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Plant Regeneration from Scales of the Fern *Platycerium bifurcatum* (Cav.) C. Chr.

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S u m m a r y

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A new and unique shoot regeneration from the scales of *Platycerium bifurcatum* is described, and the effect of 1, 2, or 3% sucrose on regeneration is studied. Scales detached from 3-5 month old plants grown in vitro on medium without growth regulators, were used as initial explants. After 30 days of culture, 45 to 60% of scales developed adventitious shoots. After 60 days, 90 to 100% of scales developed shoots with leaves, and some of them developed roots. Sucrose did not affect the number of shoots formed on the scales, but did affect further development of shoots, roots, and also fresh and dry weights of scales with shoots. Shoot regeneration from scales also represents a good system for studying the early stages of morphogenesis.

Introduction

Several in vitro multiplication methods are known for the *Platycerium* species (HENNEN & SHEEHAN 1978, WEE & al. 1992). However the micropropagation of *P. bifurcatum* has been described only twice (THENTZ & MONCOUSIN 1984, CAMLOH & al. 1994). As initial explants juvenile leaves, leaf fragments or shoot apexes were used.

In our previous investigation we demonstrated that scales from in vitro grown plants of *P. bifurcatum* have the potential to develop different structures, rhizoids, aposporous gametophytes or adventitious shoots (AMBROŽIČ - DOLINŠEK & CAMLOH 1997). We found that 1-3% sucrose significantly stimulated shoot organogenesis after 60 days in culture. Therefore in this work we studied in detail

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the effect of these sucrose concentrations on shoot regeneration from scales for their potential use in micropropagation. To our knowledge, scales have never been used as initial explants in any other fern species. In addition, in vitro culture of scales represents an excellent system for various morphological studies.

Materials and Methods

Scales of juvenile sporophytes of the fern *Platycerium bifurcatum* (Cav.) C. Chr. cultured in vitro were used as initial explants. Sporophytes were produced by the direct adventitious shoot initiation method without the use of growth regulators as described by CAMLOH & al. 1994, except that MURASHIGE & SKOOG's 1962 original medium (MS) was used instead of a modified MS one (HENNEN & SHEENAN 1978). Scales were detached from 3-5 months old shoots. Forty to 60 scales were placed flat on the surface of the 15 ml MS medium as described previously (AMBROŽIČ - DOLINŠEK & CAMLOH 1997). Media were supplemented with 0.8% Difco-Bacto agar and 1, 2, or 3% of sucrose. Media were adjusted to pH 5.7-5.8 and, after autoclaving, placed in plastic 7 cm Petri dishes. Cultures were kept at 23 ± 2°C, with a photoperiod of 16 ^{h} at 50-80 μmol m^{-2} s^{-1} (Osram L 58 W/77 - Fluora).

The number of shoots on scales was determined after 30 and 60 days of culture. The number of leaves and roots on the shoots was determined after 60 and 90 days. The fresh and dry weights were measured after 60 and 90 days of culture for 10 shoots together and calculated from their average weights. Shoot regeneration was examined using a stereomicroscope.

The 2 × 2 Chi-squared test (χ^2) or the Student's t-test were used for evaluating the levels of statistical significance (P) between the media with 1% sucrose and those with other sucrose concentrations. All experiments were repeated twice and the examples given represent typical results.

Results and Discussion

Scales detached from juvenile sporophytes of the fern *P. bifurcatum* produced adventitious shoots when cultured on MS medium without growth regulators (Fig. 1a). The viability and regenerative potential were comparable to our previous results (AMBROŽIČ-DOLINŠEK & CAMLOH 1997) obtained on scales grown on modified MS medium (HENNEN & SHEEHAN 1978).

