Effects of Seed Harvest Time on Embryo Development and Seed Germination in *Musa velutina* Wendl. & Drude

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The ornamental banana plant *Musa velutina* Wendl. & Drude produces many seeds, but they do not germinate. To mass seeding production using seeds, we investigated the effects of seed harvest time on embryo development and seed germinability. Seeds harvested in August contained mushroom-shaped embryos with first and/or second leaf primordia. These seeds required scarification for germination, but not gibberellic acid 3 (GA₃) treatment. Seeds harvested in November contained mushroom-shaped embryos with no leaf primordia. These seeds required scarification and 2.9 μM GA₃ treatments for germination. Seeds harvested in February contained immature globular embryos and did not germinate after both treatments, but only 30% of the globular and mushroom-shaped embryos germinated in embryo culture. These results indicate that seeds harvested in August contain well-developed embryos that synthesize endogenous GA after imbibition, but are unable to germinate because of the hard seed coat. Seeds harvested in November and February contained immature embryos that do not synthesize endogenous GA after imbibition. Embryos without leaf primordia germinated *in vitro*, but globular embryos did not. High germination percentage for mass seeding production can be obtained by harvesting seeds from August to November and treating them with scarification and GA₃.

Keywords: embryo development, embryo germinability, *Musa velutina*, seed harvest time, seed germinability

INTRODUCTION

*Musa velutina* Wendl. & Drude is popular as an ornamental plant in Japan because of its pink flowers and pericarps. It is cultured for gardening plants, pot plants or cut flower.

*M. velutina* produces many seeds, but they do not germinate (Pancholi et al., 1995). Therefore, this plant is vegetatively propagated by division of suckers. Other *Musa* species, e.g., edible bananas, generally do not produce seeds because they are sterile polyploids (Swennen and Vuylsteke, 1993). The low germination percentage of *Musa* seeds has restricted cross breeding in banana (Shepherd, 1960; Stotzky et al., 1962).

Storage of seeds in moistened vermiculite at 25°C promoted embryo development and increased the germination percentage (Nagano et al., 2008). In addition, scarification under aseptic conditions of the seeds resulted in high germination percentage after 8–10 months of storage.
conditions or embryo culture on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) promoted germination within 14 days of culture (Nagano et al., 2009). Treatment of seeds with 2.9 μM GA₃ promoted endogenous α-amylase synthesis and germination (Nagano et al., 2010). These results suggested that the seeds of *M. velutina* contain immature mushroom-shaped embryos that may be able to germinate under certain conditions.

*M. velutina* flowers from spring to winter in greenhouses, producing seeds throughout the year in Japan. Generally, seeds scattered from mother plants or harvested from mature fruits have well-matured embryos that are able to germinate. In some plants, however, seeds with immature embryos that are unable to germinate are scattered from mother plants (Nakamura, 1985). Seed harvest time affects the germination of seeds and plant growth in *Bryza maxima* (Lombardi et al., 1998), *Chenopodium quinoa* (Jacobsen et al., 1999), *Styrax japonicus* (Roh et al., 2004) and *Zinnia* (Miyajima, 1997). In *Olea europaea*, the harvest time affects dry weight, moisture content, and ethylene sensitivity of the seeds (Rinaldi, 2000). Therefore, embryo development and/or seed germination in *M. velutina* also may be affected by seed harvest time.

In this study, we investigated the effects of seed harvest time on embryo development and germinability of seeds and embryos of *M. velutina*. To evaluate their germinability, the seeds were scarified and treated with GA₃, and embryos were cultured in vitro to assess their germinability. The overall aims of this research were to obtain a population of *M. velutina* seeds with a high germination percentage and to establish evaluation method for germinability of immature embryos obtained from inter-specific or -varietal incompatible crosses in banana breeding.

**MATERIALS AND METHODS**

**Plant material**

Parent plants of *M. velutina* were cultivated in a greenhouse located at Osaka Prefecture University Farm. The greenhouse was maintained at a minimum temperature of 10°C, and air temperatures in the greenhouse were recorded continuously with an electric thermometer (TR-72U; T and D Inc., Nagano, Japan). The average, highest, and lowest temperatures were calculated at 10-day intervals (Fig. 1). Only first flower cluster was hand-pollinated for using experiments and other flower clusters cut before flowering. Fruits were harvested when ripe, just before natural fruit abscission (i.e., dehiscence of the pericarp). In this study, we used seeds harvested in November 2005, February 2006, and August 2006. Table 1 summarizes the flowering time, harvest time, the period of ripening on the plant, the mean temperature during the ripening period, and the accumulated hours below 20°C. Fruits were washed under running tap water, and the flesh was removed from the seeds.

