Effects of Scarification with Sulfuric Acid and Matric Priming on Seed Germination of Seed Propagation Type of F\textsubscript{1} Hybrid Strawberry (\textit{Fragaria} \textit{×} \textit{ananassa} Duch.)

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For practical use of the seed propagation type of F\textsubscript{1} hybrid strawberry, high germination percentage and germination uniformity are necessary. The effects of scarification with concentrated sulfuric acid and matric priming on seed germination of this strawberry were investigated in order to improve the germination performance. Open-pollinated seeds of ‘Tochiotome’ and seeds from the seed propagation type of the F\textsubscript{1} hybrid strawberry cultivar ‘Chiba F-1 go’, bred by ourselves, were used. Germination tests were carried out on a temperature-gradient plate to investigate the response to temperature. The number of germinated seeds was counted for 7 days after sowing. The final germination percentage (FGP) of seeds scarified with 36 N sulfuric acid for 10 min exceeded 83\% in the optimal temperature range of around 24–27\degree C, whereas untreated seeds did not germinate. FGP increased to more than 82\% in the wide temperature range of 18–32\degree C with matric priming in vermiculite (−1.5 MPa) for 12 days after scarification. The effect of scarification time on germination was investigated for the seeds of ‘Chiba F-1 go’. The highest percentage (84\%) was obtained in the seeds scarified for 35 min. Furthermore, the germination speed and uniformity increased with extension of the duration of matric priming from 0 to 14 days. Germination performance of the seeds of ‘Chiba F-1 go’ was improved for practical use by scarification with concentrated sulfuric acid and scarification plus matric priming.

Key Words: breaking of hard seed for germination, germination performance, germination temperature, germination uniformity, seed treatment.

Introduction

In conventional strawberry cultivation, transplants are propagated by using runner plants. However, raising of transplants is labor-intensive and time-consuming, and runner plants may often become infected with diseases. If seed propagation could be achieved in strawberry transplant production, some of these problems could be solved by using the seed propagation type of strawberry F\textsubscript{1} hybrid cultivars (Bentvelsen et al., 1997; Ishikawa et al., 2005; Maruo et al., 2007). In the present paper, the term “seed” is used to mean achene.

Recently, a seed propagation type of F\textsubscript{1} hybrid strawberry (\textit{Fragaria} \textit{×} \textit{ananassa} Duch.) from a June-bearing cultivar was developed (Ishikawa et al., 2009). However, many problems remain to be solved for practical use, including, seed production, seed preparation, seed germination, raising of seedlings, etc. (Maruo et al., 2007). Generally, the germination performance of strawberry seeds is very low and non-uniform (Nakamura, 1972; Scott and Ink, 1948; Yanagi et al., 2004), and strawberry germination is usually very slow.

Several chemical treatments have been reported for enhancing strawberry seed germination, including the use of gibberellin, ethrel, thiourea, potassium nitrate, or sulfuric acid (Nakamura, 1972; Thompson, 1969; Yamakawa et al., 1987; Yanagi et al., 2004). Scarification of strawberry seeds with sulfuric acid can also be
performed to further enhance the germination performance, compared with the use of other chemical treatments. Several studies on scarification of strawberry seeds have been carried out, noting differences in the effective time of scarification with concentrated sulfuric acid (El Hamdouni et al., 2001; Nakamura, 1972; Scott and Ink, 1948; Yamakawa et al., 1987). In the present study, the effective scarification time was investigated more precisely.

Priming is a well-known seed treatment for the improvement of seed germination, especially under unfavorable germination conditions. Guttridge and Bright (1978) reported that osmotic priming accelerated and synchronized the germination of strawberry seeds. This technique was used for small lots of seeds. Matric priming is applied for the treatment of large number of strawberry seeds.

For the practical use of the seed propagation type of F$_1$ hybrid strawberry, a germination percentage of more than 80% within 7 days is necessary. Therefore, the effects of scarification with concentrated sulfuric acid and scarification followed by matric priming on the seed germination of this strawberry were investigated in order to enhance the germination performance. We constructed a temperature-gradient plate to investigate the germination reaction to a wide range of temperatures.

The objective of the present study was to enhance the strawberry seed germination performance for practical use of the seed propagation type of F$_1$ hybrid strawberry.

