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Seed Priming Effect on Symbiotic Germination and Seedling Development of *Orchis palustris* Jacq.

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Abstract. **Seeds of *Orchis palustris* Jacq. were primed for 1- to 5-day in polyethylene glycol (PEG-6000) solutions at –0.5, –1.0 or –1.5 MPa. The seeds were symbiotically germinated with BNR 8-3 mycorrhizal fungus on oatmeal agar at 22 °C. In general, priming hastened rapid germination. At –1.5 MPa water potential, the first to germinate was eight days compared to 18 days for the control. Percentage germination increased as priming water potential decreased, and the percentage germination was 55%, 58%, and 65%, at –0.5, –1.0, and –1.5 MPa, respectively, versus 43% for the nonprimed control. Priming duration from 1 to 5 days had little effect on germination performance. The best germination percentage (68%) was obtained from 1 day at –1.5 MPa treatment.**

Seeds of terrestrial orchid are dust-like, minute (0.07 to 0.40 mm width and 0.11 to 1.97 mm length) and difficult to handle and germinate (Rasmussen, 1995). Orchid seeds do not have endosperm, and terrestrial orchid seeds require mycorrhizal fungi as an energy source (mycotrophy) in a symbiotic relationship (Rasmussen, 1995) to initiate seed germination and seedling development (Arditti et al., 1984). Unfortunately, only a few seeds out of millions produce this symbiotic relationship in nature (Arditti et al., 1984). In addition, in vitro symbiotic seed germination and seedling development is lower than those of many other angiosperm seeds (Markovina and McGee, 2000; Stewart and Zettler, 2002; Zettler and Hofer, 1998).

Seed priming, a process in which seeds are imbibed to a desired moisture content to arrest radicle emergence, followed by drying (McDonald, 2000), has resulted in more rapid and uniform germination in numerous plant species (Capron et al., 2000; Chen and Sung, 2001; Pill and Kilian, 2000). A range of osmotic substances has been used for priming seeds, including mannitol, glycerol, sucrose and inorganic salts of K, Na and Mg (Heydecker and Coolbear, 1977; Parera and Cantliffe, 1994), but most studies have been conducted with polyethylene glycol (PEG), a high-molecular-weight organic compound (Capron et al., 2000; Pill and Kilian, 2000). To date seed priming techniques have not been applied to symbiotic germination of orchids. In the present study, we determined the effect of priming with different concentrations of PEG-6000 for varying periods on symbiotic seed germination and seedling development of *Orchis palustris* Jacq.

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Materials and Methods

Seed and fungal collection. Mature, nondehisced yellowing capsules of *Orchis palustris* from the Erzurum district in Turkey were collected in June 2002. After collection, seeds were dried in air at room temperature and 50% to 55% relative humidity (RH) for 2 d and stored in sealed, sterile glass vials at 2 °C in total darkness for 9 months. BNR 8-3 mycorrhizal isolate, recovered from the root-like organs of native *Dactylorhiza urvilleana* in Artvin, Turkey, was used because of its effectiveness in inducing seed germination and seedling development of *Orchis palustris*.

Priming treatments. Seeds were imbibed in polyethylene glycol (PEG-6000) solutions at three water potentials (–0.5, –1.0, and –1.5 MPa) according to Michel and Kaufmann (1973), for 1, 2, 3, 4, or 5 d. The volume of PEG solutions used for imbibition was 3 mL in 50-mm-diameter petri dish. Seeds were kept in darkness during priming at 25 ± 1°C. The imbibition solutions were not refreshed during incubation. Seeds were dried at 25 ± 1°C after imbibition for 1 d.

Seed sowing, fungal inoculation and germination assessment. Following priming, seeds were surface-sterilized for 1 min in a 1:1:1 (by volume) mixture of absolute EtOH, 5.25% NaOCl, and deionized (DI) water, followed by three 1-min rinses in sterile DI water. Then seeds were sown immediately according to the procedure described by Debeljak et al. (2002); 100 to 250 disinfested seeds for per petri dish were spread on the surface of 20 mL of oatmeal agar (2.5 $g \cdot L^{-1}$ rolled oats and 7.0 $g \cdot L^{-1}$ agar in 1 L DI water at pH 6.0 before autoclaving at 121 °C for 15 min) contained within 90-mm-diameter petri dishes. Each treatment was replicated five times. Each dish was then inoculated with about 1 cm^3 block of agar containing mycelium of the BNR 8-3 isolate. The petri dishes were sealed with Parafilm and incubated in white light (warm white fluorescent) of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of 16 h photoperiod at constant 22 ± 1°C for 90 d. Seed germination was monitored daily for first germination time. Seedling development were scored for a duration of 90 d on a scale of 0 to 5, where 0 = no germination; 1 = production of rhizoid (i.e., germination); 2 = rupture of testa of the enlarged embryo; 3 = appearance of promeristem; 4 = appearance of the first true leaf; and 5 = elongation of the first true leaf and the formation of branched roots (Stewart and Zettler, 2002; Zettler and Hofer, 1998).

A germination (G) index was calculated to take into consideration all the germinated seeds in different stages of seeding development (Scale 1 to 5) at the end of 90 d. The formula used was as follows:
G index = [germination % of stage 1 × 1 (scale 1) + germination % of stage 2 × 2 (scale 2) + ... + germination % of stage 5 × 5 (scale 5)]/100.

