Seed Germination of Three Flower Species following MatricGconditioning under Various Environments

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Abstract. The effect of preplant conditioning on germination of three flower seeds, Bupleurum griffithii (Tourn.) L. (thorough-wax), Ammi majus L. (greater ammi), and Cirsium japonicum DC. Per. (Japanese thistle), were studied. Seeds were osmoconditioned with -1.2 MPa polyethylene glycol 8000 (PEG) solution and matricconditioned with moist Micro-Cel E (ratio of 2 seed : 0.6 carrier : 3 water by weight for Bupleurum and Cirsium; for Ammi the ratio was 2.1:4:6) and moist expanded vermiculite #5 (the ratio was of 2 seed : 0.6 carrier : 2 water for Bupleurum). In some treatments, water in the matricconditioning mixture was replaced with 1 mM gibberellin A4, (GA) or 0.2 % KNO3. In Bupleurum, matricconditioning with Micro-Cel E was generally superior to matricconditioning with vermiculite or osmoconditioning with PEG. A 4-day matricconditioning with Micro-Cel E and germination in the dark reduced the period required for 50% (T50) of final germination by 4 days and improved the percentage germination at 20°C (73% vs. 95%), compared to nonconditioned seeds germinated in the dark. The treatment also improved the percentage of germination at 15°C (68% vs. 95%) and effectively removed the theromimhibition of germination at 25 and 30°C. Germination was inhibited to a greater extent for seeds kept in the light during matricconditioning and germination than for seeds conditioned in darkness and germinated in light or conditioned in light and germinated in darkness. Nitrate added during conditioning in light prevented inhibition of germination, provided seeds were kept in darkness during germination. In A. majus, germination in light after 4-day matricconditioning reduced the T0 by ~2 days, but had little effect on percentage germination. Both GA and irradiance equally promoted germination when added during osmoconditioning, with nitrate having no effect. In C. japonicum, a 4-day matricconditioning or a 7-day osmoconditioning reduced the T0 of germination by ~2 days and improved the percentage germination to some extent. Neither irradiance nor nitrate had any significant effect.

Low water potential seed hydration or conditioning has been used widely to reduce the germination period, synchronize emergence, and increase stand size and yield. Osmoconditioning (priming) refers to conditioning in media with high solute content, while matricconditioning describes conditioning with moist solid carriers with high water adsorptive capillary forces (Khan et al., 1992).

Osmoconditioning has proved effective in improving the performance of small seeded vegetables and flower seeds (Heydecker, 1973, 1974; Heydecker and Coolbear, 1977). Carpenter (1989; 1990) has shown that osmoconditioning in polyethylene glycol 8000 (PEG) can improve the ability of flower seeds to germinate at temperatures that would have been too high for germination of nonconditioned seeds. Irradiation has been found to promote osmoconditioning of seeds with phytochrome control of germination (Khan et al. 1980/81). More recently, matricconditioning has proved effective in improving the performance of vegetable and flower seeds, including Impatien and Primula (Khan et al., 1990; 1992).

There is considerable interest in combining the advantages of various chemical, physical, and biological factors with conditioning to further enhance the benefits to seeds and seedlings (Khan, 1992). These factors include irradiation, moist-chilling (before conditioning), addition of pesticides and beneficial microbes, seed coating, and inclusion of hormones. Nitrates at low levels with low or negligible water potentials (0.001 to 0.02 MPa) have been extensively used to promote the germination of seeds (Evenari, 1965; Hilhorst and Karssen, 1988). The promotive effect of nitrates on germination when applied during seed osmoconditioning or matricconditioning, when germination is suspended, has not been tested.

