Clipping and Gibberellin Treatments Promote Germination in Dormant Grape Seeds

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Abstract. **'Crimson Cabernet' grape (*Vitis vinifera*) seeds showed physiological dormancy and germinated at ~60% after 60 days of chilling stratification. Fresh seeds harvested after physiological maturity and sown without drying failed to germinate after 30 days when sown on agar. In agar-sown fresh seeds cut at the distal seed end or intact seeds treated with gibberellic acid (GA), the seeds germinated at ~20% after 30 days. The highest germination percentages after 30 days were 63% to 83% in fresh, agar-sown seeds that were cut and treated with GA at 5000 mg·L^{-1} regardless of stratification time. Similar results were seen in seeds allowed to dry before sowing. Seeds cut and treated with GA at 5000 mg·L^{-1} germinated at 79% after 30 days. However, dry seeds sown on germination paper showed lower germination after cutting and GA treatment compared with agar-sown seeds. The highest germination percentages after 30 days in dry, cut seeds on germination paper treated with GA at 2000 and 5000 mg·L^{-1} were 33% and 55%, respectively, compared with agar-sown seeds, which germinated at 76% and 79%, with the same treatments. Results from this study provide a system that reduces the need for chilling stratification for grape seed germination by using partial seedcoat removal and GA treatment.**

Grapes (*Vitis* sp.) are commercially important both as a table fruit and a processed fruit for raisins, juice, jams, and wine. Weather patterns around the world are changing and there is a need to breed new adapted cultivars of a variety of traditional crops, including grapes. There are predictions for dramatic reductions in suitable wine grape acreage in the United States—up to 81% by the late 21st century (White et al. 2006).

Grape seeds have physiological dormancy and usually require 3 to 4 months of chilling stratification to relieve dormancy (Baskin and Baskin 2014; Davies et al. 2018; Lin et al. 2009; Schopmeyer 1974). Although 3 to 4 months of stratification is usually successful in promoting high germination percentages (Ellis et al. 1983; Wang et al. 2009), several studies have shown variable and lower germination percentages after stratification in certain grape types (Selim et al. 1981; Singh 1961). Gan et al. (2008) demonstrated that stratification requirements for high germination percentages could be as long as 6 months, depending on genotype.

Seed germination is an important step in traditional breeding programs as well as accelerated crop breeding programs using novel genetic approaches for crop improvement. Extending the time between genetic crosses to seedling production decreases breeding efficiency. One obvious issue is the extended stratification periods required in many woody perennial fruit crops, such as the 3 to 4 months observed in grapes.

There are several surgical methods to bypass seed physiological dormancy in woody perennials, including embryo removal from the seed, or disruption of seed or fruit coat integrity (Geneve 1991). This has been observed in several fruit crops, including strawberry [*Fragaria ×ananassa* (Miller et al. 1992)], blackberry [*Rubus* sp. (Ke et al. 1985)], and grape (Wang et al. 2022). Hormones, primarily gibberellic acid (GA), can also substitute for chilling stratification to satisfy dormancy (Baskin and Baskin 2014). There is also significant anecdotal evidence that using fresh seeds that have not gone through the desiccation process show less dormancy compared with dried seeds in several woody perennials, including alder (*Alnus* sp.), persimmon (*Diospyros* sp.), and eucalyptus (*Eucalyptus* sp.) (Schopmeyer 1974).

Systems to bypass the need for chilling stratification to expedite seedling production could reduce breeding cycles and facilitate novel accelerated breeding programs. Therefore, the objective of this study was to investigate the impact of partial seedcoat removal and gibberellin treatment on the germination of freshly harvested and stored grape seeds.

Materials and methods

Plant material. Seeds were extracted from ripened 'Crimson Cabernet' grape (*Vitis vinifera*) fruit selected randomly from field-grown plants produced at the University of Kentucky Horticulture research farm, Lexington, KY, USA. 'Crimson Cabernet' is a cross between 'Cabernet Sauvignon' and 'Norton' (Dressel 2020). Fruit was crushed physically by hand, the pulp removed from seeds by rubbing it between paper towels, and the remaining pulp was rinsed from seeds. Cleaned seeds were either used immediately as fresh, undried seeds (~35% moisture) or were air-dried to ~4% moisture and stored in an airtight container at 10 °C.