Table 1. Effect of sucrose on shoots regeneration, shoot number and root regeneration.

sucrose (%)	scales with shoots (%)		number of shoots regenerated per scale		shoots with roots (%)	
	30 d	60 d	30 d	60 d	60 d	90 d
1	48	82	1	1.1	5	34
2	53	83	1	1.2	3	26
3	59	94	1	1.1	13	44

Sucrose affected shoot regeneration. With increasing sucrose concentration from 1 to 3%, the percentages of scales with shoots and especially the number of shoots formed per scale insignificantly increased (Table 1). This was already

evident after 30 but especially after 60 days of culture. The appearance of leaves was comparable irrespective of the sucrose concentrations in the medium (Fig. 1b). However, sucrose especially at a concentration of 3% slightly promoted root formation (Table 1). Although the effect of sucrose on shoots regeneration was not significant, concentration of 3% sucrose significantly increased fresh and dry weights of shoots after 90 days of culture compared to the medium with 1% of sucrose (Fig. 2). After 60 days of culture 90% of scales developed at least one shoot and first roots were also observed (Table 1). Sometimes on the basal parts of primary shoots secondary shoots developed (Fig. 1c, d). It is interesting that the morphology of secondary shoots was similar to shoots regenerated directly on scales (Fig. 1d). Thus, the possibility exists that they originated from scales of primary shoots, indicating the high regenerative capacity of *P. bifurcatum* in vitro without growth regulators as was also reported for leaf cultures (CAMLOH & al. 1994). Interestingly, initial divisions of protoplasts isolated from juvenile leaves occurred in growth regulator-free media even in this fern (CAMLOH & ŽEL 1995).

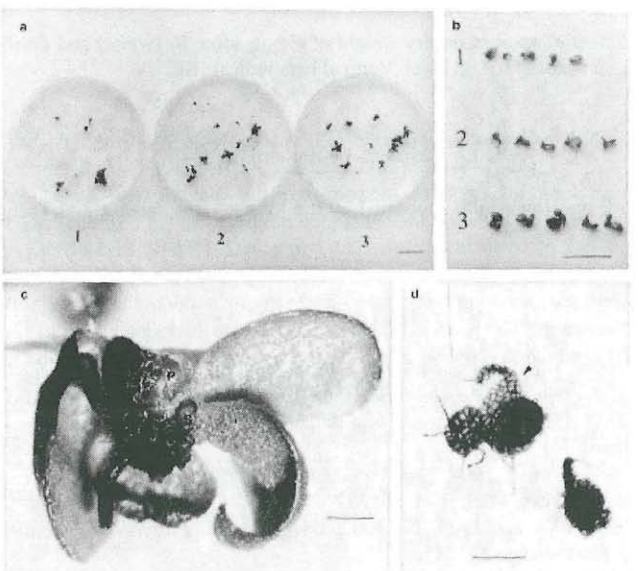

Fig. 1a-d. a, Regeneration of shoots after 60 days of culture. Bar = 1 cm. b, Regeneration of shoots after 90 days of culture. Bar = 1 cm. c, Primary shoot with regenerated secondary shoots after 90 days of culture. Bar = 1mm. d, Secondary shoots after detachment from parent plant. Note the shoot surrounded tissue and its similarity with scale morphology. Bar = 0.5 mm. P = primary shoot, S = secondary shoot.

After 3 months, adventitious shoots 2-4 mm long, were convenient for elongation and rooting. Although a *P. bifurcatum* micropropagation protocol has already been described (THENTZ & MONCOUSIN 1984, CAMLOH & al. 1994), this

procedure, starting from scales, is a relatively simple alternative with fewer subcultures and is also convenient for studying different aspects of morphogenesis.

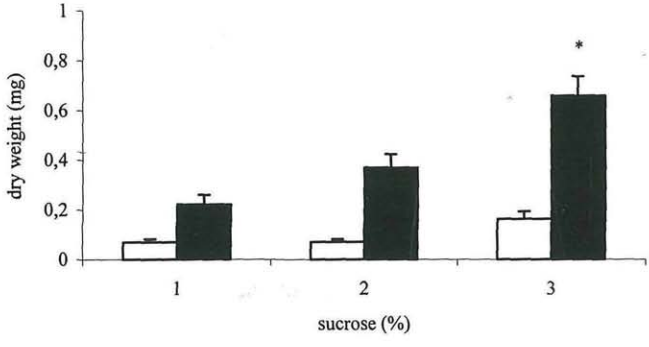

Fig. 2. Effect of sucrose on dry weight of shoots after 30 (white) and 60 (black) days of culture. *, significant at the $P < 0.05$ level. Vertical bars indicate SE.

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