The cut flower industry in Zimbabwe is still in its infancy. About 90% of the flowers produced in Zimbabwe are sold at the Dutch auction. In 1990-91, Zimbabwe was the largest supplier of Ammi majus and Bupleurum griffithii at the Dutch auction. Another flower that is gaining in importance on the Dutch auction is Cirsium japonicum. The seeds of these flowers are produced in Holland. Under Zimbabwean conditions, the germination of these seeds is low and highly erratic. Attempts are being made to produce these seeds locally in Zimbabwe and develop procedures that would improve seed germination and uniformity of stand.

The objectives of this study were to determine if preplant seed conditioning with liquid and solid carriers would improve subsequent germination of B. griffithii, C. japonicum, and A. majus seeds, and to ascertain if light, darkness, GA, and KNO3 would influence germination through the conditioning process.

Materials and Methods

Seeds. Seeds of B. griffithii, C. japonicum, and A. majus were produced at the Univ. of Zimbabwe Crop Science field plots and harvested from Aug. to Oct. 1991. Seeds were stored at 4°C and 35% relative humidity (RH) until 1 week before use.

Preplant physiological seed conditioning. During osmoconditioning, seeds were placed in 9-cm petri plates on two
layers of Whatman no. 1 filter paper moistened with 7.5 ml of -1.2 MPa polyethylene glycol 8000 (PEG) solution supplemented with 0.2% thiram with or without the addition of 1 mM gibberellin A₄ + gibberellin A₇ (GA). Osmoconditioning was at 15°C in continuous light (9 µmol·m⁻²·s⁻¹) or in darkness for 4 or 7 days. After conditioning, seeds were thoroughly washed and dried back to the original weight by forced air at 25°C (required ≈ 2h) and germinated. For matriconditioning of *Bupleurum* and *Cirsium*, seeds were mixed with the carrier Micro-Cel E at the ratio of seed to carrier to water in the conditioning mixture of 2 g seeds : 0.6 g carrier : 3 g water; using expanded vermiculite #5 as the carrier, at 2 g seeds : 0.6 g carrier : 2 g water. Corresponding ratios for matriconditioning of *Ammi* seeds using Micro-Cel E was 2 g seeds : 1.4 g carrier : 6 g water. In some cases, the water in the matriconditioning mixture was replaced with the same volume of 1 mM GA₄₇, or 0.2% KNO₃ solution. Seeds were mixed with the carrier and water in 0.5 liter glass jars, loosely capped, and then transferred to 15°C in light (9 µmol·m⁻²·s⁻¹) or in darkness for 4 or

<table>
<thead>
<tr>
<th>Treatment¹</th>
<th>T₅₀ (days)²</th>
<th>Final germination (%)³</th>
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<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
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<tr>
<td>1. G (L)</td>
<td>6.4</td>
<td>33</td>
</tr>
<tr>
<td>2. G (D)</td>
<td>6.0</td>
<td>73</td>
</tr>
<tr>
<td>Osmoconditioned</td>
<td></td>
<td></td>
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<tr>
<td>3. PEG (L), G (L)</td>
<td>4.5</td>
<td>51</td>
</tr>
<tr>
<td>4. PEG (D), G (D)</td>
<td>4.6</td>
<td>92</td>
</tr>
<tr>
<td>5. PEG (D + GA), G (D)</td>
<td>3.0</td>
<td>87</td>
</tr>
<tr>
<td>Matriconditioned</td>
<td></td>
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<tr>
<td>6. MC (D), G (D)</td>
<td>2.1</td>
<td>95</td>
</tr>
<tr>
<td>7. MC (D), G (L)</td>
<td>2.1</td>
<td>89</td>
</tr>
<tr>
<td>8. MC (L), G (D)</td>
<td>2.1</td>
<td>76</td>
</tr>
<tr>
<td>9. MC (L), G (L)</td>
<td>2.0</td>
<td>68</td>
</tr>
<tr>
<td>10. MC (D + N), G (D)</td>
<td>2.1</td>
<td>94</td>
</tr>
<tr>
<td>11. MC (D + N), G (L)</td>
<td>1.9</td>
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<tr>
<td>12. MC (L + N), G (L)</td>
<td>1.9</td>
<td>83</td>
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<tr>
<td>13. MC (L + N), G (D)</td>
<td>1.9</td>
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<tr>
<td>14. MC (D + GA), G (D)</td>
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<tr>
<td>15. MC (L + GA), G (L)</td>
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</tr>
<tr>
<td>16. VER (L), G(L)</td>
<td>3.8</td>
<td>76</td>
</tr>
</tbody>
</table>

