Establishment of a Collection of *Rosa* Species through in Vitro Embryo Culture

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Abstract. To update and complete a collection of wild roses in the Montreal Botanical Garden, Canada, in vitro embryo culture was used to propagate several *Rosa* species that are only available as seeds and difficult to germinate conventionally. Using embryo culture, it was possible to overcome seed dormancy and to rapidly increase the number of species in the collection, and this from a very limited number of seeds obtained from botanical institutions located around the world.

The Montreal Botanical Garden possesses a rich collection of more than 100 species of the genus *Rosa* coming from all over the world. These species are typically available only as seeds. Thus, the growth of the collection is slowed by the difficulty of germinating seeds. To update and increase the number of species in the collection, embryo culture was used to obtain several species of *Rosa* that could not be germinated conventionally.

Through embryo culture, it is possible to study embryonic development, bypass seed dormancy, test seed viability, and rescue immature hybrid embryos from otherwise incompatible crosses (Hu and Wang, 1986). Embryo culture has been applied to many plant species (Collins and Grosser, 1984). In roses, where the thick pericarp and growth inhibitors in the achene cause dormancy of the seeds, breeders use embryo culture to bypass dormancy and to shorten the breeding cycles (Asen, 1948; Lammerts, 1946; Marchant et al., 1994; Skirvin et al., 1990). In the present work, rose embryo culture was used to develop a collection of typically noncultivated wild species of *Rosa*.

Materials and Methods

For all experiments, seeds were stored at 4 °C upon arrival from contributors. Depending on the date of arrival, seeds were stored from 6 to 12 months before disinfection. Seeds were surface-sterilized for 1 min in 70% ethanol, followed by 45 min in 2.4% (w/v) sodium hypochlorite (NaOCl) solution with 0.1% Tween 20 (Fisher Scientific, N.J.), then rinsed three times in sterile water. Seeds were not

imbibed before embryo excision. Embryos were aseptically excised (removal of the pericarp and seedcoat) with scalpel and forceps under a dissecting microscope. The germination medium consisted of Murashige and Skoog (1962) microelements, half-strength macroelements and vitamins, sucrose at 30 g·L⁻¹, and agar at 7 g·L⁻¹ (Difco Bacto, Detroit). Seeds or embryos were cultured in 150 × 25-mm glass tubes at 23 ± 2 °C with a 16-h photoperiod at 65 μmol·m⁻²·s⁻¹ provided by cool-white fluorescent lamps.

Seeds of *Rosa nitida* Willd., *R. acicularis* Lindl., and *R. carolina* L. (species for which sufficient amount of seeds allowed for in vivo and in vitro germination experiments) were sown in a soil mixture composed of 3 peat : 1 vermiculite : 1 perlite (by volume). In addition, intact seeds and isolated embryos of the three species were cultured in vitro (see Table 1). Embryos of several other species (with limited quantities of seeds available) also were cultured in vitro (see Table 2). The percent germination of seeds was recorded 3 weeks after sowing. Three-week-old in vitro plantlets were planted into a mixture of 3 peat : 1 perlite : 1 fine vermiculite (by volume) and transferred into a greenhouse with a photoperiod of 16 h at 50 μmol·m⁻²·s⁻¹ and 23 ± 2 °C. They were transferred into an acclimatization module in which the relative humidity was slowly reduced from saturation to 40%.

Results and Discussion

Germination of *R. nitida*, *R. acicularis*, and *R. carolina* was only possible when the embryo was removed from the seed and cultured in vitro (Table 1). Isolated embryos of *R. nitida* and *R. acicularis* germinated at 100% and 93%, respectively, while embryos of *R. carolina* germinated at only 60%. The low germination percentage of *R. carolina* embryos may be due to poor seed conservation conditions before arrival at the Montreal Botanical Garden or because fruit were collected before complete maturation. In vitro plantlets of the three species were successfully acclimatized at 93% to 100% (Table 1).

Given these successful results, embryo culture of several other species of the genus *Rosa* was attempted. Many species were successfully germinated in vitro, acclimatized, and transferred into the rose garden (Table 2). Germination percentage and acclimatization success varied from one species to another, but the very small quantity of seeds received (sometimes <10) from the various botanical institutions made any statistical analyses of these percentages unreliable. Furthermore, some seeds could not be used because their embryos had aborted or been replaced by insects. The small quantity of seeds available for each species also made it inadvisable to soften the seedcoat by chemical or enzyme treatments as previously reported (Skirvin et al., 1990; Yambe and Takeno, 1992). As the hardness of the achenes was not the same for all species, several more achenes than had been provided would have been needed to optimize these treatments for each species. Mechanical excision was thus the most appropriate way to isolate the embryos from the achenes. However, good technical skills and patience are needed for species having achenes with a very hard pericarp.