**Effect of harvest time on embryo development and seed germination (Expt. 1)**

At each harvest time, ten seeds were cut with a wire cutter and observed under a microscope (SZ-PT; Olympus, Tokyo, Japan) to determine the percentage of seeds containing embryos.

And ten embryos removed from seeds at each harvest time were used to measure width and length, and then they were fixed in 5:5:90 (v:v:v) formaldehyde: acetic acid: 70% (v v⁻¹) ethanol (FAA). Embryos were prepared by paraffin sectioning, and then sections (8 μm in thickness) were stained with Delafield’s hematoxylin (Yamaguchi, 2000) and observed under a microscope (BX50; Olympus, Tokyo, Japan) to examine embryo development. As described previously, mushroom-shaped embryos were classified into various leaf primordia developmental stages from 0 to 3 (Nagano et al., 2008).

To study germinability of *M. velutina* seeds, the seeds were sown on filter paper moistened with distilled water in Petri dishes (90 mm diameter × 20 mm; INA-OPTIKA, Osaka, Japan) and kept at 25°C in the dark for 56 days. Seeds were judged to have germinated when the emerging leaf bud reached 3 mm in length. Ten seeds were sown in each dish and three dishes were used.
Fig. 1 Air temperatures during the experimental period in a greenhouse (maintained at min. temperature of 10°C).

Table 1 Flowering time, harvest time, and temperature during ripening period for tested seeds of *M. velutina*.

<table>
<thead>
<tr>
<th>Flowering time</th>
<th>Harvest time (^a)</th>
<th>Ripening period on tree (^b) (Days)</th>
<th>Mean temperature during ripening period (^c) (°C)</th>
<th>Accumulated hours below 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 Aug. 2005</td>
<td>6 Nov. 2005</td>
<td>67</td>
<td>23.6</td>
<td>676</td>
</tr>
<tr>
<td>30 Sept. 2005</td>
<td>7 Feb. 2006</td>
<td>130</td>
<td>14.9</td>
<td>2223</td>
</tr>
<tr>
<td>8 June 2006</td>
<td>20 Aug. 2006</td>
<td>73</td>
<td>28.3</td>
<td>20</td>
</tr>
</tbody>
</table>

\(^a\) Ripening time of the first fruit in the fruit cluster.

\(^b\) Days from flowering time to harvest time.

for each treatment.

*Effects of treatments with scarification and GA\(_3\) on seed germination (Expt. 2)*

Immediately after harvest, seeds were immersed in 70% (v v\(^{-1}\)) ethanol for 10 s, then in 10% (v v\(^{-1}\)) sodium hypochlorite for 10 min, and finally rinsed three times with sterile distilled water. The seed coat was scarified with a wire cutter and the seeds were sown immediately on filter paper moistened with 10 ml of 0 (distilled water) or 2.9 μM GA\(_3\) solution in Petri dishes. The dishes were maintained at 25°C in the dark for 2 weeks. Seeds were judged to have germinated when the leaf bud emerging from the seed reached 3 mm in length. Ten seeds were sown in each dish and three dishes were used for each treatment. The number of days until germination, the cumulative germination percentage at 14 days after sowing, the number of days to reach 50% germination (T\(_{50}\)), and the number of days from 10% to 90% germination (T\(_{90}\)–T\(_{50}\)) were recorded as described by Furutani et al. (1985).

*Effects of harvest time on embryo germinability in vitro (Expt. 3)*

Immediately after harvest, seeds were sterilized as described for Expt. 2. Embryos were removed from seeds using a wire cutter under aseptic conditions and cultured in a plastic vessel (60 × 60 × 100 mm) on MS medium (Murashige and Skoog, 1962), containing 30 g L\(^{-1}\) sucrose and 8 g L\(^{-1}\) agar. Ten embryos were sown in each vessel and were cultured for 14 days at 25°C in the dark. We recorded the number of days until germination, the cumulative germination percentage
at 14 days after sowing, T<sub>n</sub>, and T<sub>n</sub>–T<sub>i</sub>.