Significance

1 vs. 2 ** **
1 vs. 3 ** **
2 vs. 4 ** **
3 vs. 4 NS **
3 vs. 5 ** **
4 vs. 6 ** NS
6 vs. 7 NS *
6 vs. 8 NS **
6 vs. 9 NS **
8 vs. 9 NS *
6 vs. 10 NS NS
10 vs. 11 NS *
11 vs. 12 NS **
12 vs. 13 NS *
6 vs. 13 NS NS
5 vs. 14 ** **
6 vs. 14 NS **
14 vs. 15 NS NS
9 vs. 16 ** NS

¹Treatment notations: G (L) = germination in light. G (D) = germination in darkness. PEG (L) = conditioning in PEG in light. PEG (D) = conditioning in PEG in dark. PEG (D + GA) = conditioning in dark with GA₄₇ added. MC (D) = conditioning with Micro-Cel in dark. MC (L) = conditioning with Micro-Cel in light. MC (D + N) = conditioning with Micro-Cel in dark with KNO₃ added. MC (L + N) = conditioning with Micro-Cel in light with KNO₃ added. MC (D + GA) = conditioning with Micro-Cel in dark with GA₄₇ added. MC (L + GA) = conditioning with Micro-Cel in light with GA₄₇ added. VER (L) = conditioning with vermiculite in light.

²Time for 50% of final percentage germination at 20°C.

³Mean separation following transformation to arcsin square root percent, P = 0.05.

**,**NS Significant at P = 0.05 or 0.01 or nonsignificant, respectively.
7 days. Following matriconditioning, seeds were rinsed to remove the carrier and dried back to the original weight by forced air at 25°C (required ≈ 2h). All manipulations in darkness were performed in green safe light (0.15 µmol·m⁻²·s⁻¹).

Germination. Three replicates of 50 seeds each of conditioned and nonconditioned seeds were germinated at 20°C in 5.5 cm petri plates lined with two layers of Whatman no. 1 filter paper and moistened with 3.5 ml water. Nonconditioned seeds were also germinated in 3.5 ml of 0.2% KNO₃ solution. Germination was evaluated at various constant temperatures (15, 20, 25, 30, and 35°C) and in continuous light (7 to 9.5 µmol·m⁻²·s⁻¹) or in darkness. Germination also was evaluated at the alternating temperature regime of 15°C (8 h darkness) and 25°C (16 h with light). The time required for 50% (T₅₀) of final germination was computed from the germination data. The data from selected B. griffithii seed treatments were pooled and subjected to analysis of variance (ANOVA) to determine the statistical significance of mean differences in T₅₀ of final germination and final germination percentage. Percentage germination values were transformed to arcsin square root percent before ANOVA.

Results and Discussion

Bupleurum griffithii. Germination of this seed was greatly influenced by temperature, light, and KNO₃ (Fig. 1). Seeds germinated better in darkness than in light at 20°C. At 25°C, germination was completely inhibited in darkness, while some seeds germinated in light. Nitrate had relatively little effect in the dark at all temperatures. In the light, it greatly stimulated germination, with large increases in germination percentages at 20°C (33% vs. 73%) and 25°C (8% vs. 60%). There was little difference in germination at 15°C in light or darkness. Germination at 15/25°C (data not shown) was similar to that at 20°C in both light and darkness, with nitrate influencing germination in light to a greater extent than germination in darkness.