**Materials and Methods**

**Experiment 1: Effect of temperature on germination of scarified seeds or scarified plus matric-primed seeds**

In the present experiment, seeds were taken from mature fruits of ‘Tochiotome’ produced by open-pollination in May, 2006. The seeds were prepared by the method of industrial enzyme treatment (Maruo and Ito, 2008) and then were stored in a desiccator containing silica gel at 25°C.

1. **Scarification**

The seeds were scarified with 36 N sulfuric acid for 10 min at 23°C. They were then rinsed in running tap water for 1 min and sterilized with a sodium hypochlorite solution containing 2.5% of available chlorine for 5 min. After then, the seeds were rinsed in running distilled water for 1 min, and immediately dried at 25°C. The moisture content of the seeds after drying was 8.7%.

2. **Matric priming**

All the seeds were scarified with 36 N sulfuric acid for 10 min before matric priming. Fine vermiculite with particle size of 250 to 500 μm, dried in an oven at 180°C for 120 min, was used as matric priming medium. One gram of dried fine vermiculite was put into a 50 mL centrifuge tube with seal caps (Neptune, CLP, San Diego, USA) and 100 μL of distilled water was added into the medium with thorough mixing. The initial water potential of this medium was −1.5 MPa. The water potential was measured using a HR-33T Dew Point Micro-voltmeter and C-52 Sample Chamber (Wescor Inc., Utah, USA) at 25°C. Five hundred scarified seeds were mixed with the wet vermiculite. The tubes were stored for 12 days at 25°C. During the treatment, the tubes were shaken once a day. After the treatment, the seeds were separated from the medium in 710 μm mesh sieves and the water potential of the medium was −7.8 MPa. The seeds were dried again until the initial moisture content of 8.7% was reached, and stored in a desiccator containing silica gel at 25°C for 2 days.

3. **Construction of temperature-gradient plate**

Three copper plates (180 cm long, 14 cm wide, and 0.2 cm thick) with both ends bent downward over 20 cm, were laid on a styrene foam board. One side was dipped into a cool water bath and the other side into a hot water bath. Each bath was insulated with a 2 cm thick styrene foam board and it contained 33 L of water. The cold bath was kept at 15 ± 0.5°C using a chiller (COOLPUMP-150R, Taitec Co., Ltd., Saitama, Japan), and the hot bath was kept at 35 ± 0.1°C using an electric heater with a controller (UT150-RR, Yokogawa Electric Co., Ltd., Tokyo, Japan). The water in each bath was stirred constantly by an air pump. The three copper plates were laid side-by-side to achieve a total copper plate size of 140 cm × 42 cm, as shown in Figure 1. Four lines of 15 plastic petri dishes, 90 mm in diameter, could be set on the plate for four replications at the same temperature. The temperature at the center bottom of each petri dish was measured with a thermocouple and recorded with a data logger (CR1000, Campbell Scientific Inc., Utah, USA) at 1 min intervals. This system was installed in a temperature-controlled room at 25°C.

4. **Germination test**

Germination tests were carried out on the temperature-gradient plate within a temperature range of 18 to 32°C. The seeds were sown on two layers of filter paper (No. 2, ADVANTEC Toyo, Ltd., Tokyo, Japan) in plastic petri dishes, 90 mm in diameter, moistened with 5 mL of water.
distilled water. Four plastic petri dishes were used for replications (50 seeds per petri dish). Two white fluorescent lamps were used for continuous illumination at an average photon flux density of about 50 μmol·m⁻²·s⁻¹. Seeds with a radicle protruding over a 1 mm length were considered to have germinated. The number of germinated seeds was counted every day for 7 days. The germinated seeds were discarded every day. The final germination percentage (FGP), the days to 50% of final germination percentage (T50), and days from 10 to 90% of final germination percentage (T10–90) were calculated. The germination percentages were transformed to arcsine square roots before statistical analysis. All the data were subjected to an analysis of variance (ANOVA) and mean separation was performed by Tukey’s multiple range test at P=0.05.

Experiment 2: Effect of scarification or scarification plus matric priming on seed germination of seed propagation type of F1 hybrid strawberry

In this experiment, seeds from the seed propagation type of the F1 hybrid strawberry cultivar ‘Chiba F-1 go’ (Chiba Prefectural Agriculture and Forestry Research Center, Japan) were taken from mature fruits harvested in March 2007. The F1 hybrid seeds were produced by cross-pollination with ‘8-17’ × ‘IS5’. The seeds were prepared by the method of industrial enzyme treatment (Maruo and Ito, 2008) and then stored in a desiccator containing silica gel at 25°C.