RESULTS

**Effect of harvest time on embryo development and seed germination (Expt. 1)**

There were no differences in fruit size (data not shown). Moreover, there were no differences in width (approx. 0.8–0.9 mm) and length (approx. 1.0 mm) of mushroom-shaped embryos among the different harvest times. However, the developmental stage of the embryos differed among the harvest times. Seeds harvested in November contained mushroom-shaped embryos at Stage 0, but those harvested in August contained embryos at Stage 1 or Stage 2 (Table 2). Of the seeds harvested in February, only 30% of seeds containing embryos and 80% of those were at the globular stage. The globular embryos were less mature than mushroom-shaped embryos. Seeds containing mushroom-shaped embryos had mature mealy endosperm, but those containing globular embryos had degraded and liquefied endosperm tissues.

None of the seeds germinated during the 56-day experimental period, regardless of the harvest time.

**Effects of treatments with scarification and GA<sub>3</sub> on seed germination (Expt. 2)**

For the seeds harvested in August, non-scarified seeds did not germinate, while 100% of the scarified seeds germinated, regardless of the GA<sub>3</sub> treatment. For seeds harvested in November and February, scarification and imbibition with distilled water were insufficient for germination, but imbibition with 2.9-μM GA<sub>3</sub> solution after scarification resulted in 100% germination of seeds harvested in November. The seeds harvested in February scarcely germinate, regardless of scarification and GA<sub>3</sub> treatments.

In germinated seeds, there were no significant differences in T<sub>n</sub>, and T<sub>n</sub>–T<sub>i</sub> among the various scarification and GA<sub>3</sub> treatments. All of the intact (non-scarified) seeds did not germinate until 14 days after sowing (Table 3).

**Effects of harvest time on embryo germinability in vitro (Expt. 3)**

For seeds harvested in November and August, 100% of mushroom-shaped embryos germinated within 14 days of embryo culture (Table 4). However, for the seeds harvested in February, only 30% of embryos germinated in vitro, and all of them were mushroom-shaped embryos at stage 0. There were no significant differences in T<sub>n</sub>, and T<sub>n</sub>–T<sub>i</sub> among the seeds harvested at different times.

DISCUSSION

In this study, seeds of *M. velutina* were harvested in different seasons from mother plants cultivated in a greenhouse. The seeds harvested in November, February, and August contained embryos at various developmental stages. Seeds harvested in August contained well-developed

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Percentage of seeds containing embryo</th>
<th>Distribution of embryo development (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Globular</td>
</tr>
<tr>
<td>6 Nov. 2005</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7 Feb. 2006</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>20 Aug. 2006</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Ten embryos were analyzed at each harvest time.

* Stages of mushroom-shaped embryo development are as described in Nagano et al. (2008), as follows: embryo with 0 (Stage 0), 1 (Stage 1) 2 (Stage 2) or 3 (Stage 3) leaf primordia.
Table 3  Effects of scarification and GA₃ treatment on seed germination of *M. velutina* seeds harvested at different times.

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Scarification</th>
<th>Concentration of GA₃ (µM)</th>
<th>Percentage germination</th>
<th>Days till germination</th>
<th>Tₛ</th>
<th>Tₛ−Tₒ⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Nov. 2005</td>
<td>−</td>
<td>0.0</td>
<td>0 a'</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2.9</td>
<td>0 a</td>
<td>6.9 a</td>
<td>7.0 a</td>
<td>2.3 a</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.0</td>
<td>0 a</td>
<td>2.9</td>
<td>10 b</td>
<td>14.0</td>
</tr>
<tr>
<td>7 Feb. 2006</td>
<td>−</td>
<td>0.0</td>
<td>0 a</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2.9</td>
<td>0 a</td>
<td>2.9</td>
<td>10 b</td>
<td>14.0</td>
</tr>
<tr>
<td>20 Aug. 2006</td>
<td>−</td>
<td>0.0</td>
<td>0 a</td>
<td>2.9</td>
<td>100 c</td>
<td>9.3 b</td>
</tr>
</tbody>
</table>

Ten seeds with three replications were used for each harvest time.

− indicates intact embryos, + indicates scarified seed.

⁺ Different letters represent significant differences (Tukey’s multiple range test, *P* = 0.05).

* Indicates weeks to reach 50% relative germination.

* Indicates weeks from 10% to 90% relative germination.

Table 4  Effect of harvest time on embryo germination in *M. velutina*.

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Percentage germination</th>
<th>Days till germination</th>
<th>Tₛ</th>
<th>Tₛ−Tₒ⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Nov. 2005</td>
<td>100 a'</td>
<td>8.0 a</td>
<td>8.0 a</td>
<td>6.0 a</td>
</tr>
<tr>
<td>7 Feb. 2006</td>
<td>30 b</td>
<td>8.0 a</td>
<td>7.0 a</td>
<td>5.3 a</td>
</tr>
<tr>
<td>20 Aug. 2006</td>
<td>100 a</td>
<td>7.5 a</td>
<td>7.3 a</td>
<td>1.7 a</td>
</tr>
</tbody>
</table>

Ten embryos with three replications were used for each harvest time.

