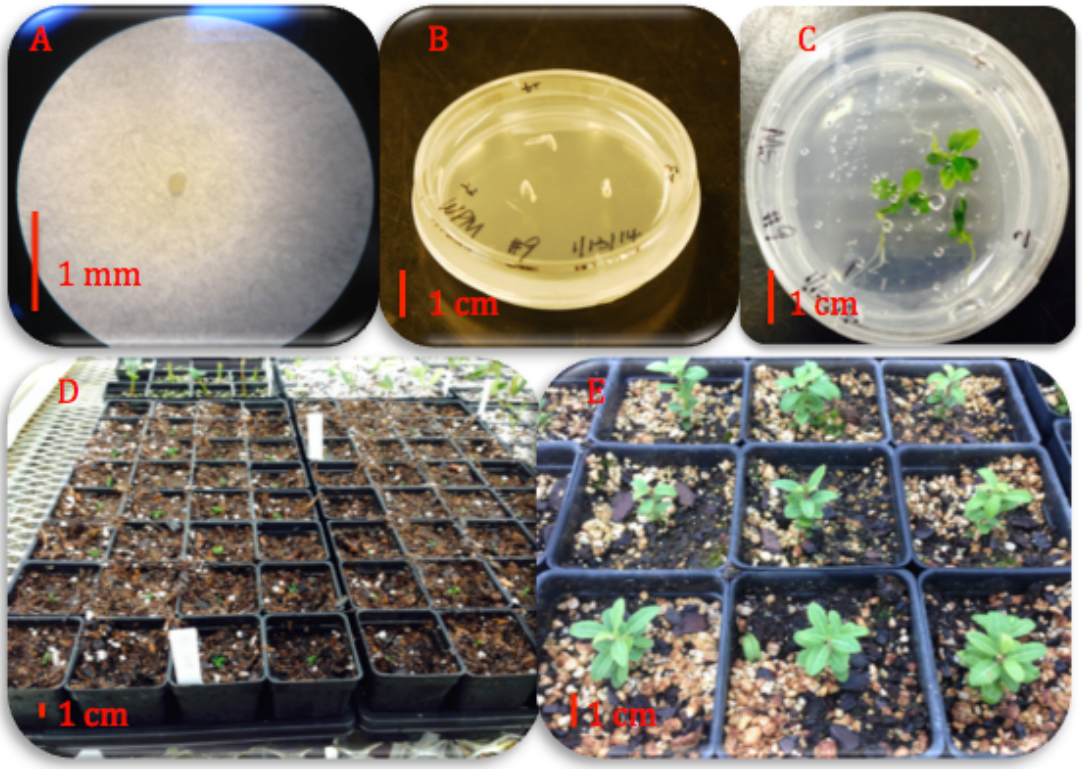

Fig. 1. *Ilex crenata* embryo (A); Embryo germination (B); Seedlings after growing four weeks in chamber (C); Transferred seedlings in greenhouse (D); Two-month old seedlings after growing (E).

Medium Selection for Embryo Germination
To determine the optimal conditions for embryo germination, quarter-strength Murashige and Skoog (1962) (MS) (¼ MS) (1962), half-strength MS (½ MS), full-strength MS, and Woody Plant Medium (WPM) (Lloyd and McCown, 1981) were tested in this experiment.

Experimental Design and Statistical Analysis
Randomized complete block design was employed in all experiments. Each treatment with nine embryos (subsamples) was repeated three times. Analysis of Variation (ANOVA) and General Linear Model (GLM) were performed using Statistical Analysis Systems (SAS Version 9.2; SAS Institute, Inc., Cary, North Carolina).

RESULTS AND DISCUSSION

Protocol for Holly Embryo Germination
Instead of 2-3 years to germinate holly seeds, embryo germination only needs 2-3 weeks. The time you saved is very significant. Generally, we should collect fruits and harvested immature embryos from July to December. Embryos were excised under a stereomicroscope, and inoculate on embryo germination media. In 2-3 weeks, embryos germinated. The entire procedure is not difficult as we normally think. What you need is a

hood, a clean room, a stereomicroscope, and an autoclave. If you don't have an autoclave, you could buy sterilized customer designed media for your embryo germination.

Effect of Media on Germination Rate

We took germination data on 28 Jan. (Germination Rate 1) and on 1 Mar. 2014 (Germination Rate 2), respectively (Fig. 2). The embryo germination rate was from 45.8% to 79.2% in January. Four weeks later, the germination rate ranged from 58.3 to 91.7%. Obviously, majority of embryos germinated in 2 weeks and this trend still continued in the next 4 weeks. The highest germination rate, 91.7%, was obtained under the treatment of ¼ MS. It was significantly higher than that of ½ MS at 57%. From our observation, the culture media didn't have significant difference on germination rate in the first 2 weeks. But they had significant difference on germination rate after 6 weeks. From the above result, we concluded that the embryo germination of *I. crenata* 'Sky Pencil' took 2-3 weeks.

