IN VITRO CULTURE USED FOR WOODY FERN *CYATHEA AUSTRALIS* (R. Br.) DOMIN VEGETATIVE PROPAGATION

KATARZYNA GOLLER and JAN J. RYBCZYŃSKI

The Botanical Garden of the Polish Academy of Sciences,
02-973 Warsaw, ul Prawdziwka 2, Poland

(Received: May 21, 1994. Accepted: December 3, 1994)

ABSTRACT

Experiments have been carried out on vegetative multiplication of *Cyathea australis*. Cultures were initiated from spores collected from sporangium of mature fronds. Spores were sterilized in 3% chloramine with Tween and sown on Anderson (1984) medium supplemented with 80.0 mg/l of Ads and solidified by 8.0 g/l of agar. After three months of spore germination overgrowth of prothallia was observed. Multiplication of prothallia was stimulated by MS (1962) medium supplemented with 0.25 mg/l BAP, 0.50 mg/l IBA, 0.50 mg/l IAA, 1.00 mg/l GA_3, 40.00 mg/l Ads and sucrose 30.0 g/l. Small drops of water were placed on the basal part of gametophyte in order to help ovary fertilization. After the next few weeks the first very fragile, small and green crosier emerged from the base of gametophyte. Perlite culture stimulated root formation and plant hardening to soil conditions.

KEY WORDS: woody fern, spore germination, prothallium culture, plant hardening, vegetative propagation, *Cyathea australis*.

INTRODUCTION

Ferns included in the *Cyatheaceae* family live in the tropics and subtropics. They grow in very humid, shadowed tropical forests. They form multi-individual sits or are spread among other plants in the lower level of the forest.

Culture of woody ferns in indoor conditions is limited because of their intensive growth and height of greenhouses. Here in the Botanical Garden of the Polish Academy of Sciences the *Cyathea australis* ronds reached the greenhouse roof and stopped growing, forcing us to develop a system for their propagation by spore culture.

Generally, two methods of fern propagation have been developed: sexually and asexually by offshoots. The former consists in sterile spores germination on the medium. Many of the *Platycerium* species reproduce asexually by offshoots from roots or rhizome quite readily and are easily increased by the removal of these shoots (Hennen and Sheehan 1978). Knauss (1976) developed a partial tissue culture method of fern propagation. Some modification of this method was used for *Platycerium* and *Davallia* propagation by Cooke (1979). *In vitro* metod for spore germination and prothallium storage of nineteen fern species was tested (Zenkteler 1992). Recently published data of a new technique of fern culture consists in successful growth of fern gametophytes, initiated from spores, which were immobilised in reticulate polyurethane foam in liquid shake culture (Douglas and Sheffield 1990).

The aim of this paper is to give the description of vegetative propagation by means of spores as the initial explant in *Cyathea australis* culture.

Abbrevations:

IAA – indole-3-acetic acid,
IBA – indole-3-butyric acid,
GA_3 – gibberellic acid, Ads- adenine sulphate,
BAP – 6-benzylaminopurine,
Kin – kinetin,
MS medium – Murashige and Skoog 1962,
0.5 MS – half concentration of mineral salts of MS (1962) medium

MATERIALS AND METHODS

Material sterilization

Fragments of fronds were isolated from greenhouse-grown mature individuals. Initially, fronds were immersed in 70% ethanol for 30 secs. Later the fronds were transferred to 3% chloranine with two drops of Tween 80. Conical flasks with green material were shaken for 30 mins. Afterwards the fronds were washed three times with sterile distilled water during 1,5 h.

Spore culture

One half of the frond spores was filtrated and plated on agar MS medium (Murashige and Skoog, 1962). The second half was plated on Anderson's (1984) medium supplemented with 80.0 mg/l Ads, solidified with 8.0 g/l of agar and ajusted to pH 4.5. Sporangia were scraped off and plated on media. Cultures were hold in growth chamber with 23 ± 1°C with day/night photoperiod 16/8 hrs. 0.6 m^2 was lighted by five tubes of white fluoresence light of 40 Watts. The culture conditions mention above were common for all steps of culture.

