Chapter 8

In Vitro Regeneration Systems of Platycerium

Marjana Camloh and Jana Ambrožič-Dolinšek

8.1 Introduction

The staghorn fern genus, Platycerium Desv., belongs to the family Polypodiaceae. It is an epiphytic genus of pantropical distribution, consisting of 15–18 species, cultivated worldwide because of their unique appearance (Hoshizaki 1972; Tryon and Tryon 1982; Hoshizaki and Price 1990; Hoshizaki and Moran 2001; Poremski and Biedinger 2001; Darnaedi and Praptosuwiryo 2003; Fernández and Vail 2003; Pemberton 2003). It has been stated that staghorns are the aristocrats of the cultivated ferns (Hoshizaki and Price 1990). Some Platycerium species, e.g., P. grande (Amoroso and Amoroso 2003) and P. ridleyi (Wee et al. 1992; Rodpradit 2003), are considered as endangered. These plants are distinguished from other ferns, among other characteristics, by the differentiation of the leaves into base fronds or mantle leaves and forked fertile leaves. Because of their great economic value, and their special place among ferns, these plants frequently have been used in cytological, morphological, developmental, physiological, and phylogenetic studies (e.g., Hoshizaki 1970; Nagmani and Raghavan 1983; Kwa et al. 1995a, b, Kwa et al. 1997a, 1997b; Camloh, et al. 1996, 1999; Teng and Teng 1997; Ambrožič-Dolinšek et al. 2002; Kreier and Schneider 2006; Espinosa-Matías et al. 2007; Janssen et al. 2007; Rut et al. 2008; Aspiras 2010).

Platycerium species are conventionally propagated by the sexual and vegetative methods. The first method involves raising plants from spores. The vegetative method of propagation, typical of at least some species of the genus Platycerium, is the development of new plants through root bud initiation (Hoshizaki 1970; Richards et al. 1983; Hoshizaki and Moran 2001). For some staghorn ferns (e.g., P. grande), it has been established that their spores are difficult to germinate in natural conditions (Amoroso and Amoroso 2003). As shown in studies of in vitro culture of spores, the duration of the process from spore to sporophyte can vary.

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from 1 to 8 months in different ferns (Fernández et al. 1999). In *Platycerium* species, the length of this process, determined by using *in vitro* culture of spores, varies from 3 months in *P. bifurcatum* (Camloh et al. 2001), and 5 months in *P. ridleyi* (Rodpradit 2003) to 7 months in *P. grande* (Amoroso and Amoroso 2003). However, in a recent report on *P. coronarium* and *P. grande*, sporophyte development occurred only when gametophytes from the *in vitro* culture were transferred to sterilized potting medium (Aspiras 2010). Similarly, it was shown for *P. andinum* and *P. wandae* that sporophytes were not observed even 100 days after spore sowing in the medium (Espinosa-Matías et al. 2007).

It is known that the conventional propagation of ferns from spores is slow, while vegetative propagation is also hampered by low multiplication rates, and both observations are valid for staghorns also. Therefore, plant propagation methods using *in vitro* culture techniques have been intensively studied in ferns in recent decades (reviewed by Hegde and D’Souza 2000, Fernández and Revilla 2003, Somer et al. 2010). *Platycerium* species were also used for *in vitro* studies, and the great morphogenetic potential of these plants was revealed using different experimental systems (Wee et al. 1992, Camloh 2006 and references therein).

Besides mass propagation, *in vitro* cultures also have a role in the *ex situ* management of endangered species (Pence 2004; Soare 2008), a category to which some staghorns belong. Additionally, cryopreservation was studied in detail for *P. ridleyi* (Rodpradit 2003).

In this review, we provide an overview of research on *Platycerium* species using *in vitro* techniques, with special emphasis on the regeneration patterns obtained on sporophytic tissue. Regeneration systems in *Platycerium* are compared to those of other fern species and discussed with respect to their applicability to mass propagation. Furthermore, some similarities in regeneration patterns between *Platycerium* and seed plants are emphasized.

Firstly, the process of organogenesis regarding the initial explants, media, plant growth regulators, etc., is summarized. It has been established that various culture treatments can be used to induce and optimize organogenesis in seed plants and ferns; however, these factors are often manipulated in different ways (Sugiyama 2000; Philips 2004; George and Debergh 2008; Somer et al. 2010).