For all species tested, good shoot development was obtained on germination medium, but root apices became necrotic after a few weeks in culture. However, it was possible to acclimatize all species by transferring the plantlets into soil after 3 to 4 weeks of culture.

Thus, we were able, from a very limited number of seeds, to use embryo culture to overcome seed dormancy and to obtain several species of the genus *Rosa* that could not be germinated by conventional procedures. Embryo culture resulted in an increase of 29% (from 108 to 139) of the number of species of the genus *Rosa* in the Montreal Botanical Garden rose collection. Since rose seeds are

Table 1. In vivo and in vitro germination and acclimatization of three *Rosa* species.

Species of <i>Rosa</i>	In vivo seed germination	In vitro seed germination	In vitro embryo germination	Acclimatization ^z
<i>nitida</i>	0/45 (0%)	0/15 (0%)	30/30 (100%)	14/15 (93%)
<i>acicularis</i>	0/45 (0%)	0/15 (0%)	28/30 (93%)	15/15 (100%)
<i>carolina</i>	0/45 (0%)	0/15 (0%)	18/30 (60%)	15/15 (100%)

^zAcclimatization success for 15 germinated embryos (see Materials and Methods for details).

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Table 2. Species of the genus *Rosa* obtained through in vitro embryo culture.

Species	Source of seeds (botanical institution)	Geographical distribution
<i>R. serafinii</i> Viv.	Germany (Botanischer Garten Technische Universität, Dresden)	Mediterranean region
<i>R. marrettii</i> Lév.	Poland (Hortus Plantarum Medicinarum Acad. Wroclaw)	Russia
<i>R. vosagiaca</i> Desport.	Switzerland (Conservatoire et Jardin Botaniques, Chambésy, Geneva)	Northern Europe
<i>R. heckliana</i> Tratt.	Tzechoslovakia (Arboretum Novy Dvur, Steborice)	Southern Europe
<i>R. nitida</i> Willd.	Belgium (Hortus Botanicus Antverpiensis, Antwerpen)	USA
<i>R. sempervirens</i> L.	Italy (Dipartimento di Biologia Ambientale, Orto Botanico- Università di Siena, Siena)	Southern Europe, northern Africa
<i>R. nitidula</i> Bess.	Italy (as above)	Europe
<i>R. luciae</i> Franch. & Rochebr.	Japan (Ofuna Botanical Garden, Kanagawa-Ken)	East-Asia, Japan
<i>R. zalana</i> Wiesb.	Hungary (Hortus Botanicus Soroksarensis, Budapest)	Europe, Austria
<i>R. orientalis</i> Dupont. ex Ser.	Hungary (Hortus Botanicus, Vacratot)	Greece, Albania
<i>R. achburensis</i> Chrshan.	Hungary (as above)	Central Asia
<i>R. montana</i> Chaix.	France (Conservatoire National des Plantes, Milly-La-Forêt)	Central and southern Europe
<i>R. pouzinii</i> Tratt.	Spain (Real Jardín Botánico-CSIC, Madrid)	Mediterranean region
<i>R. stylosa</i> Desv.	Spain (as above)	Western Europe
<i>R. stellata</i> Woot.	Spain (as above)	USA
<i>R. longicuspis</i> Bertol.	China (Hortus Botanicus, Inst. Botanici Kunminensis, Kunming)	China
<i>R. xodorata</i> (Andr.) Sweet.	China (as above)	China
<i>R. banksiae</i> Ait.	China (as above)	China
<i>R. gigantea</i> Coll. ex Crép.	China (as above)	China
<i>R. maracandica</i> Bge.	USA (Joseph Halda Seeds, Englewood, Colo.)	Tadjikistan
<i>R. ecae</i> Aitchis.	USA (The Botanical Garden of Smith College, Northampton, Mass.)	Afghanistan

difficult to germinate under conventional methods, it took more than 20 years to obtain the first 108 species. However, it took only 1 year to obtain 31 new species through in vitro embryo culture.

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