Prothallium culture

After spore germination the growing prothallia were transferred from tubes to jars containing MS medium sup-

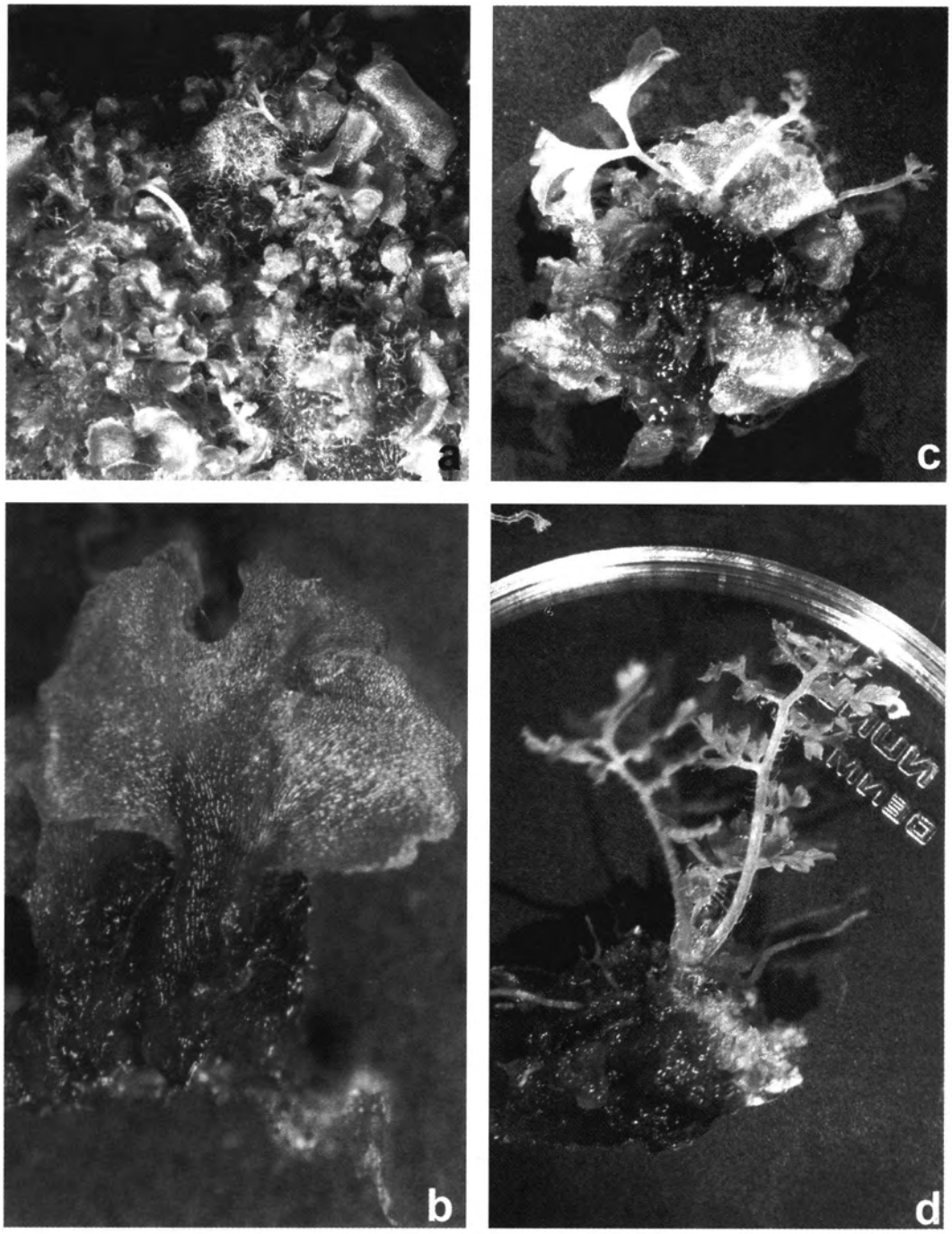

Fig. 1. Prothallium culture and initial stages of *Cyathea australis* plant formation.
a. Numerous prothallia (gametophytes) of *C. australis* after 3 months of culture on MS (1962) modified for fern culture medium Culture initiated from one spore.
b. Individual prothallium with rhizoids after 4 months of culture.
c. Young sporophyte with three fronds located on the base of prothallium on MSO medium after one year of maintaining culture.
d. Young plantlet with tightly rolled tip of the youngest frond with retained parts of prothallium.