In ferns, regeneration on sporophytic tissue is usually initiated by growth regulators, although their usage is minimal (reviewed by Hegde and D’Souza 2000; Fernández and Revilla 2003). For some *Platycerium* species, it was reported that organogenesis of sporophytic tissue using different experimental systems was achieved without any growth regulators in the media (i.e., Wee et al. 1992; Camloh et al. 1994; Ambrožič-Dolinshek and Camloh 1997; Teng 1997; Rodpradit 2003). Furthermore, a high regenerative capacity was reported for some *Platycerium* species (Wee et al. 1992; Camloh 2006 and references therein). Regarding high regenerative capacity and simple culture requirements, a simple regeneration system applicable to mass propagation will be presented for *P. bifurcatum*.

In addition, the reports on apospory and apogamy, which were both studied in many ferns (reviewed by Fernández and Revilla 2003; Camloh 2006 and references therein, Martin et al. 2006; Somer et al. 2010 and references therein), including
staghorns (Kwa et al. 1995a; Teng and Teng 1997; Ambrožič-Dolinšek et al. 2002), are summarized for Platycerium species.

Secondly, we will point out the applicability of in vitro systems of Platycerium species, to obtain new insight into the effects of various culture treatments on regeneration patterns. For some Platycerium species, in addition to high regenerative capacity, high morphogenetic plasticity was claimed (Kwa et al. 1997a; Camloh 2006). For different in vitro systems of P. bifurcatum, e.g., the excised-leaf culture system, bud scale culture, and leaf cell suspension culture, it has been demonstrated that they can be easily manipulated for different morphogenetic responses: bud organogenesis, aposporous gametophyte development, rhizoid development, etc., by the practice of either wounding or cutting the explants, the concentration of sucrose, and jasmonic acid (JA) in the medium or cell aggregate size (Ambrožič-Dolinšek and Camloh 1997; Teng and Teng 1997; Camloh et al. 1999; Ambrožič-Dolinšek et al. 2002; Wee et al. 1992). Furthermore, in the case of the P. coronarium cell suspension culture, initiated from a gametophyte-derived callus, it was reported that morphogenesis into either gametophytes or sporophytes occurred without growth regulators, depending on the type of callus used (Kwa et al. 1997a). Thus, such systems are excellent tools for studying factors affecting morphogenesis.

8.2 In Vitro Regeneration Systems of Sporophytic Tissue

In seed plants, in vitro propagation is achieved either from pre-existing meristems (axillary buds), or from somatic cells through organogenesis (direct or indirect) or by somatic embryogenesis. A well-balanced mineral nutrient composition, an adequate supply of carbohydrates, and appropriate levels of growth regulators in the medium are major factors in morphogenetic expression in vitro (Ziv and Chen 2008 and references therein).

In ferns, when using sporophytic tissue, regeneration through axillary buds and direct or indirect organogenesis was reported (reviewed by Hegde and D’Souza 2000; Fernández and Revilla 2003). In ferns, homogenized cultures of gametophytic or sporophytic tissue are often used as propagation method (Bertrand et al. 1999, Fernández and Revilla 2003, Somer et al. 2010) and recommended for species with a short life cycle (Fernández et al. 1999). In several ferns, micropropagation was reported also through the proliferation of green globular bodies (GGBs) on different sporophytic explants, usually on media with cytokinin; these GGBs later develop into sporophytes (Higuchi et al. 1987; Amaki and Higuchi 1991; Fernández et al. 1996; Bertrand et al. 1999). The term GGB was first used by Higuchi et al. (1987) for the rapidly proliferating tissue developed on rhizome segments of Nephrolepis cordifolia cultured on a 6-benzylaminopurine (BA) containing medium. Somatic embryogenesis in Pteridophyta has rarely been described; it was reported for Lycopodiella inundata (Atmane et al. 2000) and Huperzia selago (Szyputa et al. 2005).
Two other morphogenetic processes have often been studied in ferns using in vitro techniques: apospory, the development of gametophytes on sporophytic tissue and apogamy, the development of sporophytes directly from the gametophytes without sexual fusion (reviewed by Fernández and Revilla 2003; Camloh 2006 and references therein, Martin et al. 2006 and references therein).

