Combined Proceedings IPPS 70:81-86. 2020.

Effects of Gibberellic Acid and Cold Stratification on Sparkleberry (*Vaccinium arboreum*) Germination Under Different Collection Times

Ping Yu[1], Lin Li[2], Qiansheng Li[3] and Mengmeng Gu[3]

[1]Department of Horticulture Sciences, Texas A&M University, College Station, TX, 77843, USA; [2] College of Architectural Arts, Guangxi Arts University, Nanning, Guangxi 530007, China; [3]Department of Horticulture Sciences, Texas A&M AgriLife Extension Services, College Station, TX, 77843, USA

yuping520@tamu.edu

Keywords: Blueberry rootstock, seed germination

Summary

Sparkleberry has the potential to be used as commercial blueberry's (*Vaccinium spp.*) rootstock due to its wider adaptation to the environment, tolerance to higher pH, and its singular architecture, which can reduce blueberry yield loss during mechanical harvesting. There is little information in the literature on seed germination of sparkleberry. We report that gibberellic acid and cold stratification work synergistically to increase sparkleberry germination for seed collected in Texas during November and December. The optimal germination treatments for sparkleberry seeds collected from November was 500 mg L^{-1} gibberellic acid (GA_3) followed by cold stratification for 9 weeks, which had a 70.4% emergence percentage.

INTRODUCTION

Sparkleberry (*Vaccinium arboretum*) is a shrub in *Vaccinium* family native from climatic zone seven to nine in the United States. Sparkleberry can be found from southern Virginia to southeastern Nebraska, from Florida to eastern Texas (Dirr, 1990). Sparkleberry has the potential to be used as commercial blueberry's (*Vaccinium spp.*) rootstock due to its wider adaptation to the environment (Griffin and Blazich, 2008; Casamali et al., 2016a). Sparkleberry has a broader tolerance to soil pH and requires lower soil organic matter than blueberry. Also, unlike branchy blueberry, sparkleberry has a singular architecture, which can reduce blueberry yield loss during mechanical harvesting (Brooks and Lyrene, 1998; Yang et al., 2014; Casamali et al., 2016b).

How can we propagate sparkleberry to fulfill its potential as blueberry rootstock? It is hard to root sparkleberry either via soft-wood cuttings or hardwood cuttings. Lyrene (1998) reported difficulty in propagating sparkleberry via softwood cuttings. In our previous trials, none of sparkleberry hardwood cuttings or softwood cuttings from plants in the native habitat rooted.

Little information about sparkleberry seed germination can be found in the literature. The only study on sparkleberry seed germination in recent years reported that the highest seed germination rate was 43%, which was obtained by cold stratification (the process of subjecting seeds to both cold and moist conditions) for 90 days (Yang et al., 2014). Treating *Vaccinium* species with gibberellic acid (GA_3) could increase the germination of *Vaccinium* species in some cases (Griffin and Blazich, 2008).

Therefore, this study was to evaluate the effects of GA_3 and cold stratification on germination of sparkleberry seeds collected during different times. The goal of this study was to establish guidelines and provide valuable information for sparkleberry mass production in nurseries for blueberry rootstock.

MATERIALS AND METHODS

Plant Material

Sparkleberry seeds were collected from ripe berries on plants grown in natural areas of the Woodland Hills Park (College Station, TX, USA) in November and December, 2018. Viable seeds were obtained by washing seeds several times and selecting seeds at the bottom of water as CIORDIA et al. (2006) found that floating seeds had much-reduced viability. Seeds were surface sterilized with 5% regular Clorox bleach for 10 minutes, then rinsed with deionized water (DI water) and dried in open air at room temperature for two days.

Experimental Treatment

After being dried, for each treatment, 50 seeds were put into 10 cm petri dish filled with 20 mL of (GA_3) solutions at 500 and 1,000 mg L^{-1} for 24 h, and the same amount of DI water was used as GA_3 0 mg L^{-1} treatment (Fig. 1). Seeds were then rinsed with DI water and sown in 4-in. pots (dimensions: top 7.5cm, bottom 6cm, depth 8.2cm with four holes underneath) filled with commercial germination mix (Pro-mix HP

with Mycorrhizae, BWI Companies, USA) on April 10, 2019. After adding a thin layer of media on the top of the seeds, pots with GA_3 treated seeds were put either into the greenhouse (0 week of cold stratification) or the cold storage for cold stratification for 3, 6 and 9 weeks. After cold stratification, pots were then taken out and placed in the greenhouse. Pots were watered with DI water as needed throughout the experiment.