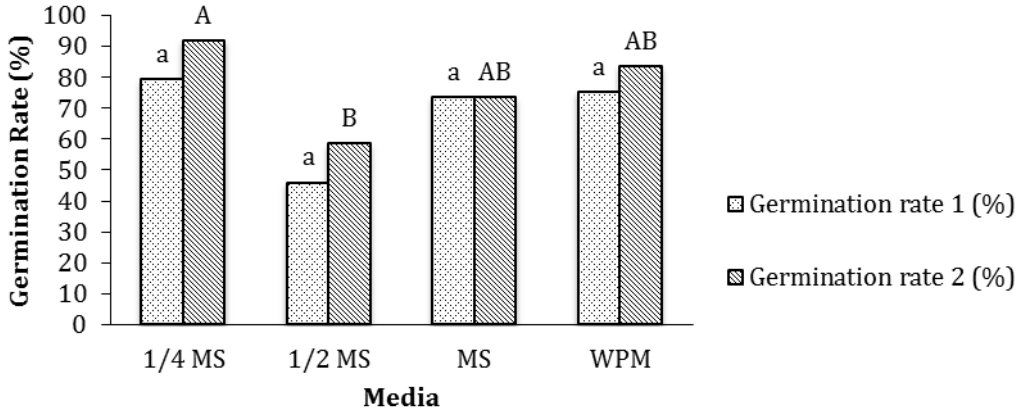

Fig. 2. Effect of media on germination rate. (Different letters mean significant differences at α=0.05).

Effect of Media on Seedling Growth

We also took data of seedling height on 28 Jan. (Seedling Height 1) and on 1 Mar. 2014 (Seedling Height 2), respectively (Fig. 3). As shown in Figure 3, culture media had a significant difference on seedling height. The fastest growth of seedlings was under the treatment of ¼ MS. The tallest average seedling height was also under the treatment of ¼ MS on 1 Mar. 2014. In addition, the culture media had significant difference on number of leaves and number of roots (Fig. 4). Both under ¼ MS and WPM, we could get much better growth of 'Sky Pencil' seedlings.

Effect of Media on Seedling Survival Rate

Two months after transplanting (Fig. 1E), the survival rate was recorded. The culture media had no significant difference on 'Sky Pencil' seedling survival rate. All of treatments had high survival rate from 87.5 to 90.9%.

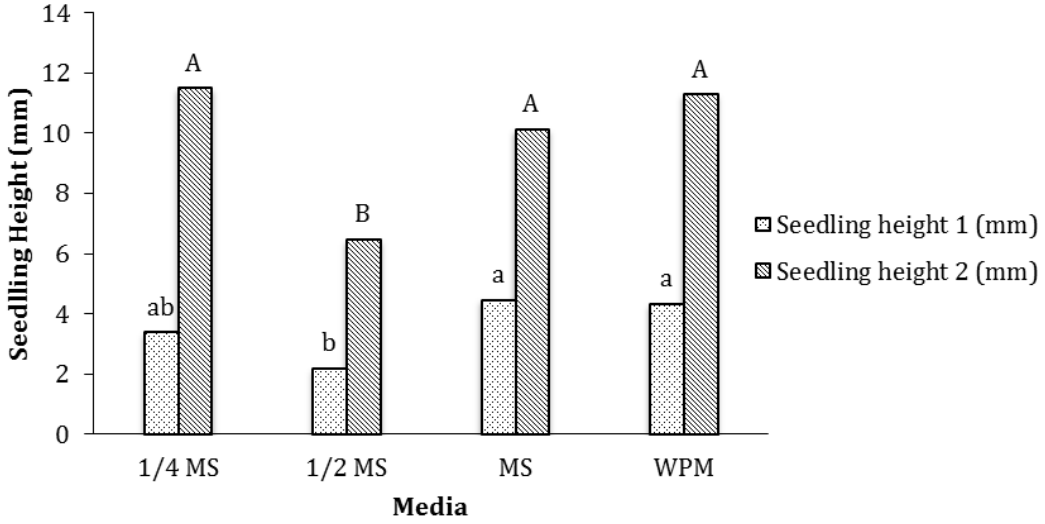

Fig. 3. Effect of media on seedling height (mm). (Different letters mean significant differences at α=0.05).

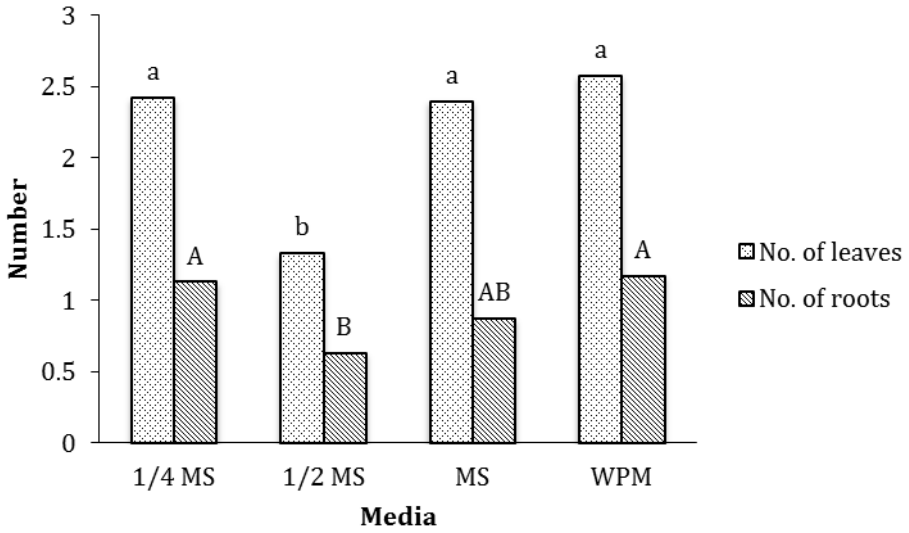

Fig. 4. Effect of media on quality of seedlings. (Different letters mean significant differences at α=0.05).

CONCLUSION

A rapid seed propagation protocol for *I. crenata* 'Sky Pencil' is: collected embryo from surface sterilized fruits, inoculated on¼ MS medium with 3% sucrose, and grow them under dark condition. After embryo germination (Fig. 1B), moved them to light and grew 4 more weeks (Fig. 1C), and then washed seedlings and transplanted them into growing media (Fig. 1D).

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