In *Platycerium* species, organogenesis has mainly been achieved directly. The regeneration of plantlets from homogenized leaf tissue (Cooke 1979; Teng and Teng 1997, 2000) and through the initiation of GGBs has also been described. Apospory and apogamy developments have also been studied. The reports carried out to date on in vitro regeneration of *Platycerium* from sporophytic tissue are summarized in Table 8.1; those already summarized by Hegde and D’Souza (2000) are not included.

### 8.2.1 Direct Shoot Organogenesis

In seed plants, direct shoot initiation on explants is rarely observed and is even unknown in many plant genera (George and Debergh 2008), while in ferns, it has been reported for several species (reviewed by Hegde and D’Souza 2000). In fern micropropagation through direct shoot organogenesis, rhizome tips or pieces, runner tips and section of fronds were mainly used as initial explants, and these explants were most commonly cultured on Murashige and Skoog (1962) and B5 media (Gamborg et al. 1968) or their modifications (reviewed by Hegde and D’Souza 2000).

In *Platycerium* species, direct shoot development was reported on the following explants: shoot tips (Hennen and Sheehan 1978; Thentz and Moncousin 1984); rhizome pieces (Wee et al. 1992); fragments of young leaves (Thentz and Moncousin 1984; Wee et al. 1992; Rodpradit 2003); entire juvenile leaf (Camloh and Gogala 1991; Camloh et al. 1994); shoots (Pevalek-Kozlina 1996); bud scales (Ambrožič-Dolinšek and Camloh 1997; Ambrožič-Dolinšek et al. 1999), and through GGB proliferation directly on pieces of juvenile leaves (Jámbor-Benczúr et al. 1995).

The following sections on direct shoot organogenesis in *Platycerium* are based on the initial explants used.

#### 8.2.1.1 Shoot and Rhizome Culture

Micropropagation using shoot tips from greenhouse-grown plants as initial explants was first described by Hennen and Sheehan (1978) for *P. stemaria*. They also used this technique successfully for *P. wandae*, *P. veitchii*, and *P. wallichii*. Shoot tips, 2–3 mm³ in size, were placed on modified MS with adenine sulfate (80 mg/L) and indole-3-acetic acid (IAA) (85.6 μM). Adventitious bud development occurred at the base of the explants, on the roots, and on leaves that were in contact with the medium. Thentz and Moncousin (1984) used shoot tips cultured on Linsmaier and
Table 8.1  The reports on in vitro regeneration of *Platycerium* from sporophytic tissue carried out to date (those reports already summarized by Hegde and D’Souza (2000) are not included)

<table>
<thead>
<tr>
<th><em>Platycerium</em> species</th>
<th>Initial explant</th>
<th>Media</th>
<th>Type of regeneration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. bifurcatum</em></td>
<td>shoot tip, leaf fragments</td>
<td>MS, LS, different conc. of sucrose, agar, pH, 0.5-2.5 μM IBA and 4.4 μM BA (shoot tips), 8.9-13.3 μM BA (leaf fragments)</td>
<td>Sporophytes,</td>
<td>Thentz and Moncousin 1984</td>
</tr>
<tr>
<td><em>P. coronarium</em></td>
<td>Rhizome pieces, juvenile leaf fragments</td>
<td>MS, 5.4 μM NAA (<em>P. coronarium</em>), no growth regulators (<em>P. ridleyi</em>)</td>
<td>Sporophytes</td>
<td>Wee, Kwa and Loh 1992</td>
</tr>
<tr>
<td><em>P. ridleyi</em></td>
<td>Rhizome pieces, juvenile leaf fragments</td>
<td>MS, Different conc. of sucrose, agar, pH, MS, 0.54 μM NAA and 9.3 μM KIN</td>
<td>Shoot multiplication</td>
<td>Pevalek-Kozlina 1996</td>
</tr>
<tr>
<td><em>P. bifurcatum</em></td>
<td>Juvenile leaf fragments</td>
<td>MS, with variations in strength, 0.54 μM NAA and 0.46 μM KIN</td>
<td>Sporophytes through GGBs</td>
<td>Jámbor-Benczúr et al. 1995</td>
</tr>
<tr>
<td><em>P. bifurcatum</em></td>
<td>Leaf cell suspension culture</td>
<td>1/2 MS, 5.4 μM NAA and 4.4 μM BA</td>
<td>Sporophytes</td>
<td>Teng 1997</td>
</tr>
<tr>
<td><em>P. bifurcatum</em></td>
<td>Leaf cell suspension culture, scales</td>
<td>MS,</td>
<td>Sporophytes (from cell aggregates &gt;500 cells) or aposporous gametophytes (from cell aggregates &lt;100 cells)</td>
<td>Teng and Teng 1997</td>
</tr>
<tr>
<td><em>P. bifurcatum</em></td>
<td>Scales</td>
<td>MS, no growth regulators</td>
<td>Sporophytes, apospory</td>
<td>Ambrožič-Dolinšek and Camloh 1997</td>
</tr>
<tr>
<td><em>P. bifurcatum</em></td>
<td>Scales</td>
<td>MS, no growth regulators</td>
<td>Sporophytes</td>
<td>Ambrožič-Dolinšek, Camloh and Žel 1999</td>
</tr>
<tr>
<td><em>P. bifurcatum</em></td>
<td>Leaves, shoot base, homogenized sporophytes</td>
<td>MS, no growth regulators or 0.54 μM NAA</td>
<td>Sporophytes</td>
<td>Teng and Teng 2000</td>
</tr>
<tr>
<td><em>P. bifurcatum</em></td>
<td>Juvenile leaves</td>
<td>MS, low sucrose, wounding</td>
<td>Sporophytes, apospory</td>
<td>Ambrožič-Dolinšek et. al. 2002</td>
</tr>
<tr>
<td><em>P. ridleyi</em></td>
<td>Pieces of juvenile leaves</td>
<td>MS, no growth regulators</td>
<td>Sporophyte</td>
<td>Rodpradit 2003</td>
</tr>
</tbody>
</table>