1. Scarification

The seeds were scarified with 36 N sulfuric acid for 0 (control), 10, 15, 20, 25, 30, 35, 40, 45, and 50 min at 23°C. Then, the seeds were quickly rinsed sterilized, and dried as described in Experiment 1. The moisture content of the seeds after drying was 9.6%.

2. Matric priming

All the seeds were scarified with 36 N sulfuric acid for 35 min before matric priming. Matric priming was conducted as described in Experiment 1 for 4, 6, 8, 10, 12, and 14 days at 25°C. After separation from the medium, the seeds were dried again until the initial moisture content of 9.6% was reached and stored in a desiccator containing silica gel at 25°C for 2 days.

3. Germination test

The germination test was carried out in a temperature-controlled room at 25°C. Other conditions were the same as those described in Experiment 1.

Results

Experiment 1: Effect of temperature on germination of scarified seeds or scarified plus matric-primed seeds

The temperature range of the temperature-gradient plate was approximately 18 to 32°C, as shown in Figure 2. The temperature difference between neighboring petri dishes was approximately 1°C.

The final germination percentage (FGP) of the seeds of ‘Tochiotome’ increased to more than 83% at 7 days after sowing in the temperature range of 24 to 27°C in the scarified seeds (Table 1). There were no significant differences in the FGP values in the temperature range of 21 to 30°C. The FGP of the non-scarified (control) seeds was 0% in the temperature range of 18 to 32°C. In the scarified seeds, a low FGP with high T10–90 was recorded at the low and high temperatures. The T50 tended to gradually increase from 18 to 32°C.

The final germination percentage of the scarified plus matric-primed seeds increased to more than 82% in the wide temperature range of 18 to 32°C. There were no significant differences in the FGP values in the temperature range from 25 to 32°C. The T50 of the scarified seeds was lower than that of non-primed seeds in the range from 18 to 32°C. The T10–90 of the primed seeds was lower than that of the non-primed seeds in the range from 21 to 32°C.

Experiment 2: Effect of scarification or scarification plus matric priming on seed germination of seed propagation type of F1 hybrid strawberry

Scarification exerted a significant effect on the FGP of the seeds of ‘Chiba F-1 go’ (Fig. 3). The FGP of the non-scarified seeds was 0%. The FGP increased gradually to reach a peak (84%) at 35 min, and then decreased until 50 min. There were no significant differences in the FGP values in the time range from 30 to 40 min.

There were no significant differences in the FGP values between the primed and non-primed seeds (Table 2). Lower T50 and lower T10–90 values were recorded in the primed seeds. When the priming time was increased from 0 to 14 days, the T50 and T10–90 values decreased with increasing number of priming days, and the germination speed and uniformity were improved. Both the significantly lowest T50 (2.7 days) and T10–90 (1.7 days) values were obtained in the seeds.
primed for 14 days, although, in the case of $T_{10–90}$, no significant differences were found in the priming range from 8 to 14 days. The primed seeds germinated almost one day earlier than the non-primed ones (data not shown).

### Discussion

Scott and Ink (1948) reported that the germination rate of strawberry seeds increased by scarification with 36 N sulfuric acid for 10 to 15 min, and decreased by treatment for more than 20 min. Nakamura (1972) reported that scarification of the seed coat for 2 to 3 min was effective for germination. Yamakawa et al. (1987) reported that scarification for 10 min increased the germination rate of ‘Toyonoka’, ‘Haruyoi’, and ‘Kurume 47 go’ seeds. These reports indicate that the effective time of scarification for germination of strawberry seeds varies with the strain and/or seed lot. In addition, it appears that the seed moisture content affects the scarification time. The endocarp thickness may affect the germination, and strawberry seeds are hard seeds (Nakamura, 1972; Yamakawa et al., 1987). The

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**Table 1.** Effect of temperature on germination of scarified or scarified plus matric-primed ‘Tochiotome’ seeds.