Statistical analysis. The experimental design used was a completely randomized with five replications. Germination percentage, germination index, first germination time and seedling development data were analyzed using analysis of variance (ANOVA), and mean comparisons were made using Duncan's multiple range test and orthogonal contrast.

Results and Discussion

All primed seeds had greater germination and seedling development than nonprimed (control) seeds (Table 1). Germination percentages were increased to an average of 65% for all –1.5 MPa water potential treatments, 58% for all –1.0 MPa treatments, and 55% at all –0.5 MPa treatments compared to 43% in the control. Reducing the water potential to –1.5 MPa increased the germination index from 1.1 in the control to 2.1. At –0.5 and –1.0 MPa, the germination index averages were 1.6 and 1.7, respectively. All priming treatments also induced faster germination (first to germinate) as compared to the nonprimed seeds (control), which took 18 d to germinate (Table 1). Seed priming with PEG-6000 at –1.5 MPa increased the germination rate 22% compared to the control.

The water potential of PEG treatments had significant effects on seedling development ($P < 0.001$, Table 1). There was no difference between the control and the –0.5 MPa treatments in seedling development (with score 5) but in the –1.0 MPa treatments there was an increase

Table 1. Effects of seed priming on germination and seedling development of *Orchis palustris*

Priming Ψ (MPa) duration (d)		FGT[z] (d)	Seedling development (%) Stage[y] 0	1	2	3	4	5[x]	Germination (stages 1–5) (%)	Germination index
–0.5	1	15	46	0	25	12	12	6	55	1.6
–0.5	2	16	44	1	25	13	12	5	56	1.6
–0.5	3	13	44	0	27	14	9	6	56	1.6
–0.5	4	14	45	0	29	12	8	5	55	1.5
–0.5	5	17	44	0	28	14	9	5	56	1.6
Mean		15 a[w]						5.6 c	55.4 b	1.59 c
–1.0	1	12	42	0	20	16	16	6	58	1.8
–1.0	2	11	41	1	29	13	11	6	59	1.7
–1.0	3	8	44	0	22	14	12	8	56	1.8
–1.0	4	10	42	0	22	15	12	8	58	1.8
–1.0	5	9	43	1	27	15	10	6	57	1.7
Mean		10 b						6.8 b	57.7 b	1.75 b
–1.5	1	7	32	0	16	19	17	17	68	2.4
–1.5	2	7	35	1	17	16	19	13	65	2.2
–1.5	3	8	33	0	16	20	21	10	67	2.3
–1.5	4	8	35	0	24	19	15	7	65	2.0
–1.5	5	10	39	0	24	16	13	8	61	1.9
Mean		8 c						10.9 a	65.4 a	2.15 a
Control		18	57	1	16	7	9	5	43	1.1
LSD 0.01		1.73						0.43	2.43	0.05
Ψ (MPa)										
Linear		***						***	***	***
Quadratic		*						*	***	***
Priming duration (d)										
Linear		NS						**	NS	*
Quadratic		NS						NS	NS	NS
Qubic		NS						NS	NS	NS
Quartic		NS						NS	NS	NS

[z]FGT = first germination time.
[y]Scale 0 to 5, where 0 = no germination, 1 = production of rhizoid (i.e., germination), 2 = rupture of testa of enlarged embriyo, 3 = appearance of promeristem, 4 = appearance of first true leaf, 5 = elongation of the first true leaf and formation of branched root.
[x]Percentage of advanced seedling at 90 d after sowing.
[w]Means in columns followed by a different letter differ significantly.
[NS,*,**,***]Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

of 29% and in the –1.5 MPa treatments 106% as compared seedlings with elongated first true leaf and branched roots from the control seeds (Table 1).

This is the first published study to demonstrate that seed priming can increase symbiotic germination and seedling development in orchid species such as *Orchis palustris*. Ali et al. (1990) reported that extending priming duration from 1 to 4 d at –0.58 MPa increased germination percentages, but decreasing water potential of PEG-8000 solution to –1.49 MPa decreased germination percentage in tomato (*Lycopersicon esculentum* L.) and onion (*Allium cepa* L) seeds. Danneberger et al. (1992) studying ryegrass found that decreasing water potential of PEG-8000 solutions from –0.1 to –1.1 MPa increased germination percentage, but at –1.2 and –1.4 MPa, germination percentage decreased. In another study, Durrant et al. (1983) found no differences in sugar beet seed response to osmopriming using salt, as long as the osmotic potential of the salt solution was in the range of –1.0 to –2.0 MPa. In general, Khan (1992) reported that water potentials between –0.8 and –1.6 MPa gave optimal germination in many plant species.

There is little information on the effect of seed priming on orchid germination and seedling development. The beneficial effects of priming may be attributed to the softening of the seed coat, reduction of the seed coat adherence, or the enhancement of enzyme activities in various plant species (Chen and Sung, 2001).

In conclusion, seed priming of *Orchis palustris* had positive effects on symbiotic germination. Priming at low water potentials (–1.5 MPa) with PEG-6000 solution for short priming durations (1 to 3 days) under in vitro conditions reduced the time to first germination, and improved final germination rate and orchid seedling development.

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