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Short Communication

Seedling establishment characteristics of *Paeonia ostii* var. *lishizhenii*

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The viability of *Paeonia ostii* var. *lishizhenii* seed used in this investigation was around 85% when tested under optimum conditions of tissue culture. Seedling establishment embodied radicle protrusion and breaking of epicotyl dormancy. The seed coat, an endosperm extract, and GA_3 significantly influenced embryo emergence. The seed coat is not an appreciable barrier to water uptake but exhibited a degree of mechanical resistance. Both the coat and endosperm extract inhibited germination. Removal of the radicle end of the testa and soaking in GA_3 (100–200mgl^{-1}) promoted radicle growth at 15°C. Epicotyl dormancy was broken by soaking seeds in GA_3 or maintaining excised embryos on Woody Plant Medium containing 1mgl^{-1} GA_3. Chilling at 5°C for 30 days was required to break epicotyl dormancy of emerged embryos. The seed structures surrounding the embryos impose mechanical and chemical restraints on germination. The embryo itself appears to be lacking growth promoters as exogenous GA_3 promoted epicotyl growth.

Paeonia suffruticosa, the Chinese tree peony, is a valuable ornamental predominantly propagated vegetatively by grafting on to other plants such as *Paeonia ostii* var. *lishizhenii*, which is cultivated from seed. Germination and establishment of tree peonies takes approximately 18 months (Ignatenko 1987) due to an inhibition of epicotyl elongation, the breaking of which appears to be dependent on a specific autumn/spring regime (Lee 1992, Wang 1997). Sowing seeds in open ground in autumn (September–October) at least 30 days before the onset of the cold winter accelerates germination in spring. Failure to meet this required regime leads to the maintenance of seed dormancy or the emergence of stunted seedlings.

While it would appear that peony seeds exhibit embryo dormancy very little is known of the effect of the hard seed coat. Similarly the causes for poor epicotyl elongation remain largely unknown. That plant hormones are implicated is suggested by the fact that application of GA_3 to stunted seedlings after embryo emergence leads to epicotyl elongation in the absence of a cold moist period (Barton and Chandler 1957, Jing *et al*. 1995, Zheng *et al*. 1995). In this investigation attention was given to the interrelationship between coat and embryo dormancy and establishing means to optimise and accelerate the germination process of the subshrubby peony.

Mature, ripe seeds of *Paeonia ostii* T.Hong et J.X.Zhang var. *lishizhenii* B.A.Shen were collected in autumn from field grown plants in Heze, China. They were subsequently stored at 5°C. Their average moisture content was 12% and the average mass for 1 000 seeds was 238g. Experiments were conducted with both intact seeds and excised embryos. All germination tests with intact seeds were conducted in petri dishes containing 40g acid washed sand (BDH, England, 40–100 mesh) moistened with 10ml distilled water and replenished when necessary. For all germination treatments four replicates of 10 seeds each were used. Radicle protrusion was monitored at 3 day intervals. All data were subjected to statistical analysis to test for least significant differences. In the case of factorial experiments data were subjected to an analysis of variance (ANOVA).

The effect of the seed coat on water uptake was recorded by weighing 20 individual seeds, incubated at different temperatures, daily for 20 days. The effect of temperature on seed germination was established by incubating seeds on sand at 5°C, 10°C, 15°C and 20°C. In addition the effects of embryo excision and various degrees of scarification, the effect of light, stratification, and plant growth regulators were investigated. Seeds were incubated on sand moistened with various concentrations of gibberellic acid (GA_3) (100mgl^{-1}, 200mgl^{-1}, 300mgl^{-1}), benzyladenine (BA) (0.1mgl^{-1}, 1mgl^{-1}, 5mgl^{-1}), and abscisic acid (ABA) (1mgl^{-1}, 10mgl^{-1} and 50mgl^{-1}) at 5°C and 15°C respectively, to establish their effects on germination.

An endosperm extract was prepared by extracting ground

endosperm material as outlined by Zhang and Tardieu (1996). Five ml distilled water were added to the ground endosperm from 10 seeds and the suspension shaken in the dark at 4°C for 24h, filtered and centrifuged (1 000g) at 4°C for 15 minutes. The solution derived from the equivalent of endosperm material of 10 seeds was used to moisten the sand in each petri dish and 10 seeds then incubated in each dish. Each treatment was replicated 3 times.