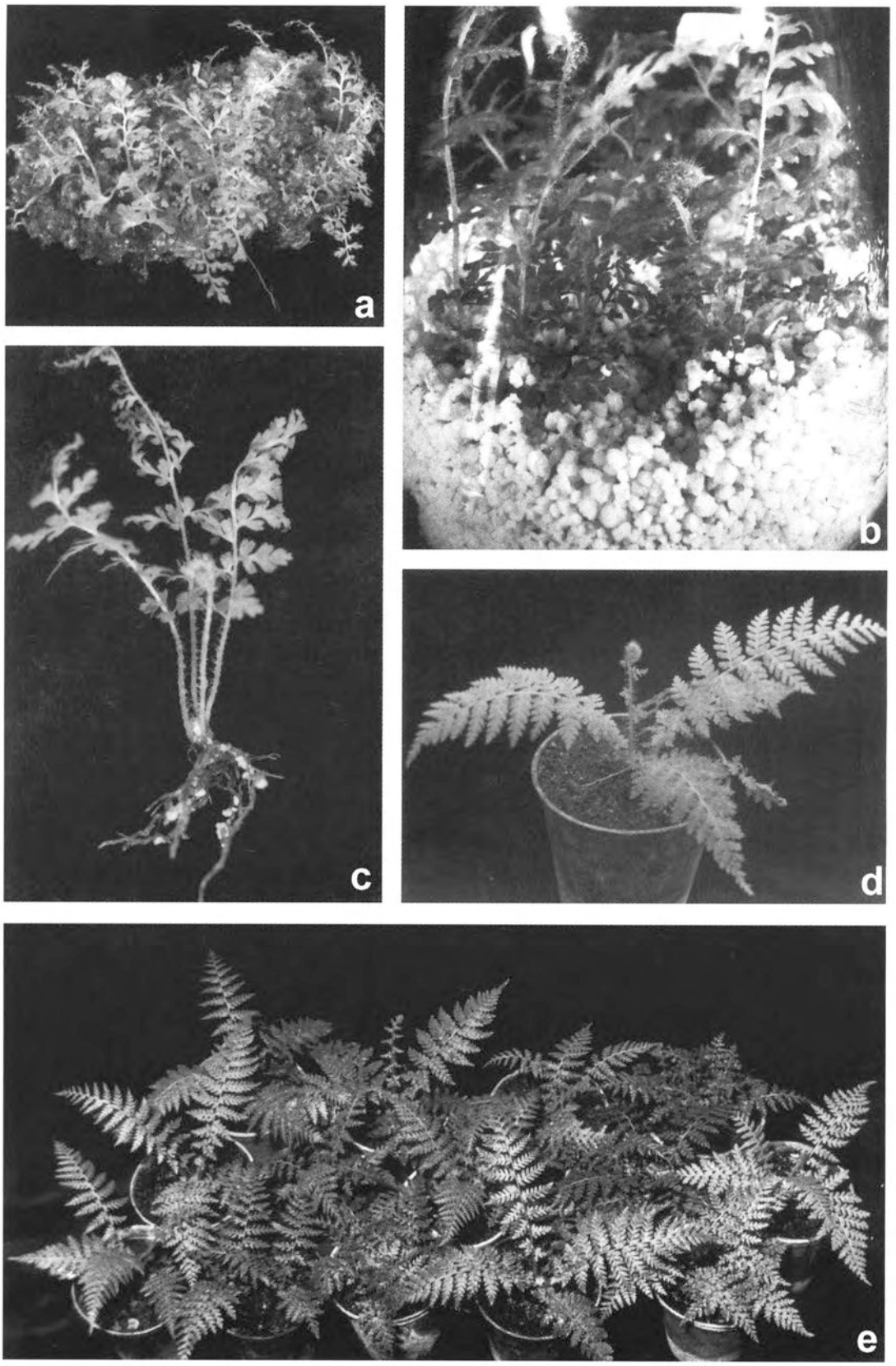

Fig. 2. Plantlet hardening of *C. australis* to greenhouse conditions
a. Overgrown prothallia with multifrond plants isolated from agar medium culture.
b. Perlite culture for better root formation on 0.5 MS liquid medium.
c. Good developed plant with four fronds and roots.
d. Five fronds plant in the pot.
e. Pot culture with numerous plants of *C. australis* in the greenhouse.

plemented with 0.25 mg/l BAP, 40.0 mg/l adenine sulphate, 0.5 mg/l IBA, 0.5 mg/l IAA, 1.0 mg/l GA_3 and 30.0 g/l sucrose.

Fertilization

Healthy prothallia were transferred to MS (1962). Drops of sterile water were placed on the basal part of prothallium to help sperm cells to swim from the antheridium to the neck of the archegonium.

Plantlet growing and plant hardening

After one month the first fragile fiddleheads were emerged from cultured prothallia. Later a few frond plants possessing roots were cultured on 0.5 MS medium with perlite, in large conical flasks sealed by cotton plugs. Plant hardening concists in taking off the plug for extending periods of time, up to completely opened conical flasks. Later on the plants were plated in pots containing the mixture of perlite, pith and soil and cultured in the greenhouse.

RESULTS AND DISCUSSION

The culture was initiated by spore plantation on MS hormone free and Anderson media. Culture initiation was not successful when isolated spore sterilization was applied, because of high fungus infection. Better results were obtained when whole leaves were sterilized and then the sori with sporangia were taken off from the leaf blade with a scalpel. Anderson's medium supplemented with Ads appeared to be sufficient for spore germination and numerous gametophytes were observed in cultures two months later (Fig. 1b).