A 4-day osmoconditioning in darkness with PEG and subsequent germination in darkness at 20°C reduced the T₅₀ by ≈2 days and improved the final germination percentage compared to the untreated seeds germinated in darkness (treatments 2 and 4, Table 1). The germination percentage of these seeds was higher than that of the seeds conditioned and germinated in the light (92% vs. 51%) (treatments 3 and 4, Table 1). Addition of GA during a 4-day osmoconditioning in darkness reduced the T₅₀ of germination, but was completely inhibited in darkness, while some seeds germinated in light. Nitrate had relatively little effect in the dark at all temperatures. In the light, it greatly stimulated germination, with large increases in germination percentages at 20°C (33% vs. 73%) and 25°C (8% vs. 60%). There was little difference in germination at 15°C in light or darkness. Germination at 15/25°C (data not shown) was similar to that at 20°C in both light and darkness, with nitrate influencing germination in light to a greater extent than germination in darkness.

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had no effect on final germination (treatments 4 and 5, Table 1). No additional improvements were obtained by a 7-day osmoconditioning with or without GA (data not shown).

A 4-day matricconditioning with moist Micro-Cel E in darkness followed by germination at 20°C in darkness was the best treatment (Fig. 2). The $T_{50}$ was reduced by $≈4$ days, and the final germination percentage increased to 95%, compared to 73% for the nonconditioned seeds (treatments 2 and 6, Table 1). The reduction in the $T_{50}$ was greater than that achieved by osmoconditioning with PEG (treatments 4 and 6, Table 1). The $T_{50}$ of seeds conditioned and germinated in the light was similarly reduced, and the final percentage germination reached only 68% compared to 33% for the nonconditioned seeds germinated in light (treatments 1 and 9, Table 1). Intermediate germination percentages were obtained in seeds conditioned in darkness and germinated in light (89%) and seeds conditioned in light and germinated in darkness (76%) (treatments 7 and 8, Table 1). A 7-day matricconditioning with Micro-Cel did not give additional improvements over the 4-day conditioned seeds. Matricconditioning with expanded vermiculite #5 appeared to be somewhat inferior to matricconditioning with Micro-Cel E in reducing the $T_{50}$ of germination (treatments 9 and 16, Table 1).

In a previous study with seeds of a related medicinal species, *Bupleurum falcatum* L., presoaking in running tap water for 4 days or 4 weeks of stratification (at 0 to 4°C) reduced the $T_{50}$ from $≈9$ days (untreated seeds) to 5 and 4 days, respectively, at 16 and 24°C (Huang and Liu, 1987). The best conditioning treatment of *B. griffithii* seeds, in the present study, reduced the $T_{50}$ to $≈2$ days (treatments 2 and 6, Table 1), greatly improved the germination at 15 or 20°C, and alleviated the thermoinhibition of germination at 25°C (0% vs. 91%) and 30°C (0% vs. 82%) (Figs. 1 and 3). An extension of the range of temperature tolerances may be valuable in germinating seeds under abnormally high or low temperature. Seed osmoconditioning has been shown previously to alleviate thermoinhibition in flower seeds (Carpenter, 1989; 1990).

