SOMATIC EMBRYOGENESIS FROM IMMATURE ZYGOTIC EMBRYOS OF ROSA BOURBONIANA DESP.

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SUMMARY

A protocol for the induction of somatic embryogenesis from immature zygotic embryos of Rosa bourboniana, a scented rose species, was established. Somatic embryos were induced after 8 wk of inoculation of zygotic embryos on MS medium supplemented with different concentrations of 2,4-dichlorophenoxy acetic acid (5–15 μM). In addition to 2,4-dichlorophenoxy acetic acid concentrations, somatic embryogenesis was also influenced by the month of collection of the explant and the stage of maturity of the hip. Maximum embryogenic response (16.6%) was observed using 2,4-dichlorophenoxy acetic acid (15 μM) from green hips in the month of September. The use of L-proline (800 mg l⁻¹) was found to be optimum for secondary embryogenesis. On periodic subculturing, the cultures formed somatic embryos sustainably over a period of 18 mo. For somatic embryo germination, 6-benzylaminopurine (5 μM) was found to be most suitable. Rooted plants were transferred successfully to soil and appear morphologically normal under greenhouse conditions. Transfer of plants for hardening was most suitable during the active growth period between June and September.

Key words: 6-benzylaminopurine; 2,4-dichlorophenoxyacetic acid; histology, plant growth regulators; L-proline; scented rose.

INTRODUCTION

Rosa bourboniana Desp., commonly known as Edward rose, is a natural hybrid between Rosa chinensis and Rosa damascena. It has long blooming period and is cultivated on a commercial scale in some parts of India for its fragrant flowers for the preparation of rose water. Presently, the improvement of rose varieties mostly relies on conventional breeding. However, narrow genetic base, sexual incompatibility, high degree of sterility, low seed set and germination, and the perennial nature of the crop are some of the major impediments in this direction (Firoozabady et al., 1994; van der Salm et al., 1997; Marchant et al., 1998). In view of these, crop improvement through in vitro genetic manipulations using an efficient regeneration system such as somatic embryogenesis is much warranted. Furthermore, somatic embryogenesis is regarded as the in vitro system of choice for large-scale propagation. It is also amenable to mechanization making the production process cheaper and more efficient. More importantly, the embryogenic cultures are an attractive target for genetic modification.

There are a few reports on somatic embryogenesis in ornamental rose using different explants such as leaf (de Wit et al., 1990), leaf and stem segments (Rout et al., 1991), anther filaments (Noriega and Sondahl, 1991), roots (van der Salm et al., 1996) and zygotic embryos (Kunitake et al., 1993). Until now no report is available on in vitro regeneration of R. bourboniana, a scented rose species. The development of somatic embryogenesis protocol from immature zygotic embryos holds immense value due to the premature abortion of embryos during the rose breeding program (Lammerts, 1976). Hence, the present study reports a complete protocol of somatic embryogenesis using immature zygotic embryos as explants, supported by histological studies in R. bourboniana. The effect of different developmental stages of hips on somatic embryogenesis is also emphasized.