<table>
<thead>
<tr>
<th>Temperature ($^\circ$C)</th>
<th>Cont. seeds</th>
<th>Scarified seeds</th>
<th>Scarified plus matric-primed seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FGP (%)</td>
<td>$T_{50}$ (days)</td>
<td>$T_{10–90}$ (days)</td>
</tr>
<tr>
<td></td>
<td>FGP (%)</td>
<td>$T_{50}$ (days)</td>
<td>$T_{10–90}$ (days)</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>6.1 a</td>
<td>2.4 d</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>6.0 a</td>
<td>2.4 d</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>5.8 ab</td>
<td>2.6 bcd</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>5.5 bc</td>
<td>2.5 cd</td>
</tr>
<tr>
<td>22</td>
<td>0</td>
<td>5.4 bc</td>
<td>2.7 abcd</td>
</tr>
<tr>
<td>23</td>
<td>0</td>
<td>5.1 cde</td>
<td>2.7 abcd</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>4.9 efg</td>
<td>3.0 ab</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>4.6 g</td>
<td>2.7 abcd</td>
</tr>
<tr>
<td>26</td>
<td>0</td>
<td>4.6 g</td>
<td>2.9 abc</td>
</tr>
<tr>
<td>27</td>
<td>0</td>
<td>4.7 fg</td>
<td>3.0 ab</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>4.5 g</td>
<td>2.8 abc</td>
</tr>
<tr>
<td>29</td>
<td>0</td>
<td>4.5 g</td>
<td>2.9 abc</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>4.6 g</td>
<td>3.1 a</td>
</tr>
<tr>
<td>31</td>
<td>0</td>
<td>5.0 def</td>
<td>3.1 a</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>5.4 bc</td>
<td>3.0 ab</td>
</tr>
</tbody>
</table>

$^a$ Seeds were scarified for 10 min with 36 N sulfuric acid.

$^b$ Seeds were primed by vermiculite for 12 days at 25°C. The water potential of the vermiculite was $-1.5$ MPa on the first day and $-7.8$ MPa after 12 days.

$^c$ $FGP$, $T_{50}$ and $T_{10–90}$ indicate final germination percentage, days to 50% of $FGP$, and days from 10 to 90% of $FGP$. Data were obtained at 7 days after sowing. For four replications, 50 seeds were used per petri dish.

$^d$ Mean separation within columns by Tukey’s multiple range test at the 5% level.

**Table 2.** Effect of scarification plus matric priming on germination of ‘Chiba F-1 go’ seeds.

<table>
<thead>
<tr>
<th>Days of priming</th>
<th>FGP (%)</th>
<th>$T_{50}$ (days)</th>
<th>$T_{10–90}$ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>85 a</td>
<td>4.5 a</td>
<td>2.5 ab</td>
</tr>
<tr>
<td>4</td>
<td>79 a</td>
<td>3.8 b</td>
<td>2.5 ab</td>
</tr>
<tr>
<td>6</td>
<td>80 a</td>
<td>3.6 bc</td>
<td>2.7 a</td>
</tr>
<tr>
<td>8</td>
<td>84 a</td>
<td>3.2 d</td>
<td>2.2 abcd</td>
</tr>
<tr>
<td>10</td>
<td>82 a</td>
<td>3.1 de</td>
<td>1.9 cd</td>
</tr>
<tr>
<td>12</td>
<td>84 a</td>
<td>3.0 ef</td>
<td>1.9 bcd</td>
</tr>
<tr>
<td>14</td>
<td>80 a</td>
<td>2.7 g</td>
<td>1.7 d</td>
</tr>
</tbody>
</table>

$^a$ Seeds were scarified for 35 min with 36 N sulfuric acid. Seeds were primed by vermiculite for 0–14 days at 25°C. The water potential of the vermiculite was $-1.5$ MPa on the first day and $-7.8$ MPa when priming treatment was complete.

$^b$ $FGP$, $T_{50}$ and $T_{10–90}$ indicate final germination percentage, days to 50% of $FGP$, and days from 10 to 90% of $FGP$. Data were obtained 7 days after sowing. For four replications, 50 seeds were used per petri dish.

$^c$ Mean separation within columns by Tukey’s multiple range test at the 5% level.
dormancy induced by a water-impermeable testa and pericarp is referred to as forced dormancy. In the present study, scars were observed on the seed coat (Fig. 4) by treatment for 20 to 50 min, and the outer layer of the pericarp disappeared or the seed coat became thinner. It is essential that the whole seed coat should become uniformly thinned for the improvement of germination. Seeds will not germinate if only part of the seed coat is injured to facilitate the absorption of water. No abnormal seedlings were observed after scarification for 35 min.

