Seed Germination of Three Flower Species following Matriconditioning 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 matriconditioned 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 matriconditioning mixture was replaced with 1 mM gibberellin A₄+7 (GA) or 0.2 % KNO₃. In *Bupleurum*, matriconditioning with Micro-Cel E was generally superior to matriconditioning with vermiculite or osmoconditioning with PEG. A 4-day matriconditioning 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 20C (73 % vs. 95%), compared to nonconditioned seeds germinated in the dark. The treatment also improved the percentage of germination at 15C (68% vs. 95%) and effectively removed the thermoinhibition of germination at 25 and 30C. Germination was inhibited to a greater extent for seeds kept in the light during matriconditioning 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 matriconditioning reduced the T₅₀ 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 matriconditioning or a 7-day osmoconditioning reduced the T₅₀ 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 matriconditioning 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, matriconditioning has proved effective in improving the performance of vegetable and flower seeds, including *Impatiens* 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 matriconditioning, 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 4C 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

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Table 1. Effect of preplant seed conditioning on germination of *Bupleurum griffithii* seeds.

Treatment ^z	T ₅₀ (days) ^y	Final germination (%) ^x
Untreated		
1. G (L)	6.4	33
2. G (D)	6.0	73
Osmoconditioned		
3. PEG (L), G (L)	4.5	51
4. PEG (D), G (D)	4.6	92
5. PEG (D + GA), G (D)	3.0	87
Matricconditioned		
6. MC (D), G (D)	2.1	95
7. MC (D), G (L)	2.1	89
8. MC (L), G (D)	2.1	76
9. MC (L), G (L)	2.0	68
10. MC (D + N), G (D)	2.1	94
11. MC (D + N), G (L)	1.9	89
12. MC (L + N), G (L)	1.9	83
13. MC (L + N), G (D)	1.9	96
14. MC (D + GA), G (D)	2.0	72
15. MC (L + GA), G (L)	2.1	78
16. VER (L), G(L)	3.8	76
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

^zTreatment 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 GA₄₊₇ 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 GA₄₊₇ added. MC (L + GA) = conditioning with Micro-Cel in light with GA₄₊₇ added. VER (L) = conditioning with vermiculite in light.

^yTime for 50% of final percentage germination at 20C.

^xMean separation following transformation to arcsin square root percent, *P* = 0.05.

*, **, NS: Significant at *P* = 0.05 or 0.01 or nonsignificant, respectively.

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 A₁ + gibberellin A₃ (GA). Osmoconditioning was at 15C 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 25C (required ≈ 2h) and germinated. For matricconditioning 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 matricconditioning 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 matricconditioning mixture was replaced with the same volume of 1 mM GA₄₊₇ 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 15C in light (9 μmol·m⁻²·s⁻¹) or in darkness for 4 or

Fig. 1. The effect of temperature and nitrate on germination of *Bupleurum griffithii* seeds in (A) light and in (B) darkness. L = light, N = 0.2% KNO_3 . Bars on the curves larger than the symbols denote SE.

7 days. Following matriconditioning, seeds were rinsed to remove the carrier and dried back to the original weight by forced air at 25C (required ≈ 2 h). All manipulations in darkness were performed in green safe light ($0.15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

Germination. Three replicates of 50 seeds each of conditioned and nonconditioned seeds were germinated at 20C 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% KNO_3 solution. Germination was evaluated at various constant temperatures (15, 20, 25, 30, and 35C) and in continuous light (7 to $9.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or in darkness. Germination also was evaluated at the alternating temperature regime of 15C (8 h darkness) and 25C (16 h with light). The time required for 50% (T_{50} 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 T_{50} 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 KNO_3 (Fig. 1). Seeds germinated better in darkness than in light at 20C. At 25C, germination

Fig. 2. Reduced germination time and enhancement in germination percentage by matriconditioning of *Bupleurum* seeds with Micro-Cel E. After conditioning, seeds were germinated at 20C. MC (D), G (D) = matriconditioning in darkness and germination in darkness. MC (L), G (L) = matriconditioning in light and germination in light. G (D) = germination in darkness. G (L) = germination in light. Bars on the curves larger than the symbols denote SE.

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 20C (33% vs. 73%) and 25C (8% vs. 60%). There was little difference in germination at 15C in light or darkness. Germination at 15/25C (data not shown) was similar to that at 20C 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 20C reduced the T_{50} 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 T_{50} of germination, but

Fig. 3. Relief of thermoinhibition by matriconditioning with Micro-Cel E in *Bupleurum* seeds. Following matriconditioning in the dark seeds were germinated in the dark. Bars on the curves larger than the symbols denote SE.

Fig. 4. Interaction between light and nitrate during matriconditioning in promoting germination of *Bupleurum* seeds. Seeds were germinated at 20C. MC (D), G (D) = matriconditioning with Micro-Cel E in darkness and germination in darkness. MC (L), G (L) = matriconditioning in light and germination in light. MC (L + N), G (D) = matriconditioning in light with 0.2% KNO₃ and germination in darkness. MC (L + N), G (L) = matriconditioning in light with 0.2% KNO₃ and germination in light. Bars on the curves larger than the symbols denote SE.

Fig. 5. Improvement in germination by preplant conditioning of *Ammi majus* seeds. After osmoconditioning with PEG solution and matriconditioning with Micro-Cel E, seeds were germinated at 20C. G (L) = germination in light. G (D) = germination in darkness. PEG (L), G (L) = osmoconditioning in PEG solution in light and germination in light. MC (L), G (L) = matriconditioning with Micro-Cel E in light and germination in light. Bars on the curves denote SE.

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 matriconditioning with moist Micro-Cel E in darkness followed by germination at 20C 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 matriconditioning with Micro-Cel did not give additional improvements over the 4-day conditioned seeds. Matriconditioning with expanded vermiculite #5 appeared to be somewhat inferior to matriconditioning 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 4C) reduced the T_{50} from ≈ 9 days (untreated seeds) to 5 and 4 days, respectively, at 16 and 24C (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 20C, and alleviated the thermoinhibition of germination at 25C (0% vs. 91%) and 30C (0% vs. 82%) (Figs. 1 and 3). An extension of the range of temperature tolerance 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 matriconditioning 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).

Fig. 6. Improvement in germination by preplant conditioning of *Cirsium japonicum* seeds. After osmoconditioning with PEG solution and matriconditioning with Micro-Cel E, seeds were germinated at 20C. G (L) = germination in light. G (D) = germination in darkness. PEG (L), G (L) = osmoconditioning with PEG in light and germination in light. PEG (D), G (D) = osmoconditioning in darkness and germination in darkness. MC (L), G (L) = matriconditioning in light and germination in light. Bars on the curves larger than the symbols denote SE.

What is the basis for photoinhibition of germination during conditioning and/or germination of *B. griffithii* seeds? Photoinhibition and photodormancy has 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_{fr}/P_{total} ratio (Taylorson, 1982). This ratio may be influenced by light-temperature interaction, seedcoat pigmentation, and other factors. The P_{fr}/P_{total} photoequilibrium established at 20C 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 matricconditioning 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 matricconditioning 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 matricconditioning accounted for the poor response to GA.

Ammi majus. Preliminary studies established 20C 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 ≈ 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 matricconditioning was combined with germination in light; the treatment reduced the T_{50} of germination by ≈ 2 days. A 4-day matricconditioning 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-ccl 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 to 25C 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_{50} by ≈ 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 matricconditioning 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.

Matricconditioning 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. Matricconditioning, 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.

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