MATERIALS AND METHODS

Explant sterilization and initiation of embryogenic callus. Open pollinated fruits (hips) of R. bourboniana, grown and maintained in the experimental farm of the Institute, were collected at different stages of maturity (green, greenish red, dark red, and dark red with wrinkled pericarp; Fig. 1) throughout the year except in the months of April and May as the plant was in full bloom and there was very low seed set, which was observed only in the last fortnight of May. Seeds were dissected out from hips and were first washed with TEEPOL solution [1% (v/v); Qualigens, India Ltd, Mumbai] for 5 min and later with running tap water for 2–3 min. Thereafter, the seeds were treated with Bavistin (0.1%; BASF, India Ltd, Mumbai) and streptomycin sulfate (0.1%) for 25–30 min and rinsed with distilled water. These seeds were then disinfected with 70% (v/v) ethanol for 30 s, followed by treatment with mercuric chloride [0.04% (w/v)] with 1–2 drops of Tween-20 for 5–10 min. After 3–4 washings with autoclaved-distilled water, the testa was removed and embryos were excised aseptically. These embryos were inoculated on MS medium (Murashige and Skoog, 1962) supplemented with varying concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D: 5.0, 10.0, 15.0 and 20.0 μM), sucrose (3%) and agar (0.5%); bacteriological grade; Qualigens, India Ltd). The experiment was designed with three replicates, each with eight embryos per Petri dish (90 mm Petri dish with 25 ml culture medium). Explants grown on MS medium without the plant-growth regulators (PGRs) served as control. The cultures were maintained initially in the dark for 3–4 wk at 25 ± 2°C and later transferred to the light with photosynthetic photon flux density...
The cultures were initially kept in the dark for 1 wk, and then转移到含 5 ml 月桂基酸液中的 10
2%(含 0.8%) 使用。子叶胚芽产生没有根的幼苗。这样的幼苗
备不育的结构，随后进一步胚芽发育。最初，胚性组织 (1.0–1.5 g FW)
和最终的干重记录在 4 周。
胚芽诱导和胚性胚芽形成。从种子到无胚根胚芽的胚芽诱导
calin 含有 2,4-D 后 3–4 周。随后，幼苗被直接移植到 8-in 盆里填
的沙子(1:1) 和保持在温室条件下。

Results and Discussion

Callus induction and somatic embryogenesis. Green-colored 品系分为绿色和绿色
变为绿色，然后转移到含有 2,4-D 的 MS 基础液中。这些组织
化和正常胚芽在 MS 基础液中 5 μM 2,4-D 后和不胚芽的组织
胚芽诱导和胚性胚芽形成。从种子到无胚根胚芽的胚芽诱导
2,4-D 含量 (Fig. 2)。一个类似的 2,4-D

Proliferation and maturation of somatic embryos. The embryogenic callus with fully developed embryonic stems derived from zygotic explants was transferred to MS medium supplemented with different concentrations of 6-benzylaminopurine or with different concentrations of L-proline (600–1000 mg l

Somatic embryo germination. For germination, somatic embryos from the cotyledonary stage were transferred to MS medium with 5 μM 2,4-D. In all these cases, sucrose (3%) and agar (0.5%) was used. Germinated embryos produced shoots without roots. Such shoots (single shoot/culture tube) were transferred to culture tubes, each containing 5 ml liquid medium containing 10 μM indole-3-butyric acid (IBA). The cultures were initially kept in the dark for 1 wk, and then transferred to liquid medium without PGRs and with the same concentration of sucrose for rooting as reported previously (Pati et al., 2001). In this medium, cultures were maintained for 4 wk at 25 ± 2°C.

Histological studies. Embryogenic calluses at different developmental stages of somatic embryos were fixed in FAA [18:1:1 (v/v/v), 50% ethanol/glacial acetic acid/formalin], dehydrated in ethanol/tert-butyl alcohol series, and embedded in paraffin wax. Sections 10 μm thick were cut and stained with Safranin-Fast green and mounted in DPX mountant (Pati et al., 2004).

Hardening and acclimatization. The rooted plants were removed from the culture vessels and washed thoroughly in lukewarm water. These plants with stem diameter of 0.5–2.5 mm, shoot length of 1.0–5.5 cm and root length of 1.0–7.0 cm were initially transplanted in 4-in (diameter) plastic pots containing sand and kept in a greenhouse covered with plastic jars for 3–4 wk. Subsequently, plants were transferred to 8-in pots filled with sand:soil (1:1) and maintained under greenhouse conditions.

FIG. 1. Somatic embryogenesis from immature zygotic embryos of R. bourboniana. a, Hips at different developmental stages (bar = 5 cm); b, somatic embryos (bar = 2 cm); c, germinating somatic embryos (bar = 2 cm); d, hardened plants in pots.

FIG. 2. Effect of different concentrations of 2,4-D and collection time of explants on somatic embryogenesis of R. bourboniana.