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Short communication

Establishment of efficient regeneration protocol from leaf explants of *Iris pumila* shoots cultured in vitro

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Abstract

An efficient plant regeneration protocol via somatic embyogenesis by leaf base culture of in vitro grown *Iris pumila* shoots was developed. Induction of embryogenic calli was achieved on MS media supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin (4.5 μM, each) and some additives (L-proline, casein hydrolysate, adenine sulphate and tyrosine). Further differentiation of embryogenic calli was achieved on MS hormone-free media, and on media supplemented with either BAP (4.5 μM) or BAP + zeatin (4.5 and 0.2 μM, respectively), which allowed somatic embryos, as well as shoot-like structures to form. Fully developed somatic embryos germinated on an MS hormone-free medium. An anatomical study confirmed that shoot-like structures represented early germinating stages of somatic embryos. Acclimatization of plants derived from somatic embryos was 64% after 1 year and no morphological variation was observed.
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Keywords: Leaf base culture; *Iris pumila*; Somatic embryos; Organogenesis

1. Introduction

Iris pumila L. is a wild dwarf tail bearded plant, which has been given the status of a closely protected rare species. It is distributed throughout Central and Eastern Europe and Turkey (Dykes, 1913), and its brilliant flowers, have earned it popularity as a garden plant.

In vitro plant regeneration of *I. pumila* via somatic embryogenesis in mature zygotic embryo culture has been done in the past (Radojević et al., 1987), while plant regeneration from suspension-derived calli has been reported recently (Jevremovic and Radojevic, 2002). One of the main problems with somatic embryogenesis is a loss of morphogenetic potential during extended subculturing. Explant source is one of the most important factors in the induction of morphogenetic response of in vitro culture, especially in monocots. For *Iris* species, plant regeneration has been reported from different explants (Laublin et al., 1991; Gozu et al., 1993; Shibli and Ajlouni, 2000).

Here we report the establishment of a plant regeneration protocol by somatic embryogenesis from leaf base explants of *I. pumila* shoots cultured in vitro. Anatomical data relating to the origin of shoot-like structures are also provided.

2. Materials and methods

Mother stock shoot cultures of *I. pumila* were maintained for many years on a medium for shoot multiplication (Radojević and Subotić, 1992).

Leaf bases (1 cm) were cultured for 6–8 weeks on solid A_0–A_6 media with MS (Murashige and Skoog, 1962) mineral solution, 0.7% agar, 3% sucrose and (in mg L^{-1}): inositol 100, pantothenic acid 10, nicotinic acid 5, vitamin B_1 2, vitamin B_6 1 and 2,4 dichlorophenoxyacetic acid (2,4-D, 4.5 μM), kinetin (4.5 μM, A_0) and additives L-proline 250 (A_1 and A_3), casein hydrolysate 250 (A_2 and A_3), adenine sulphate 80 (A_4 and A_6) and tyrosine 100 (A_5 and A_6).

Embryogenic calli were transferred to solid B_0–B_2 media supplemented with MS mineral solution, 0.7% agar, 3% sucrose and (in mg L^{-1}): inositol 100, pantothenic acid 10, nicotinic acid 5, vitamin B_1 2, vitamin B_6 1, casein hydrolysate 250 (B_0), and BAP 4.5 μM (B_1), or zeatin 0.2 μM and BAP 4.5 μM (B_2). Isolated somatic embryos were germinated on a hormone-free medium (B_0).