Our results showing long-term spore germination, even up to two months, are quite different to those described for any other species (Camoloh and Gogala 1992). Comparing the media used for gametophyte multiplication, it is nessecary to stress, that ours were richer both in mineral salts and plant growth hormones than previously published. The general tendency presented by the earlier published data indicates the application of media poor in mineral ingredients (Richards et al. 1983, Camloh and Gogala 1992). Some authors used media with mineral salts concentration reduced by 2 or 4. And in the case of *Matteuccia struthiopteris* one half of Knudson medium, composed of only four mineral salts, appeared to be the best for gametophyte growth, shown by its fresh weight (Zenkteler 1992). An alternative system of gametophytes culture is their immobilisation. This method proved to be very successful for *Anemia phyllitidis* and *Pteridium aquilinum* in terms of growth and hence as a culture system for bulk production of gametophytic tissue (Douglas and Sheffield 1990).

Gametophyte multiplication of *Cyathea australis* was reached on MS medium supplemented with BAP, IBA, IAA, GA_3 and 3.0% sucrose. After a few months unlimited prothallium multiplication was noticed. Fig. 1a presents this stage of culture. To increase fertilization possibilities on the surface of particular prothallium, a drop of water was placed on its basal part. An artificial increase in humidity in the jar induced the effectiveness of fertilization and the number of finally obtained plantlets. During the next subculture on MS medium supplemented with Kin the first fronds were recognized (Fig. 1c). Culture extension on MS medium stimulated the overgrowth of plantlets up to 3-4 leaf stage (Fig. 1d) during only one month of culture.

For shoot formation with comparatively few roots, in case of some fern species, a high concentration of IAA is very much required. The best example of such fern is *Platycerium stemaria* for which culture even 15.0 mg/l was used (Hennen and Sheehan 1978).