MS, Murashige Skoog medium (1962), LS, Linsmaier Skoog medium (1965), GGBs, green globular bodies, NAA 1-naphtaleneacetic acid, BA 6-benzylamino purine, KIN kinetin, IBA 3-indolebutyric acid
Skoog medium (1965) with BA and 3-indolebutyric acid (IBA) to initiate culture. The adventitious buds and rhizoids developed at the base of the explants and on the roots. Wee et al. (1992) placed rhizome pieces, 2 mm² in size, on a growth regulator-free MS medium to initiate in vitro culture of P. coronarium and P. ridleyi. All rhizome explants produced sporophytes after 2 months in the culture. In some cultures, callus developed, but differentiation into sporophytes was not observed. Their report describes the acclimatization experiments in detail. Pevalek-Kozlina (1996) used shoots obtained in vitro from spores to initiate P. bifurcatum culture. The shoots were placed on MS medium with 1-naphtaleneacetic acid (NAA) and kinetin (KIN). The study focused on the effects of sucrose and agar concentration, and the pH medium on shoot multiplication. The highest multiplication rate (12.75–14.29 shoots per explant) was observed on a medium containing 3.0% sucrose and 0.9% agar, with a pH of between 5.7 and 7.0.

8.2.1.2 Leaf Culture

To initiate Platycerium leaf culture, fragments or entire leaves were used. Thentz and Moncousin (1984) obtained buds on leaf fragments using the same medium as for shoot tip culture, except that the concentration of BA was higher. After 6 weeks in the culture, the whole explant was covered with buds and rhizoids. After 10 weeks, the explants were subdivided. On 1 cm² of the explants, up to 30 sporophytes formed. Wee et al. (1992) also used fragments of young leaves for regeneration of P. coronarium and P. ridleyi. They cultured juvenile frond strips on an MS medium with 2,4-dichlorophenoxyacetic acid (2,4-D), NAA or BA. The best sporophyte production was obtained on P. coronarium with NAA, while for P. ridleyi, NAA and BA had no effect. For both species, 2,4-D was inhibitory. Direct adventitious bud development was achieved in P. ridleyi on pieces of juvenile fronds (5 mm²) cultured on an MS medium without growth regulators (Rodpradit 2003). After 2 weeks, several tiny, green, round sporophytes developed on the frond strips. The regeneration started at the margins of the explants and eventually covered the rest of the explants. From one initial explant, 30–50 sporophytes approximately 9 mm long with 2–4 fronds were obtained after 8 weeks in the culture. Jambor-Benczúr et al. (1995) described in vitro regeneration of P. bifurcatum through the development of GGBs on fragments of juvenile leaf (0.5 mm² in size) cultured on a modified MS medium with NAA and KIN. In 12 weeks, along the cut surface of the explants, groups of GGB did develop even on the growth regulator-free medium. On the surface of the GGBs, first meristems and then shoot primordia appeared, but no development of callus was reported. For elongation, 2–3 mm colonies of GGBs were separated and placed on the same medium, without growth regulators. Rooting was achieved on a modified medium (Jambor-Benczúr and Márta-Riffer 1990) with added NAA. Regeneration and rooting proved better when active carbon was added to the medium. From GGBs, plantlets fit for rooting developed in 6 months.