Agar-sown fresh seeds. In experiments using fresh seeds, seeds were surface-disinfested for 10 min in a 10% commercial bleach solution followed by three rinses in sterile distilled water. Half the seeds were left intact and half were cut (clipped) through the seed, removing

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1	ppm	µg·g^{-1}	1
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

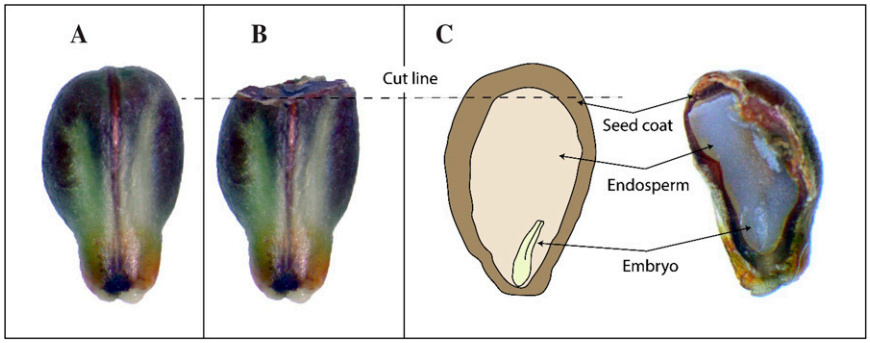

Fig. 1. Method for cutting (clipping) grape seeds through the distal rounded seedcoat and endosperm.

the distal, rounded seed portion (Fig. 1). Intact and cut seeds received a 24-h soak in sterile distilled water or in a filtered, sterilized GA (PhytoTech Laboratories, Lenexa KS, USA) solution at 2000 or 5000 mg·L^{-1}. GA-treated seeds were rinsed briefly in distilled water before moving them to plastic petri dishes (100 × 15 mm; Corning, Inc., Corning, NY, USA). In addition, seeds from each clipping or GA treatment were exposed to chilling stratification at 4 °C in the dark for 0, 30, or 60 d. Seeds were incubated in petri dishes on an agar-based (Bacto-agar; Sigma-Aldrich, St. Louis, MO, USA) Murashige and Skoog salts medium without sucrose and sealed with laboratory film (parafilm; Bemis Flexible Packaging, Shirley, MA, USA).

Dry seeds sown on agar or germination paper. Experiments with dry seeds were either used dry or hydrated for 24 h before undergoing treatment. Hydrated seeds reached about 30% moisture after soaking. Treatments included dry or hydrated seeds that were uncut or cut before being soaked for 24 h in distilled water or GA at 2000 or 5000 mg·L^{-1}. Seeds were incubated in petri dishes on agar-based (Bacto-agar) Murashige and Skoog salts medium without sucrose or placed on germination paper (grade 8001; Stults Scientific Co., Springfield, IL, USA) wetted with 5 mL distilled water.

Germination conditions. Dishes were sealed with laboratory film before being moved to a germination room at 25 °C under cool-white fluorescent lamps with a 16-h photoperiod (~40 µmol·m^{-2}·s^{-1}). For each experiment, there were 20 seeds placed per four replicate petri dishes for each treatment combination. Seed germination (radicle protrusion, 3 mm) was recorded at 10, 30, and 60 d. Mean separation was by Tukey's test at the 5% level and percentages were transformed (square of the arcsine) when appropriate before statistical analysis (SigmaPlot version 12.3; Systat Software, Inc., San Jose, CA, USA).

Seedling growth. To observe seedling growth, 10 to 15 germinated seeds from each experimental treatment were moved to cell packs (72 cells per flat) containing a greenhouse substrate (Promix BX; Premier Tech Horticulture, Quakertown, PA, USA). Flats were covered with a plastic dome and placed in a growth chamber at 25/21 °C (day/night) temperatures under cool-white fluorescent lamps with a 16-h photoperiod (~120 µmol·m^{-2}·s^{-1}). Seedling height (measured in centimeters) was recorded after 30 d.

Results

Agar-sown fresh seeds. Untreated fresh seeds sown on agar medium germinated at low percentages (<20%) and only initiated germination 60 d after sowing (Table 1). Cutting seeds before sowing increased germination in untreated seeds after both 30 and 60 d. Regardless of GA concentration, uncut GA-treated seeds germinated at similar percentages to untreated cut seeds, showing ~20% and 45% germination after 30 and 60 d, respectively. The earliest and greatest germination was observed in cut GA-treated seeds, which averaged 62% germination after 10 d and a maximal germination of ~75% averaged for GA treatments after 30 d. Seeds stratified for 30 or 60 d generally followed the same germination trends as unstratified seeds, but germination was generally initiated earlier and final percentages were higher. The greatest germination was seen in stratified cut seeds that germinated more than 69% for all treatments after 60 d (Table 1). The greatest germination was seen in cut seeds stratified for 30 d and treated with 5000 mg·L^{-1} GA (96%).