Nitrate present during matricconditioning in light completely abolished the inhibitory effect of light on germination percentage, provided the germination (in absence of nitrate) was in darkness (Fig. 4). Nitrate applied during conditioning in light was less effective in reversing the inhibition when germination was in the light. Nitrate had no effect on seeds conditioned and germinated in darkness (treatments 6 and 10, Table 1). Seeds conditioned in darkness with or without nitrate and germinated in the light were inhibited (treatments 7 and 11, Table 1), compared with seeds conditioned and germinated in darkness (treatments 6 and 10, Table 1). Thus, seeds irradiated during conditioning and/or germination might require nitrate to achieve maximum germination percentage. Although the promotion of germination by low or nonosmotic levels of nitrate applied during low water potential seed conditioning treatment in light has not been reported previously, this finding is consistent with reports that nitrate interacts with light in the promotion of germination (Evenari, 1965; Hillhorst and Karssen, 1988; Taylorson, 1982).
What is the basis for photoinhibition of germination during conditioning and/or germination of B. griffithii seeds? Photoinhibition and photodormancy have been reported for diverse seeds (Evenari, 1965). Various theories have been advanced to explain the light inhibition of germination. Inhibition by prolonged irradiation has been attributed to “high irradiance response (HIR)” and can be explained by a low $P/P_{\text{sat}}$ ratio (Taylorson, 1982). This ratio may be influenced by light-temperature interaction, seedcoat pigmentation, and other factors. The $P/P_{\text{sat}}$ photoequilibrium established at 20°C in light might be lower than in darkness, resulting in inhibition of germination. At other temperatures, light and darkness do not influence germination greatly, suggesting a similarity in the light/dark photoequilibria.

GA applied during matriconditioning appeared to mimic the light effect in inhibiting germination (treatments 8 and 14, Table 1). However, unlike light, it did not interact with KNO$_3$ in improving germination (data not shown). The reason for the adverse effect of GA on germination percentage during matriconditioning but not during osmoconditioning is unknown (treatment 5 vs. treatment 14, Table 1). Perhaps a high pH of Micro-Cel E (pH 8.4) or reduced uptake of GA during matriconditioning accounted for the poor response to GA.

Ammi majus. Preliminary studies established 20°C as the optimal temperature for germinating seeds of this species. Nitrate had no effect on germination. Irradiation improved the rate and the final percentage germination to some extent (Fig. 5). A 4-day osmoconditioning of these seeds with PEG and germination in the light reduced the germination time by $\approx 1$ day. The germination period was shortened by 0.5 day for seeds conditioned and germinated in darkness (data not shown). When GA was added during dark conditioning, dark germination improved to the level obtained in seeds osmoconditioned in light (data not shown). Maximum improvement, however, was obtained when a 4-day matriconditioning was combined with germination in light; the treatment reduced the $T_0$ of germination by $\approx 2$ days. A 4-day matriconditioning with expanded vermiculite was less successful than with Micro-Cel in improving the performance of these seeds (data not shown). The final percentage germination was not significantly affected by any of the conditioning treatments. Based on our data, conditioning with Micro-cel E in light is an effective way to shorten the germination period. Whether conditioning with Micro-Cel E in darkness in the presence of GA would be equally effective was not tested.

Cirsium japonicum. Preliminary studies found 20°C to 25°C as the optimal temperature range for germination. Nitrate and irradiation had no effect on germination (data not shown). A 7-day osmoconditioning in PEG followed by germination reduced the $T_0$ by $\approx 2$ days and slightly increased the final germination (Fig. 6). A 4-day osmoconditioning with PEG was somewhat less effective (data not shown). Irradiation during conditioning and germination had little effect on seed performance. Similarly, seeds conditioned in the presence of GA in darkness and germinated in darkness showed no additional improvement in germination over those conditioned in the absence of GA. Similar germination was found after a 4-day matriconditioning with Micro-Cel E in light and by a 7-day osmoconditioning in PEG in light. These data indicate that liquid (PEG solution) and solid media (moist Micro-Cel E) can be used to shorten the time of germination and to improve germination percentage of these seeds.

Matricotconditioning of three species of flower seeds with moist solid carrier Micro-Cel E was found to be an effective treatment in reducing germination time and, in some cases, in improving the percentage germination. It was superior to osmoconditioning in PEG solution in improving the performance of B. griffithii and A. majus seeds and was as effective as osmoconditioning in C. japonicum seed. Matricotconditioning, in addition, was effective in extending the lower and the upper temperature limit for germination in Bupleurum. The studies reveal that factors such as irradiance, darkness, nitrate, GA, and conditioning media influence the conditioning process and consequently seed performance and should be critically evaluated in all preplant seed conditioning treatments.

Literature Cited