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Dedicated to Prof. dr. sc. ZVONIMIR DEVIDÉ on the occasion of his 80th birthday

Jasmonic acid induces changes in growth and polypeptide composition of fern gametophytes

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We studied morphology of gametophytes of the fern *Platycerium bifurcatum* 20 to 45 days after spore sowing. In addition, we examined the effects of 0.01, 0.1, 1, 10 or 100 μM jasmonic acid (JA) on their growth and polypeptide composition. Gametophytes cultured for 20 days on modified Knop's medium were oblong to round in outline. 3–5 rhizoids appeared mainly on the basal cell and 2–3 unicellular hairs formed on the margins. After 30 days in culture, slightly heart-shaped gametophytes produced numerous rhizoids and hairs. Gametophytes cultured for 45 days were cordate and the first antheridia were observed on the ventral side. Hairs, frequently branched, appeared on gametophyte surface.

The effect of JA on the growth of gametophytes was age-dependent. After 20 and 30 days in culture, JA had no pronounced effect in comparison to the control (medium without JA), while after 40 days at concentrations exceeding 0.1 μM JA growth of gametophytes was inhibited. Analysis of soluble proteins from 2 month old JA-treated gametophytes revealed some alterations in polypeptide patterns when compared to the control. The most marked was an increase in a 92–93 kDa polypeptide band at 10 and 100 μM JA in the medium.

Key words: *Platycerium bifurcatum*, gametophyte, jasmonic acid, polypeptide, morphology, growth

Introduction

Fern gametophytes are used as models for studying various biological phenomena (ASHCROFT and SHEEFFIELD 2000). HICKOK et al. (1987) described their utility in developmental biology and genetics. Their morphological simplicity allows direct measurements of many growth parameters. Since they can be easily raised from spores *in vitro,* the effects of a variety of chemicals on the growth of gametophytes in a number of fern species have been tested (RAGHAVAN 1989). Spore germination and early gametophyte development were also studied in detail in *Platycerium bifurcatum* (CAMLOH 1993, 1999). Although

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gametophyte morphology has been described for many fern species (HIYAMA et al. 1992, CHIU and FARRAR 1997, and references therein) little research has been done concerning the gametophytes of *P. bifurcatum*.

Jasmonic acid (JA) and related compounds are widely distributed in the plant kingdom. When exogenously applied they produce various physiological effects in plants. Exogenous JA affects plant development and specifically alters gene expression (WASTERNACK and PARTHIER 1997). Furthermore, a role for jasmonates in plant defence has also been intensively studied (for reviews, CREELMAN and MULLET 1997, KODA 1997, STASWICK and LEHMAN 1999). In these investigations mostly angiosperm species were used, while pteridophyte species were studied only rarely.

KURIYAMA et al. (1993) reported that JA inhibited growth of gametophytic and sporophytic tissue and initiation of sporophytic shoots in *Equisetum arvense in vitro*. In our previous work on *P. bifurcatum* (CAMLOH et al. 1996) we showed that 0.1–1 μM JA in the medium significantly promotes early gametophyte development. In sporophyte protoplast culture JA at 0.01 μM stimulates the initial protoplast divisions (CAMLOH et al. 1996). In leaf culture JA promotes rhizoid and adventitious shoot development and induces changes in polypeptide patterns (CAMLOH et al. 1999).

In the present study we monitored the morphological characteristics of *P. bifurcatum* gametophytes during later developmental stages, 20 to 45 days after spore sowing. Considering the known effects of JA on development of the young gametophyte (CAMLOH et al. 1996) we examined a hypothesis that JA also affects later stages of gametophyte development.