Dry seeds sown on agar or germination paper. Untreated dry seeds sown on an agar medium failed to germinate after 30 d and dry cut seeds germinated at only 2.5% (Table 2). Agar-

Table 1. Germination in fresh 'Crimson Cabernet' grape seeds on Murashige and Skoog media following clipping and gibberellic acid (GA) treatment 10, 30, or 60 d after chilling stratification for 0 to 60 d.

			Germination (%)		
Stratification time (d)	GA (mg·L^{-1})[i]	Clipping	10 d	30 d	60 d
0	0	Uncut	0	0	18.8 c
0	0	Cut	0	13.8 b	45.0 b
0	2,000	Uncut	0	21.3 b	47.5 b
0	2,000	Cut	62.5 a[ii]	73.8 a	73.8 a
0	5,000	Uncut	0	22.5 b	46.3 b
0	5,000	Cut	66.3 a	77.5 a	77.5 a
30	0	Uncut	0	3.8 d	43.8 c
30	0	Cut	0	30.0 c	77.5 b
30	2,000	Uncut	0	76.3 b	76.3 b
30	2,000	Cut	0	83.8 ab	82.5 ab
30	5,000	Uncut	0	21.3 c	78.8 b
30	5,000	Cut	14.0 a	83.8 a	96.3 a
60	0	Uncut	0	37.5 c	57.5 b
60	0	Cut	6.3 c	57.5 ab	75.0 ab
60	2,000	Uncut	0	45.0 c	73.8 ab
60	2,000	Cut	35.0 b	51.3 b	68.8 b
60	5,000	Uncut	1.3 c	66.3 a	86.3 a
60	5,000	Cut	63.8 a	62.5 a	78.8 a

[i] 1 mg·L^{-1} = 1 ppm.
[ii] Means followed by the same letter within a column are not different at 0.05 by Tukey's test.

Table 2. Germination in dry 'Crimson Cabernet' grape seeds on Murashige and Skoog media following clipping and gibberellic acid (GA) treatment after 30 d.

GA ($mg \cdot L^{-1}$)[i]	Clipping	Germination (%)
0	Uncut	0
0	Cut	2.5 c[ii]
2,000	Uncut	15.0 b
2,000	Cut	76.3 a
5,000	Uncut	22.5 b
5,000	Cut	78.8 a

[i] $1\ mg \cdot L^{-1} = 1$ ppm.
[ii] Means followed by the same letter within a column are not different at 0.05 by Tukey's test.

sown GA-treated uncut seeds showed improved germination to between 15% and 23% compared to uncut untreated seeds. However, the combination of cutting and GA treatment for agar-sown dry seeds improved germination to 76% and 79% in seeds treated with 2000 and 5000 $mg \cdot L^{-1}$ GA, respectively.

Dry seeds sown on germination paper showed similar trends to those sown on an agar medium, but germination percentages were generally lower (Table 3). Germination percentages for GA-treated seeds averaged 32%, with the highest germination percentage of 55% in cut nonhydrated seeds treated with GA at 5000 $mg \cdot L^{-1}$ (Table 3). There was a significant difference ($P = 0.014$) between hydrated and nonhydrated uncut GA-treated seeds, but this difference was almost completely accounted for by the low germination in nonhydrated seeds treated with GA at 2000 $mg \cdot L^{-1}$. There was also a significant difference ($P = 0.042$) between hydrated and nonhydrated cut GA-treated seeds, with greater germination in nonhydrated cut seeds.

Seedling growth. Seedlings moved to a greenhouse substrate in the growth chamber developed normally. Plant height did not differ among seedlings after 1 month from seeds germinated after being treated as uncut plus GA (10.3 cm), cut plus GA (11.3 cm), or cut without GA (11.2 cm).

Discussion

Grape seeds generally require an extended period of chilling stratification for dormancy release and subsequent germination (Baskin and Baskin 2014; Davies et al. 2018; Schopmeyer 1974). Several studies have attempted to circumvent chilling stratification with various chemical or seed-cutting pretreatments. In general, pretreating seeds with GA promotes germination both with or without stratification (Ellis et al. 1983; Kachru et al. 1972; Pal et al. 1976; Yeou-Der et al. 1967). The most common form of cutting grape seeds is removal of the basal micropylar portion, or beak, of the seed (Val et al. 2010; Wang et al. 2022). Cutting or clipping grape seeds generally promoted germination. Wang et al. (2022) showed increased germination percentages in six grape cultivars by removal of the seed base. This was especially dramatic in low-germinating seed lots. Although it is not clear, it appears seed cutting occurred after stratification. Val et al. (2010) showed that seed cutting was necessary to promote germination in unstratified seeds of 'Niagara Rosada' table grapes. However, Conner (2008) found no benefit to clipping the basal end of the seeds in muscadine grapes (*Vitis rotundifolia*).