In our studies of P. bifurcatum regeneration, entire juvenile leaves, 0.8–1.2 cm in size, obtained from in vitro grown plants, were placed on the MS medium modified by Hennen and Sheehan (1978), but without adenine sulfate, so that the basal end
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and the abaxial surface were in contact with the medium (Camloh and Gogala 1991; Camloh et al. 1994). Although the effects of some growth regulators on regeneration were tested, efficient regeneration was achieved on growth regulator-free medium. Buds appeared mainly on the abaxial surface of the leaf. The development of multicellular scales and rhizoids coincides with bud induction. Scale development was also observed during in vivo root bud development (Richards et al. 1983).

After 40 days of culture, 10–35 buds developed. Eight weeks after culture initiation, the buds were excised and elongated for an additional 4 weeks. To obtain more plants, explants could first be subdivided and cultured for 4 weeks or more before bud excision and elongation. Interestingly, at the base of shoots and sometimes on their leaves, the development of new buds occurred, and as in vivo, buds were occasionally also observed on the roots. Up to 150 shoots were obtained from a single leaf after 3 months in the culture. After elongation, roots already developed on several shoots. Shoots without roots were successfully rooted on ¼-strength modified MS, and after 6 weeks the plants were planted in soil. The whole regeneration process from initial explants to plants appropriate for planting in soil took 18 weeks (Fig. 8.1). Our further studies focusing on induced apospory revealed that wounding of the initial explants does affect bud development. On wounded leaves, a higher number of buds was obtained, and their development on the adaxial surface of the leaf was promoted (Ambrožič-Dolnišek 1998).

High regenerative capacity, especially when wounded explants are used to initiate the culture, good rooting of shoots, relatively simple culture requirements, and appropriate duration of the whole process from initial explants to plants make this system suitable for mass propagation of *P. bifurcatum*.

### 8.2.1.3 Culture of Bud Scales

In mature *P. bifurcatum* plants, scales cover the buds and the entire rhizome surfaces; they protect the buds from desiccation, animals, and excess water (Hoshizaki 1970). Scales also cover the buds of *in vitro* cultured shoots, and their formation can be observed in the early stages of organogenesis (Camloh et al. 1994). During our studies on *in vitro* culture of *P. bifurcatum*, we observed different outgrowths on scales while they were still attached to the buds. Therefore, we made a detailed study of the regeneration patterns and morphogenetic potential of scales (Ambrožič-Dolnišek and Camloh 1997; Ambrožič-Dolnišek et al. 1999). To initiate scale culture, scales composed of a single layer of cells were detached from the *in vitro* grown shoots and placed flat on the same medium as used for leaf culture (Fig. 8.2). In the majority of scales, regeneration began during the first 30 days of culture as the proliferation of several cells, rarely one, at different sites on the scale. Newly proliferated zones formed three-, or occasionally two-dimensional groups of cells, which developed into buds, aposporous gametophytes, or remained undifferentiated. On the aposporous gametophytes, gametangia developed. The formation of rhizoids on the outgrowths was also observed (Fig. 8.2). Interestingly, after 90 days of culture, roots were already formed on nearly half of all developed buds. At the base of shoots, secondary shoots commonly developed, which most likely originated from scales of
the shoots. New shoots of such origin were simply detached from primary shoots and cultivated further. The described system of regeneration obtained on scales with no growth regulators in the medium additionally confirmed the high regenerative capacity of some *Platycerium* species.
George and Debergh (2008) define indirect shoot organogenesis as the formation of shoots on a previously unorganized callus, or in cell cultures. In homogenization studies of *Platycerium*, sporophytes were homogenized, and used directly to obtain regeneration. Since, no callus was observed during the regeneration, we placed these studies in the direct shoot organogenesis category. Cooke (1979) was the first to use homogenized sporophytes obtained in vitro for propagation of *Platycerium* species. Homogenized tissue was placed on a medium consisting of MS salts, sucrose, agar, inositol, and thiamine-HCl. Growth was evident in 2–3 weeks, and an excellent multiplication rate was reported. Teng and Teng (2000) used homogenized sporophytes, 1–3 cm long, to study the effects of antibiotic pulse treatment (ATP) on the regenerative ability of *P. bifurcatum*. As a control, they used nontreated homogenized sporophytes and obtained very high numbers of regenerants. Two milliliters of suspension (cell density was adjusted to 40–50 mg dry wt/mL) inoculated on a modified MS medium with or without NAA generally regenerated 800–900 and 100–200 sporophytes larger than 0.5 cm and 0.1–0.5 cm, respectively within 8–10 weeks.