Based on the results of the present study, partial degradation of the pericarp by scarification promoted germination, probably due to the increase of water permeation and oxygen influx to the embryo. In addition, scarification may have broken the physical barrier of germination. The most effective time of scarification for the seed germination of ‘Chiba F-1 go’ was 35 min. These results indicate that scarification with concentrated sulfuric acid can be recommended for seed germination of ‘Chiba F-1 go’.

Matric priming was found to be superior to chemical treatments such as gibberellin or ethrel, mainly because the use of these chemicals (plant growth regulators) is not authorized commercially for strawberry seeds in Japan, unlike matric priming. In addition, these chemical treatments did not appear to appreciably affect strawberry seed germination (Nakamura, 1972; Yanagi et al., 2004) and the effects of the chemical treatments were unstable (Nakamura, 1972). Therefore, in the present study, matric priming was performed using fine vermiculite. It is easy to separate seeds from the vermiculite by sieving, and large numbers of seeds can be primed with vermiculite. Importantly, vermiculite is a very cheap material. The non-scarified strawberry seeds did not germinate within 7 days, even if matric priming was applied from 2 to 14 days (data not shown). Therefore, all seeds should be scarified with concentrated sulfuric acid for 35 min before matric priming. When the seeds were treated by scarification with concentrated sulfuric acid, followed by matric priming in −1.5 MPa for 14 days at 25°C, it was possible to enhance the seed germination performance of ‘Chiba F-1 go’ (Table 2). Scarification followed by matric priming is recommended for the enhancement of seed germination of the seed propagation type of F₁ hybrid strawberry.

Barbour and Racine (1967) reported the importance of the good performance of a temperature-gradient bar for use in studies of seed germination. The temperature-gradient plate constructed in the present study showed a high temperature stability (Fig. 2). Germination testing could be carried out under a wide range of highly accurate temperature conditions at the same time by using a temperature-gradient plate. In the present study, the temperature range of 18 to 32°C corresponded to the conditions prevailing in the strawberry seeding season in spring. Nakamura (1972) reported that the optimal germination temperature of strawberry seeds is around 25°C. Yanagi et al. (2004) reported that the optimal germination temperature of the strawberry seeds ranged between 20 and 25°C. In the present study, the optimal germination temperature of the open-pollinated seeds of ‘Tochiotome’ ranged from 21 to 30°C (Table 1). These results were similar to those previously reported.

In the present study, scarified seeds (non-primed seeds) showed a large reduction in FGP values at low and high temperatures (Table 1). Although the FGP values of the scarified seeds decreased at temperatures below 20°C and above 30°C, they did not decrease in the scarified plus matric-primed seeds. In the primed seeds, the T₅₀ and T₁₀–₉₀ values were lower than those of the unprimed scarified seeds. The major effects of priming include promotion of seed vigor, germination uniformity, increase of the germination percentage, and shortening of the germination time under unfavorable germination conditions (Carpenter, 1991; Heydecker and Coolbear, 1977; Pill et al., 1997). Maruo and Ito (2008) reported that strawberry germination was related to the maturation stage of fruits. Seeds prepared from premature fruits displayed a low germination performance. Germination promotion due to scarification plus matric priming of the ‘Tochiotome’ seeds was more

![Fig. 4. Photomicrograph of ‘Chiba F-1 go’ seed. (A) Non-scarified seed (B) Seed scarified with concentrated sulfuric acid for 35 min, resulting in the highest FGP values. The scars on the seed coat are indicated by arrows in B.](image-url)
pronounced under unfavorable temperature conditions than at optimum temperatures (Table 1). This method could be applied for the treatment of the seed propagation type of F₁ hybrid strawberry seeds for the improvement of germination, especially under unfavorable temperature conditions.

**Conclusion**

The present study revealed that the seed germination performance of the seed propagation type of F₁ hybrid strawberry could be enhanced practically by scarification with concentrated sulfuric acid and matric priming. Moreover, these treatments improved the strawberry seed germination performance under unfavorable temperature conditions. These methods could be applied not only for the seed propagation type of F₁ hybrid strawberries but also for strawberry breeding studies.

**Literature Cited**


