In vitro germination of *Podophyllum hexandrum* seeds.

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Considerable interest has centred on the *Podophyllum*-based lignans as lead compounds for the development of new drugs. The successful introduction of the anticancer drugs etoposide® and teniposide® and the development of new derivatives such as etopophos® have created a demand for podophyllotoxin. Currently it is obtained from the rhizomes and roots of wild populations of *Podophyllum hexandrum*, and thus the availability of this natural product is limited. There is an urgent need for a maintainable supply of *P. hexandrum* plants, a rare and threatened species. In the present work, a protocol was developed for the sterilisation and germination of seeds of *P. hexandrum*. Ripe fruits were harvested from seed-derived plants cultivated at the University of Nottingham. Seeds were removed from fruits, left for 20 – 30 min in running water, and washed three times with sterile, reverse-osmosis water. Seeds were surface sterilised in (5, 10, 15 and 20%; v:v) Domestos bleach solution with 0.2% (v:v) Tween 20 for 5, 10, 15 and 20 min, followed by three washes in sterile, reverse-osmosis water. The effect of storage as a pre-treatment for the germination of seeds was also investigated. Sterilised seeds were kept in Petri dishes and maintained in the dark at 22 ± 1°C for 30 d. Ten stored seeds were cultured per dish containing a moist sterile filter paper disk. Cultures were incubated in the dark (22 ± 1°C). Seeds with emerged radicles were individually transferred onto full-strength MS medium containing IAA (0.00875 mg l^{-1}) in combination with kinetin (0.03 mg l^{-1}) and folic acid (0.01 mg l^{-1}) designated BGS medium. Seeds were also transferred onto full-strength MS medium lacking growth regulators (MSO medium). Media were supplemented with 3.0% (v:v) sucrose and solidified with 0.8% (w:v) agar. Cultures were maintained under diffuse light (3.8 μmol m^{-2} s^{-1}) at 22 ± 1°C for three weeks and then transferred to a 16 h photoperiod under fluorescent light (42 μmol m^{-2} s^{-1}) at 22 ± 1°C. Seed sterilisation was best achieved with 20% (v:v) Domestos and 0.2% (v:v) Tween 20 for 20 min. The results from these studies confirm that a post-harvest ripening period of 30 d was required for *in vitro* seed germination. If seeds are stored in moist and dark conditions, spontaneous germination occurs within 35 to 40 d. Axenic cultures were successfully established either on full-strength BGS medium with growth regulators or full-strength MS medium lacking growth regulators. However, the overall growth of plants showing a normal morphology was superior on the latter medium. The *in vitro*-grown seedlings can be used as an alternative source of plant material for tissue culture experiments.

Keywords
Podophyllum hexandrum; lignan; podophyllotoxin; *in vitro* germination; seeds.