### 8.2.2 Indirect Shoot Organogenesis

Propagation of ferns through indirect shoot organogenesis, the development of shoots from callus tissue, was rarely reported in ferns (reviewed by Hegde and D’Souza 2000). However, Hegde et al. (2006) obtained callus on rhizome tips of *Drynaria quercifolia* – a medicinal fern, when cultured on a Knop’s salt solution and different concentrations of auxins. Sporophytic regeneration occurred on the medium with 6-(γ,γ-dimethylallylamino) purine (2iP). Suspension cultures were also induced from the friable morphogenetic callus.
In *Platycerium*, Wee et al. (1992) observed callus development on some cultures of *P. coronarium* and *P. ridleyi* initiated from rhizome pieces, but differentiation of shoots was not obtained. In their further studies, successful initiation of callus cultures from gametophytes was reported (Kwa et al. 1995b, Kwa et al. 1997a, b). However, these studies were not focused on *in vitro* propagation, but on some physiological aspects of callus cultures e.g., in the report of Kwa et al. (1995b) the establishment of a photoautotrophic callus culture system and its physiological analysis were described for *P. coronarium*. They also used this system to study the activity of Rubisco and phosphoenolpyruvate carboxylase (Kwa et al. 1997b).

### 8.2.3 Apospory and Apogamy

Apospory and apogamy will be described in detail in other chapters of this book; therefore, we will focus only on studies of these morphogenetic processes performed in *Platycerium* species. Kwa et al. (1995a) described IAA-induced apogamy in *P. coronarium* gametophytes cultured *in vitro*. The gametophytes were macerated into fine pieces using a scalpel, and clumps of about 70–75 mg were cultured individually on MS medium with IAA at different concentrations. The percentage of apogamy and the total number of apogamous sporophytes produced per gametophytic clump were the highest in the presence of 40 \( \mu \text{M} \) IAA. Teng and Teng (1997) obtained aposporous gametophytes from leaf cell suspension cultures of *P. bifurcatum*, but only from single cells or aggregates of up to 100 cells. This aposporous gametophyte later gave rise to sporophytes through apogamy. For suspensions, they used MS salts supplemented by thiamine-HCl, pyridoxine-HCl, nicotinic acid, glycine, myoinositol, and NAA.

In our experiments on *P. bifurcatum*, apospory was obtained during regeneration of bud scales (Ambrožič-Dolinšek and Camloh 1997), but a detailed study was performed on leaf culture (Ambrožič-Dolinšek et al. 2002) (Fig. 8.3). On the basis of

![Fig. 8.3](image) The development of apospory on wounded leaves of *P. bifurcatum*, on modified MS medium with 0.01% sucrose. (a) aposporous gametophytes after 30 days of culture. Note rhizoids (r) and papillae (p). Bar=0.1 mm. (b) the development of aposporous gametophytes (ap) and shoots (s) after 45 days of culture, arrows indicate wounds. Bar=2mm
this study, we propose a set of conditions, which regularly and reproducibly induce apospory on most of the leaf explants of *P. bifurcatum*. Apart from the juvenility of explants, the most important factors for apospory induction are a combination of low sucrose concentration in the medium (0.01%) and wounding of the leaf.

8.3 *In Vitro* Cultures of *Platycerium* in Developmental and Physiological Studies

In vitro cultures of *Platycerium* species have often been used for different studies. In a recent review, the applicability of different experimental systems of *P. bifurcatum* to study the developmental and physiological processes was described in detail (Camloh 2006). Therefore, in this chapter, we will focus mostly on other staghorns and those studies using *in vitro* cultures of sporophytic tissue not described in the review, while studies on *P. bifurcatum* will be dealt with only briefly.