In our study, GA treatment, seed cutting, and their combination promoted seed germination in unstratified seeds. One difference compared with the previous studies described was that seed cutting was performed on the distal portion of the seeds, away from the micropylar end opposite the radicle. Potential benefits of cutting at the distal seed end is the avoidance of any potential embryo damage and a larger exposed surface for penetration of exogenous substances such as GA, as well as a greater area to allow internal substances that might be associated with dormancy maintenance to leach from the seed. It is possible that seed cutting simply reduces the mechanical resistance to radicle emergence, but distal seed cutting being equally effective as basal cutting suggests that leaching could also be a possibility. Studies (Ellis et al. 1983; Song and Ou 2003) that have shown increased germination by leaching grape seeds in running water suggest that water soluble germination inhibitors such as abscisic acid (ABA) could be reduced in cut seeds with exposed cut surfaces. Alternatively, it has been suggested that dormancy alleviation and germination promotion by hydrogen peroxide in grapes (Ellis et al. 1983) and other species is a result, in part, of increased oxygen uptake, which has a putative impact on germination-inhibiting substances, including ABA (Nambara and Marion-Poll 2005). ABA levels have been shown to be correlated with precocious germination in grape somatic embryos, suggesting ABA is associated with seed dormancy in grape (Faure et al. 1998).

Numerous studies have shown improved germination in seeds or fruit (including achenes and drupelets) sown on an agar medium compared with ex vitro conditions, including several fruit crops such a s papaya [*Carica papaya* (Bhattacharya and Khuspe 2001)], blackberry (Ke et al. 1985), strawberry (Miller et al. 1992), and grape (Generoso et al. 2019; Val et al. 2010). Germination was further improved for agar-sown seeds by seed or fruit cutting in blackberry, strawberry, and grape. Similar results are

Table 3. Germination in dry 'Crimson Cabernet' grape seeds used with or without prior hydration on germination paper following clipping and gibberellic acid (GA) treatment after 30 d.

Seed type	GA ($mg \cdot L^{-1}$)[i]	Clipping	Germination (%)
Dry	0	Uncut	0
Dry	0	Cut	5.0 c[ii]
Dry	2,000	Uncut	8.8 c
Dry	2,000	Cut	32.5 b
Dry	5,000	Uncut	20.0 b
Dry	5,000	Cut	55.0 a
Hydrated	0	Uncut	0
Hydrated	0	Cut	20.0 b
Hydrated	2,000	Uncut	21.5 b
Hydrated	2,000	Cut	23.5 b
Hydrated	5,000	Uncut	37.5 ab
Hydrated	5,000	Cut	35.0 ab

[i] $1\ mg \cdot L^{-1} = 1$ ppm.
[ii] Means followed by the same letter within a column are not different at 0.05 by Tukey's test.

reported here for 'Crimson Cabernet' grape, in which the greatest germination was observed in fresh or dry seeds cut and germinated on an agar medium. The greatest germination in our study for unstratified seeds was observed in cut seeds treated with GA and sown on an agar medium (66% to 79%; Tables 1 and 2), which is remarkably similar to the 77% observed by Val et al. (2010) for cut seeds sown on an agar medium treated with GA at 4000 $mg \cdot L^{-1}$. Again, the major differences between the two studies were grape cultivars and location of the cut on the seed.

Dormancy may be affected by maturation drying, which is the transition phase from seed development to a mature, orthodox seed. In other seeds with fleshy fruit, such as blackberry and raspberry (*Rubus idaeus*), seed drying reduces germination percentages (Dale and Jarvis 1983; Mian et al. 1995). This is possibly associated with changes in dormancy-related substances in the seed coverings as the seed dries. However, in our study, there was not a substantial difference in using fresh seeds that were not exposed to drying after removal from the fruit compared with seeds allowed to dry after cleaning and sown on an agar (Tables 1 and 2). Uncut seeds treated with GA sown on an agar medium averaged 21.9% and 18.7% germination in fresh vs. dried seeds, respectively, whereas cut seeds treated with GA sown on an agar medium averaged 75.7% and 77.6% germination in fresh vs. dried seeds, respectively. Although, our study did not investigate the relationship between cutting seeds, GA treatment, and stratification in dry seeds, the interactions among these treatments in fresh seeds sown on an agar medium showed that the significant increase in germination by cutting and GA treatment was no longer observed after 30 or 60 d of stratification (Table 1).

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