Smoke extract (SM) (Baxter *et al*. 1994) was tested by soaking seed for 1, 2, 3 or 4 days at various dilutions (0, 1:100, 1:1 000 and 1:10 000) and then incubating the seeds at 5°C, 10°C, 15°C and 20°C respectively. Combinations of 0 or 1:1 000 SM with 200mgl^{-1} GA_3 and 0.1mgl^{-1} BA were also tested. The seeds were first soaked in these solutions and thereafter incubated on sand moistened with distilled water.

In view of their vulnerability to microbial attack all experiments with excised embryos were done aseptically *in vitro*. Seeds were decontaminated with a mixture of 20% ethanol and 0.075% $HgCl_2$ containing Tween 20 (1 drop per 50ml) for 7min. They were then thoroughly rinsed in sterile distilled water whereafter the embryos were excised aseptically using a microscope. Excised embryos were placed on Woody Plant Medium (WPM) (Lloyd and McCown 1980) containing 6mM calcium. Cultures were maintained on medium (10ml) solidified with agar (8gl^{-1}, Unilab) at pH 5.8. Sub-culturing occurred every 28 days. The growth room temperature was 15°C. Light (23µmolm^{-2}s^{-1}) was provided for 16h by cool white fluorescent tubes. Germination (radicle elongation) and embryo extension were recorded every 5 days under aseptic conditions.

To establish whether or not the covering structures contain inhibitory substances seed coats were ground to a fine powder and extracted as outlined earlier for the endosperm. The aqueous extract was filter sterilised by passing through a Millipore filter (HA 0.22µm) and introducing it to the WPM cooled at 40°C, after autoclaving had taken place.

The speed at which water enters intact seeds both at 15 and 20°C incubated on wet sand suggests that the testa is not an appreciable barrier to water uptake at these temperatures (Figure 1). Although at 15°C only 43±7% of the radicles of the hard black seeds protruded, it was established, using excised embryos incubated *in vitro*, that 85±4% of the seeds used were viable. Seedling establishment took place in two distinct phases, radicle emergence followed by embryo elongation to ensure emergence from the embryo structures (Figures 2A and B) with subsequent plumule establishment. Factorial germination experiments conducted on sand (Table 1) indicated that the presence of the seed coat, application of an endosperm extract and maintaining the seed at 5°C all inhibited germination, whilst light had no effect on seed germination. From Figure 3 it can be seen that a seed coat extract significantly inhibited embryo elongation over a period of 60 days of incubation. However, transfer to 15°C following stratification at 5°C and the application of GA_3 improved germination. Germination and embryo elongation was highest and most rapid at 15°C (Figures 4 and 5).

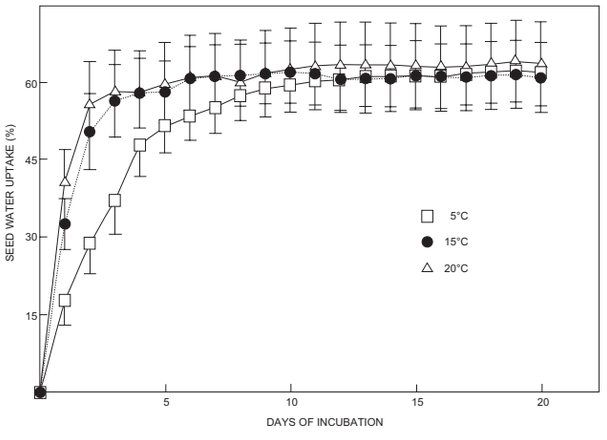

Figure 1: Water uptake of intact *P. ostii* var. *lishizhenii* seeds incubated at different temperatures

Of the hormones tested only GA_3 at 100mgl^{-1} and 200mgl^{-1} significantly improved germination from 43% to 73% and 83% respectively. BA had no effect and ABA inhibited radicle protrusion (Table 2). The application of SM had no effect on seed germination (results not shown).

After radicle emergence, the epicotyl required a further dormancy breaking treatment in order to ensure plumule elongation. The epicotyls from seeds soaked in GA_3, commenced growth when incubated at 15°C. Those soaked in BA or ABA, remained dormant during the 30 day incubation period at 15°C. This dormancy could be broken by reducing the incubating temperature to 5°C for 30 days (results not shown).