⁺ Different letters represent significant differences (Tukey’s multiple range test, *P* = 0.05).

* Indicates weeks to reach 50% relative germination.

* Indicates weeks from 10% to 90% relative germination.

Embryos, which had developed from flowers that bloomed in early summer. High temperature during midsummer might allow the embryos to develop. In contrast, seeds harvested in November and February contained immature mushroom-shaped embryos, since the temperature during autumn and winter were not enough for embryo maturation.

The ‘Stage 0’ embryo is a mushroom-shaped embryo lacking leaf primordia. This stage was the most immature developmental stage observed in our previous study (Nagano et al., 2008). In the present study, we observed globular embryos that were less mature than Stage 0 mushroom-shaped embryos in the seeds harvested in February. Furthermore, only 30% of the seeds harvested in February contained embryos, and the endosperm of the embryos was degraded and liquefied. Thus, adequate temperature is necessary for full embryo maturation.

The seed ripening period (days from flowering to fruit harvest) ranged from 67 to 130 days, and was markedly longer for seeds harvested in February than for those harvested in November and August. In the tropics, growth of edible banana plants and fruit ripening slow at 20°C and stop at 10°C (Daito, 2000). Moreover, continuously low temperature below 14°C made fruits cluster malformed. In our study, the mean temperature during the ripening period was less than 20°C only for seeds harvested in February, and accumulated hours by harvest time below 20°C were also markedly greater for these seeds (Table 1). This indicates that an extended ripening period at low temperatures delays embryo development. There were many reports about temperature for growth and fruits production in edible banana, however there were no reports that of *M. velutina*. In this study, leaf emergences stopped from November to March but malformed fruits cluster was not observed.
Our results indicate minimum mean temperature higher than 10°C might was needed for producing the germinable seeds with well-developed embryos in *M. velutina*. On the other hands, ripening period of seeds harvested in November was slightly shorter than that of seeds harvested in August. In this study, flower numbers per sucker were restricted and there were no differences in fruit size in different harvest time. Higher temperature might also promote flowering and nutrient competition between fruits cluster and flower buds initiation might make ripening later. In edible bananas, fruit thinning was necessary to produce high quality fruits production (Daito, 2000).

Since seed germination was promoted by scarification and GA3 treatments in *M. velutina* (Nagano et al., 2010), we investigated the effects of these treatments on seeds harvested in various seasons. Seeds harvested in August germinated whether or not they had been treated with GA3, whereas those harvested in November germinated after scarification and imbibition with GA3 solution. Seeds harvested in February did not germinate at all, regardless of scarification and GA3 treatments. These observations suggest that seeds harvested in the warmer seasons, in which the embryos were more fully developed, required little intervention to germinate. Thus, the mushroom-shaped embryos were able to synthesize endogenous GA and α-amylase from the stage in which the leaf primordia had formed. In contrast, mushroom-shaped embryos with no leaf primordia or globular embryos could not synthesize endogenous GA and α-amylase after imbibition.

Embryo germinability was evaluated by embryo culture (Nagano et al., 2009). The mushroom-shaped embryos removed from seeds harvested in August and November grew *in vitro*, whereas only 30% of the globular and mushroom-shaped embryos harvested in February germinated. Cell hypertrophy, but not morphogenesis, was observed in globular embryos. Mushroom-shaped embryos lacking leaf primordia developed leaf primordia during storage in moist conditions at 25°C (Nagano et al., 2008), but globular embryos did not (data not shown). From these results, we can conclude that the mushroom-shaped embryos without leaf primordia were immature but were able to germinate. In contrast, globular embryos were unable to germinate.

The hard seed coat of *M. velutina* seeds inhibits imbibition and germination (Nagano et al., 2010). Therefore, scarification of the seeds harvested in August that contained well-developed mushroom-shaped embryos was an important factor in achieving a high germination percentage. However, the seed harvest time could be prolonged until November, since seeds harvested in November can be induced to germinate by applying a GA3 treatment after scarification. This method may effective to evaluate germinability of embryos obtained from inter-specific or -varietal incompatible crosses in banana.

**REFERENCES**


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SEED GERMINATION IN ORNAMENTAL BANANA