Fig. 2a presents a culture with numerous multileaf plantlets which, when transferred to perlite, developed the root system (Fig. 2b) in four weeks. Fig. 2c shows the plants obtained. Due to very fine structure of regenerants, perlite culture was necessary to develop. With perlite cultures only, those plantlets survived which were supplied with 0.5 MS medium. Only half of regenerants survivedwhen only sterile distilled water was used.

We did not make any statistical analysis but the final number of plantlets, which were obtained from culture initiated from spores, reached 480. There were no problems with transferring and plantlet adaptation to soil and greenhouse conditions (Fig. 2d). There was no loss of plant in this stage of culture. After three months all plantlets had 8-10 leaves (Fig. 2e). The stage described, from plated spore *via* three subcultures, was reached in 21 months.

LITERATURE CITED

ANDERSON W.C., 1984. Arevised tissue culture medium for shoot multiplication of *Rhododendron*. J. Amer. Soc. Hort. Sci. 109: 343-347.

CAMOLOH M., GOGALA N., 1992. *In vitro* culture of *Platycerium bifurcatum* gametophytes. Scientia Horticulturae 51: 343-346.

COOKE R.C., 1979. Homogenization as an aid in tissue culture propagation of *Platycerium* and *Davallia*. Hort Science 14: 21-22.

DOUGLAS G.E., SHEFFIELD E., 1990. A new technique for the culture of fern gametophytes. Plant Cell Reports 8: 632-634.

HENNEN G.R., SHEEHAN T.J., 1978. *In vitro* propagation of *Platycerium stemaria* (Beauvois) Desv. Hort Science 13: 245.

KNAUSS J.F., 1976. Partial tissue culture method for pathogen-free propagation of selected ferns from spores. Proc. Fla. State Hort. Soc. 89: 363-365.

MURASHIGE T., SKOOG F., 1962. A revised medium for rapid growth and bioassays with tabacco tissue culture. Plant Physiol. 15: 473-497.

RICHARDS J.H., BECK J.Z., HIRSCH A.M., 1983. Structural investigation of asexual reproduction in *Nephrolepis exaltata* and *Platycerium bifurcatum*. Amer. J. Bot. 70: 993-1001.

ZENKTELER E., 1992. Metoda *in vitro* w rozmnażaniu i okresowym przechowywaniu chronionych oraz rzadkich i ginących gatunków paproci. Hodowla roślin i nasiennictwo 5: 20-30.

ZASTOSOWANIE KULTUR *IN VITRO* DO VEGETATYWNEGO MNOŻENIA PAPROCI DRZEWIASTEJ *CYATHEA AUSTRALIS* (R. Br.) DOMIN

STRESZCZENIE

Kultury *in vitro* drzewiastej paproci *Cyathea australis* zapoczątkowano wysiewem zarodników na pożywki agarowe wg. Murashige i Skoog (1962) oraz Anderson (1984). Zastosowane pożywki uzupełnione zostały różnymi substancjami wzrostowymi. Po upływie trzech miesięcy kultury stwierdzono występowanie obficie mnożących się gametofitów na pożywce MS (1962) uzupełnionej 0.25 mg/l BAP, 0.50 mg/L IBA, 0.50mg/L IAA, 1.00 mg/L GA_3, 40.00 mg/L Ads i 30.0 g/L sacharozy. Celem podniesienia efektywności zapłodnienia, na przedrośla nanoszono krople sterylnej wody destylowanej. Po upływie kilku tygodni kultury zaobserwowano delikatne pastorały pierwszych płonych liści. Rośliny kolejno przeniesiono do perlitu gdzie rozwinęły system korzeniowy pozwalający na łatwą adaplację do warunków glebowych w szklarni.

SŁOWA KLUCZOWE: paproć drzewiasta, kiełkowanie zarodników, kultura gametofitów, wegetatywne mnożenie *in vitro*, *Cyanthea australis*.