Table 1: ANOVA of treatments to determine the factors which influence the germination of *P. ostii* var. *lishizhenii* (** = P<0.05; * = P<0.1; NS = not significant)

SOURCE OF VARIANCE	DF	MS	F
Scarification	1	55.1	441**
Light	1	1.1	g^{NS}
Stratification	1	45.1	361**
Endosperm extract	1	21.1	169**
GA_3	1	78.1	625**
BA	1	15.1	121*
Error	1	0.1	–
TOTAL	7	–	–

Table 2: Radicle protrusion (%) of *P. ostii* var. *lishizhenii* in response to phytohormones. Asterisks indicate significance (P<0.05)

TREATMENT	DAYS OF INCUBATION			
	15	30	45	60
Control	0	23 ± 21	43 ± 17	43 = 17
GA (100mgl^{-1})	13 ± 5	43 ± 17	67 ± 19 *	73 ± 9*
GA (200mg1^{-1})	3 ± 5	50 ± 14 *	53 ± 19	83 ± 5*
GA (300mgl^{-1})	0	53 ± 13 *	67 ± 19 *	67 ± 19*
BA (1mgl^{-1})	10 ± 11	37 ± 13	50 ± 8	50 ± 8
BA (5mgl^{-1})	0	43 ± 17	50 ± 8	57 ± 9
ABA (1mgl^{-1})	3 ± 5	27 ± 18	50 ± 14	53 ± 9
ABA (50mgl^{-1})	0	17 ± 5	23 ± 5 *	43 ± 13

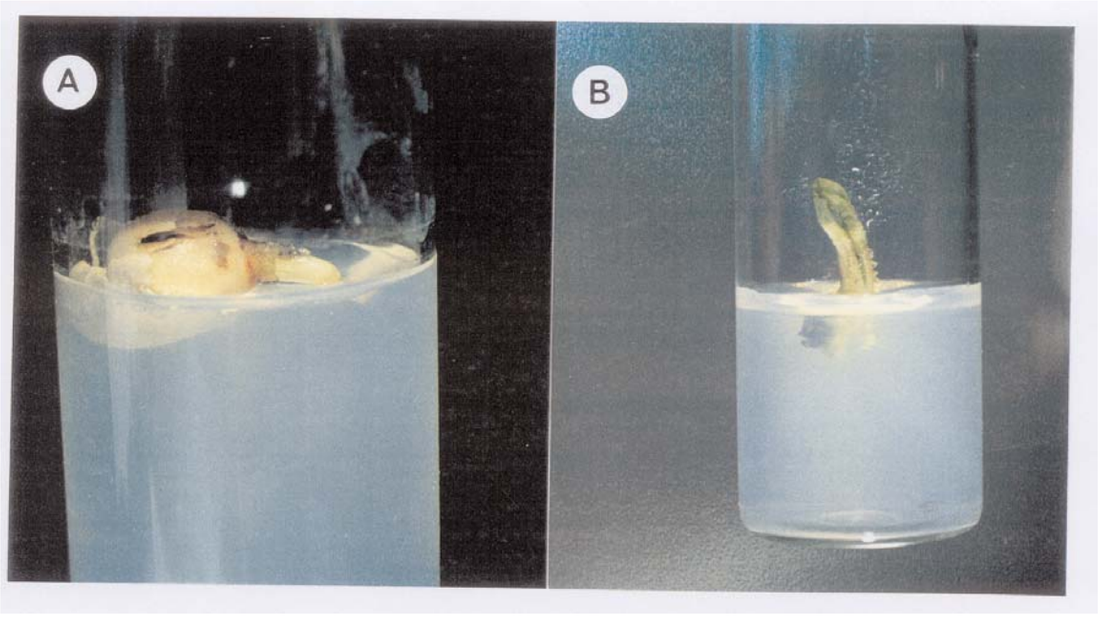

Figure 2: Radicle emergence from excised embryo of *P. ostii* var. *lishizhenii* after 20 days culture on WPM medium containing 6mM Ca, 0.5mgl^{-1} BA and 0.5mgl^{-1} GA_3 (A), followed by subsequent embryo emergence (B)

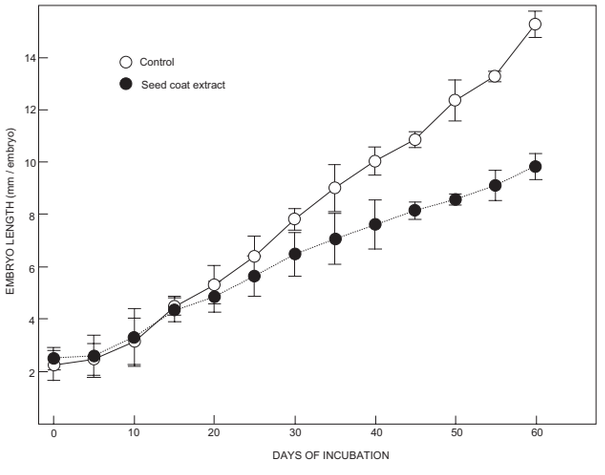

Figure 3: The effect of an aqueous seed coat extract incorporated into the WPM medium on the elongation of excised embryos of *P. ostii* var. *lishizhenii*