Figure 1. Sparkleberry (*Vaccinium arboreum*) seed collected in November and December in 2018, subjected to different gibberellic acid (GA_3) (0, 500, and 1,000 mg L^{-1}) in petri dishes (A); Sparkleberry (*Vaccinium arboreum*) seed collected in November and December in 2018, germinated after 3 weeks of cold stratification with GA_3 treated at 0, 500, and 1,000 mg L^{-1} (B).

Experimental Design

The experiment was arranged in a two-factor split-plot design with the GA_3 as the main plot and cold stratification as the subplot with five replicates for each treatment. The two factors were GA_3 concentration (0, 500, and 1,000 mg L^{-1}) and cold stratification (0, 3, 6, and 9 weeks). Each treatment in the experiment contained 50 seeds. Seeds collected in November and December were analyzed separately.

Data Collection

Data was collected by counting the numbers of germinated seeds (cotyledon emerged from the media) twice a week starting at week 0 for 0, 3, 6, and 9 weeks of cold stratification until week five when no more seeds came out. The emergence percentage (EP) was calculated by the following formula: EP = (No. of emerged seeds/total No. of seeds) × 100% and the emergence index (EI) were calculated as following: EI $=\sum_{i=1}^{n}(EPi/Ti)$, where EP_i is EP on day i ($i \geq 2$), and Ti is the number of days after sowing.

Data Analysis

Under two seed collection times (November and December), analysis of variance (ANOVA) was used to test the significance of treatments and interaction effects on germination. When the ANOVA showed difference, the multiple honest significant difference (HSD) Tukey's test was applied with the calculation of significant differences among means at $p \leq 0.05$.

RESULTS

There were two ways interactions (GA_3 and stratification) in the emergence percentage (EP) and emergence index (EI) for both November and December seeds (Table 1). GA_3 and stratification had significant impacts on EP and EI for both November and December seeds.

Table 1: A summary of the statistical significance of treatment factors on emergence percentage (EP, %), emergence index (EI, %), and number of days needed for germination. *, **, *** indicated significant difference according to Tukey HSD multiple comparison test at $p \leq 0.05$, ≤ 0.01, and ≤ 0.001, respectively.

	November		**December**	
Source	Emergence (%)	Emergence index	Emergence (%)	Emergence index
GA_3	***	***	*	**
Stratification	***	**	***	***
GA_3 × Stratification	***	***	***	***

For seed collected in November, cold stratification significantly increased seed EP (Fig. 2 A, B, C, D). For seed had been stratified for 0 week, 3 weeks, and 9 weeks, GA_3 concentration had significant influence on EP (Fig. 2 A, B, D). For seed had been stratified for 0 week and 3 weeks, 1,000 mg L^{-1} GA_3 had significantly higher EP than those without GA_3 (Fig. 2 A, B).

Seed treated with GA_3 at 1,000 mg L^{-1} for 3 weeks could reach an EP at 58.8%. There was no significant difference for 6-week treated seed among 0, 500 or 1,000 mg L^{-1} GA_3 treatments (Fig. 2 C). Seed treated with GA_3 at 500 mg L^{-1} for 6 weeks could reach an EP at 54.4%. For 9 weeks seed, 500 mg L^{-1} GA_3 had significantly higher EP than those with GA_3 at 0 or 1,000 mg L^{-1} (Fig. 2 D). Seed treated with GA_3 at 500 mg L^{-1} for 9 weeks had the highest EP (70.4%).