Each treatment was performed in 5–7 plates containing five or 10 explants and each experiment was replicated twice. All

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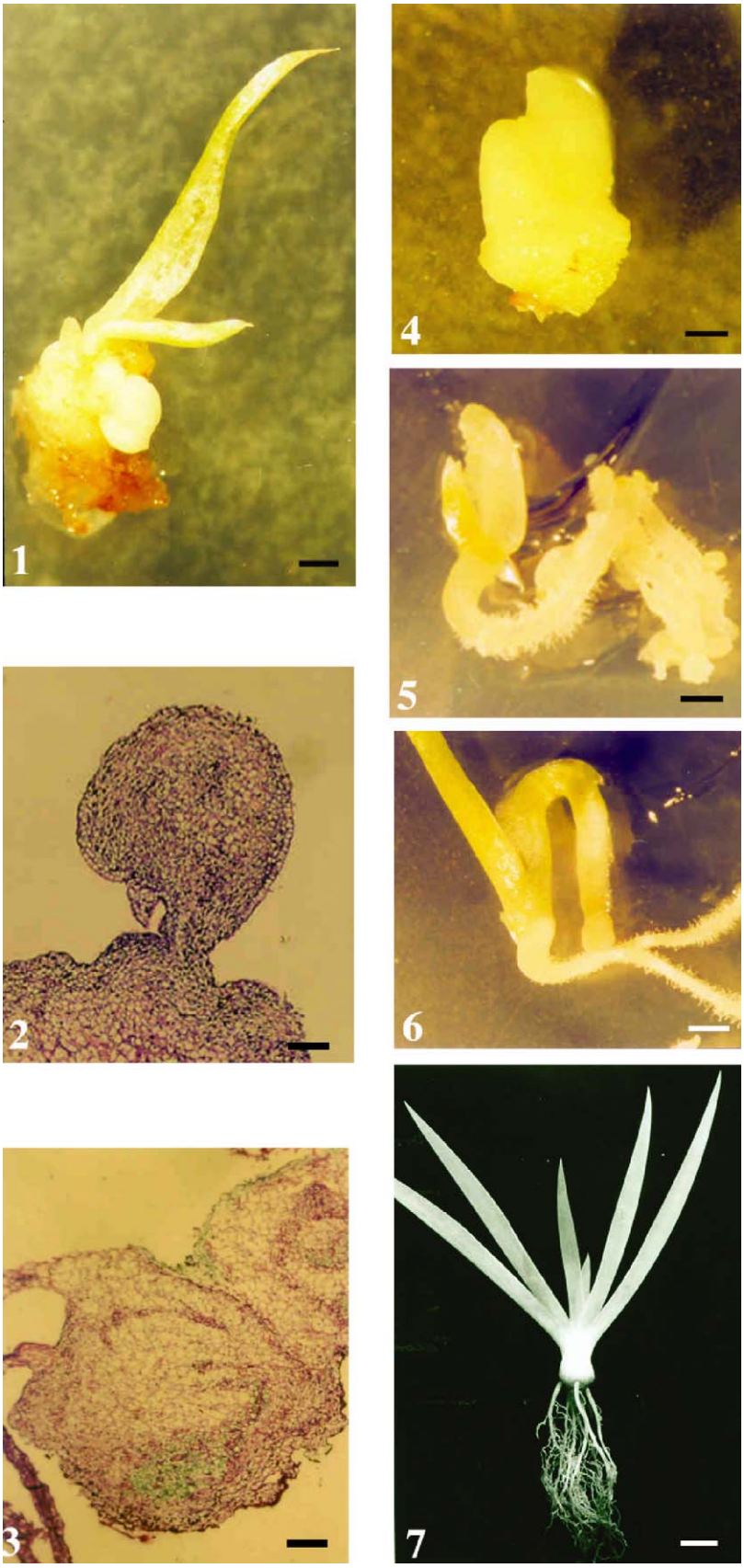

Figs. 1–7. Embryogenic calli with somatic embryos and shoot-like structures of *I. pumila* (bar 20 μm). Figs. 2 and 3. Cross-section of Fig. 1. Somatic embryo with a notch (Fig. 2) and early-germinated somatic embryo (Fig. 3, bars 10 μm). Figs. 4–6. Different phases of a single somatic embryo germination on B_0 medium. (Fig. 4, bar 10 μm; Figs. 5 and 6, bar 20 μm). Fig. 7. *I. pumila* plant derived from a somatic embryo after 1 year in a greenhouse.

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