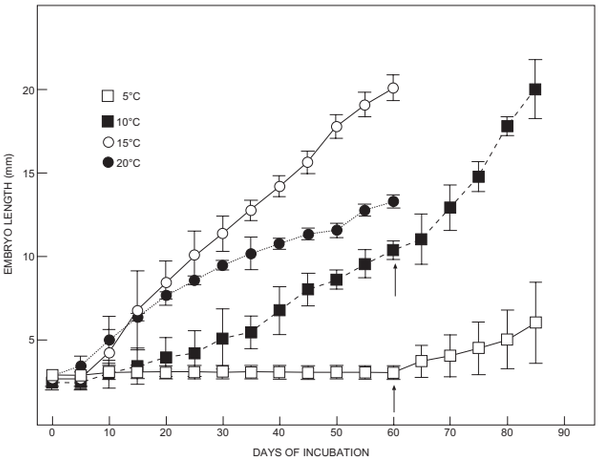

Figure 4: Elongation of excised embryos of *P. ostii* var. *lishizhenii* cultured on WPM at different temperatures. Arrows indicate time of temperature shift from 5°C and 10°C to 15°C

Tree peony establishment involves a two temperature regime, one for radicle emergence and another for epicotyl elongation. For radicle protrusion 15°C is effective, while 5°C is needed to break epicotyl dormancy. These temperature requirements explain why the black, hard seeds have to be sown in autumn otherwise they will not germinate until the next autumn. This process can be regulated by controlling the incubation temperature. Radicle protrusion is however, restricted by multiple factors. The seed covering structures can influence embryo emergence (Table 1), as in many other plants, via chemical inhibitors (Bewley and Black 1994, Dennis 1994). Removal of the radicle end of the testa significantly improved radicle growth while a testa extract inhibited embryo elongation, indicating some mechanical restriction by, and the presence of inhibiting substances in the testa.

GA_3 promoted both radicle protrusion and epicotyl elongation of tree peony. Seeds soaked in GA_3 at 5°C germinated after being shifted to 15°C. These results differ from those where GA_3 was reported to be without effect on radicle

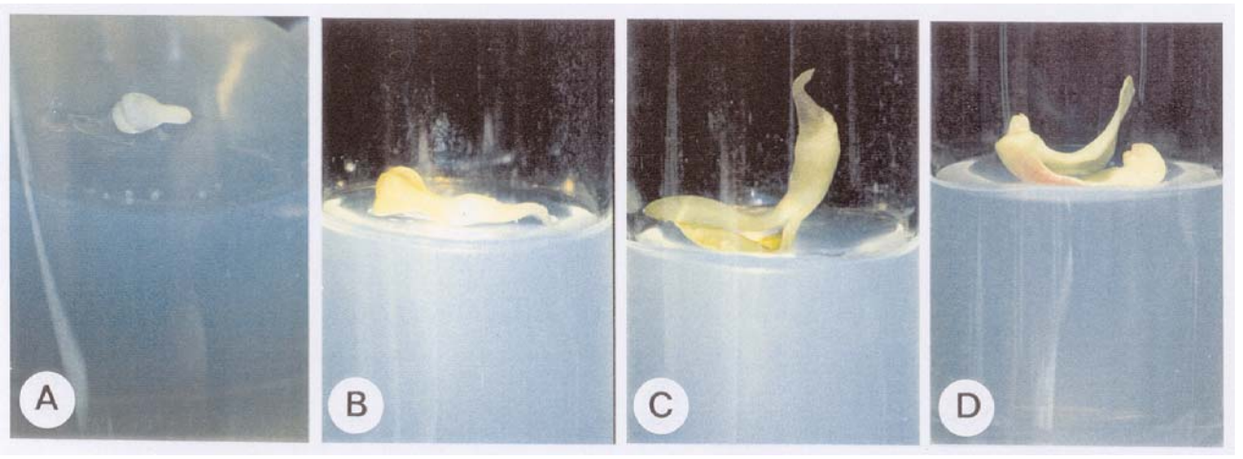

Figure 5: The effect of temperature on embryo elongation of *P. ostii* var. *lishizhenii*. A, B, C, D indicate excised embryos incubated at 5°C, 10°C, 15°C, and 20°C respectively

emergence (Jing *et al*. 1995, Zheng *et al*. 1995). However, it was similar in that GA_3 improved epicotyl growth as reported previously (Barton and Chandler 1957, Jing *et al*. 1995, Zheng *et al*. 1995). This ensures seedling establishment and normal epicotyl growth.

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