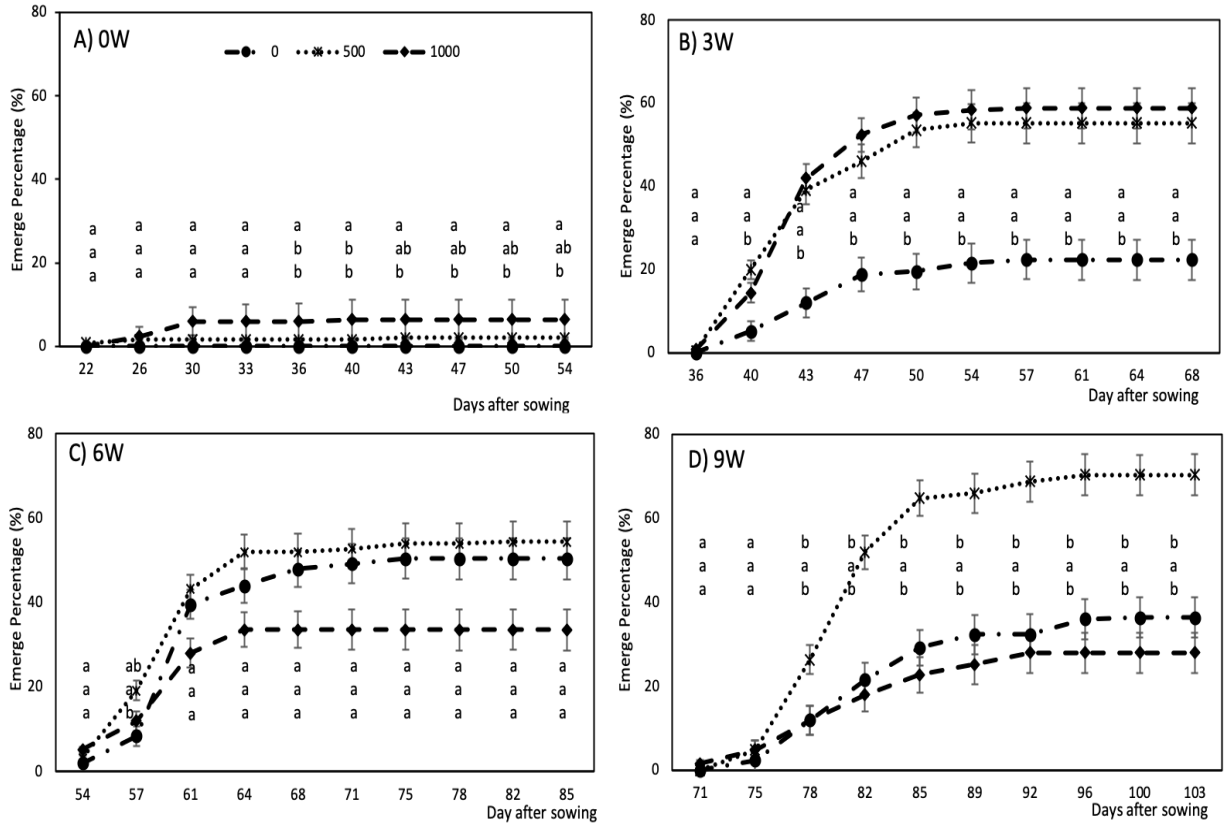

Figure 2. Emergence percentage (± standard error) for sparkleberry (*Vaccinium arboreum*) seed collected in November with cold stratification for 0 (A), 3 (B), 6 (C) and 9 (D) weeks and gibberellic acid (GA_3) at 0, 500, and 1,000 mg L^{-1} (indicated by solid line, dash line, and dash line with diamond, respectively). The same letters on the same day (in an order of 0, 500, 1,000 mg L^{-1} from the bottom to the top) indicate no significant difference among GA_3 treatments (0, 500, 1,000 mg L^{-1}) according to Tukey HSD multiple comparison test at $p \leq 0.05$.

CONCLUSION

In conclusion, we found that gibberellic acid and cold stratification could work synergistically to increase sparkleberry germination for seed collected in both November and December (Data not shown). The optimal germination treatments for sparkleberry seed collected from November was 500 mg L^{-1} GA_3 followed by stratification for 9 weeks, which had a 70.4% emergence percentage.

Literature Cited

Brooks, S.J. and P.M. Lyrene. (1998). Derivatives of *Vaccinium arboreum*× *Vaccinium* section cyanococcus: I. Morphological characteristics. Journal of the American Society for Horticultural Science *123*:273-277.

Casamali, B., J.G. Williamson, A.P. Kovaleski, S.A. Sargent, and R.L. Darnel. (2016). Mechanical harvesting and postharvest storage of two southern highbush blueberry cultivars grafted onto *Vaccinium arboreum* rootstocks. HortScience *51*:1503-1510.

Ciordia, M., M.d. Lucas, V. Mateos, L. Rodriguez, J. Garcia, and J. Majada. (2006). Optimization of germination requirement and seed production of wild-type *Vaccinium myrtillus*. Acta horticulturae *715*:85-88.

Dirr, M.A., (1990). Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses. Stripes Publishing, Champaingn, 1187 p.

Griffin, J.J. and F.A. Blazich. (2008). *Vaccinium* L. Woody plant seed manual. *US Department Agricultural and Forest Service.* Washington. EE. UU:1154-1159.

Lyrene, P.M. (1998). Germination and growth of sparkleberry seedlings (*Vaccinium arboreum* Marsh). Fruit Varieties Journal *52*:171-178.

Yang, W., H. Andrews, and A. Basey. (2014). Blueberry rootstock: Selection, evaluation, and field performance of grafted blueberry plants. Acta horticulturae *1117*:119-123.