Kwa et al. (1995c) studied the role of ethylene on *in vitro* regeneration from frond and rhizome explants of *P. coronarium* sporophytes. They found that the addition of the ethylene action inhibitor silver thiosulfate at 25 and 15 \( \mu \text{M} \) resulted in an increase in the percentage of regeneration from frond and rhizome explants, respectively. Since in further experiments inhibitors of ethylene biosynthesis (which are effective in higher plants) were ineffective, they suggested that ferns do not produce ethylene via the same pathway as angiosperms. Kwa et al. (1997a) studied the morphogenetic plasticity of callus reinitiated from cell suspension cultures of *P. coronarium*. From callus initiated from gametophytes cultured on MS medium with 2,4-D, they established cell suspension cultures. When cells from the suspension cultures were plated on semisolid MS medium containing 10 \( \mu \text{M} \) KIN, two distinct types of callus masses, distinguished by their coloration, were obtained. Morphogenesis into either gametophytes or sporophytes occurred when these callus masses were cultured on growth regulator-free medium. They proposed this system as useful for comparative studies of developmental plasticity. Teng and Teng (1997) used leaf cell suspension cultures to study regeneration patterns of *P. bifurcatum* *in vitro*. They showed that morphogenetic events can be manipulated not only by environmental factors but also by cell status, i.e., aggregate size. From single cells and aggregates of up to 100 cells aposporous gametophytes developed, while from aggregates of more than 500 cells, direct regeneration of sporophytes was observed. In another report on *P. bifurcatum* (Teng and Teng 2000), they used leaf and shoot base explants and homogenized sporophytes to study the effects of ATP on the regenerative ability of *P. bifurcatum*. The response to ATP was dependent on the duration of ATP and the cell status at which the cells were treated. They considered the reaction to ATP as measured by changes in sporophyte regeneration and morphology as stress response. The cells’ susceptibility to ATP was graded as tissue homogenized prior to the treatment > tissue homogenized after the treatment > leaf and shoot base explants.

In our experiments using the excised-leaf culture system, we found that JA affects organogenesis in *P. bifurcatum* (Camloh et al. 1999). JA stimulated development
of rhizoids and the development of adventitious shoots. Furthermore, it affects the site of shoot development. Therefore, we suggested that JA might be involved in regulating morphogenesis in this fern. We also studied the effects of wounding on organogenesis, and found that wounding has an effect on organogenesis similar to that with the addition of JA to the medium (Ambrožič-Dolinšek, 1998). In scale culture, we studied the effect of sucrose on organogenesis and found that increasing the sucrose concentration from 0 to 3% strongly increased bud development on scales, while the opposite effect of sucrose was observed on gametophyte development on scales (Ambrožič-Dolinšek and Camloh 1997; Ambrožič-Dolinšek et al. 1999).

In *P. ridleyi*, a detailed study on cryopreservation was performed using young sporophytes obtained *in vitro* from spores (approximately 2 mm in diameter) (Rodpradit 2003). Encapsulation/dehydration and encapsulation/vitrification techniques were tested. The encapsulation/dehydration technique gave a higher regrowth rate and faster regeneration. Each encapsulated young sporophyte usually develops into several new sporophytes, around 30–80 from the treated control and encapsulation/dehydration techniques, while 5–50 were yielded by the encapsulation/vitrification technique. The effect of cryopreservation on mass reproduction of *in vitro*-grown *P. ridleyi* was also studied using pieces of juvenile fronds (5 mm²) cultured on an MS medium without growth regulators. No abnormality in regrowth or morphology was observed when comparing frond cultures initiated from noncryopreserved and cryopreserved frond strips; both could produce up to 50 new sporophytes on each strip after 8 weeks in the culture.

### 8.4 Conclusion

In vitro techniques have been intensively used in different *Platycerium* species in recent decades, and several regeneration systems applicable to mass propagation have been described. Successful cryopreservation has also been performed. For some staghorns, the great morphogenetic potential and plasticity of *in vitro* cultures is evident. Organogenesis was successfully achieved on different sporophytic explants (pieces or whole juvenile leaves, bud scales, rhizome pieces) with no growth regulators in the media. Furthermore, a simple, reproducible system for apospory induction was defined. Such a system represents an excellent tool for studying the factors affecting morphogenesis, and by using them, new information on the mechanisms involved in organogenesis has been obtained. Undoubtedly, such systems also represent a promising tool for studying developmental and physiological processes in ferns.

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References


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