Materials and methods

Gametophyte culture

Spores of *Platycerium bifurcatum* (Cav.) C.Chr. collected by Dr. B. J. HOSHIZAKI, Univ. of California, Los Angeles, in September 1991 or March 1994 from the same plant were used. For spore sterilization and culture we followed the procedures already described (CAMLOH 1993, CAMLOH et al. 1996). Spores were sown in test tubes containing 5 ml of Knop's medium (MILLER and GREANY 1974) with minor modifications (CAMLOH et al. 1996) or in Erlenmeyer flasks with 10 ml of the same medium but supplied with 3% sucrose. The medium was supplemented with 0.01– 100 μM (±) jasmonic acid, (Apex Organics – UK), and adjusted to pH 5.7–5.8 before autoclaving. Medium without JA was used as the control. Cultures were kept at 23±2 °C with a photoperiod of 16 h light/8 h dark at 8–11 Wm^{-2} (Osram L 65W/20S, cool white lamps). For morphological observations gametophytes grown in the control medium in test tubes were cleared and stained with acetocarmine-chloral hydrate according to EDWARDS and MILLER (1972). For examining gametophyte growth, the length and the width of gametophytes from test tubes were determined after 20, 30 and 40 days in culture. At least 20–30 gametophytes were examined per test tube and there were 2–3 test tubes per treatment in each experiment.

Protein extraction and SDS-PAGE

Soluble proteins of 2 month old gametophytes grown in Erlenmeyer flasks were extracted, analysed with SDS polyacrylamide gel electrophoresis and silver stained according to procedures previously described (CAMLOH et al. 1999).

Statistycal analysis

Student's *t*-test was used to calculate the level of statistical significance (*P*) between the data obtained for control media and for those supplemented with JA. All experiments were performed three times with similar results.

Results and discussion

The earliest stages of gametophyte development in *P. bifurcatum* from spore germination to initiation of planar morphology in a few celled protonema are described elsewhere (CAMLOH 1993). Thereafter gametophytes increase in length and width through the activity of the apical cell. After 20 days of culture gametophytes were oblong to round in outline. 3–5 rhizoids appeared mainly on the basal cell and 2–3 unicellular hairs (papillae) appeared on the margins (Fig. 1a). The elongated shape of the gametophytes indicated that at this stage gametophytes grew faster longitudinally than transversely. Similar growth pattern, but without development of hairs, was described for *Osmunda lancea* and *O. japonica* (HIYAMA et al. 1992). 30 days after spore sowing the gametophytes were slightly heart--shaped with numerous rhizoids and hairs. Marginal pluricellular meristem replaced the single meristematic cell and the growth rate in both directions was comparable (Fig. 1b). Sometimes the meristem was initiated on the side of gametophyte leading to an unusual morphology. After 45 days gametophytes grew faster in width than in length. They were clearly cordate and beside numerous rhizoids the first antheridia were observed on the ventral side (Fig. 1c). Similar gametophyte morphology was also described for some other species of the family Polypodiaceae (CHIU and FARRAR 1997). Hairs formed on surfaces of gametophytes. Frequently some branched hairs were observed, especially on older gametophytes. Branched few-celled hairs also appeared on aposporous gametophytes developed on bud scales of *P. bifurcatum* (AMBROŽIČ DOLINŠEK and CAMLOH 1997). In some fern species two hairs were present on one gametophyte cell (CHIOU and FARRAR 1997), but in *P. bifurcatum* one hair was always produced on one cell. Although archegonia were not observed on gametophytes the first sporophytes formed after 3 months in culture. Obviously archegonia appeared in later stages in the ontogeny of the gametophyte, when the morphology was not monitored.

The effect of JA on the growth of gametophytes is age-dependent. The growth of gametophytes on JA-media was comparable to the control medium after 20 and 30 days in culture (data not shown). Surprisingly, after 40 days their growth in length and width was inhibited on media supplied with JA at concentrations exceeding 0.1 μM (Figs. 2, 3). KURIYAMA et al. (1993) obtained similar results on *Equisetum arvense* gametophytic tissue, except that the growth was significantly retarded by JA at concentrations higher than 1 μM. In our previous study we have shown that JA promoted early gametophyte development and stimulated protoplast divisions (CAMLOH et al. 1996). Probably the maturation of gametophytes leads to differences in response to JA. TAKAHASHI et al. (1994) reported that physiological age markedly affected JA-induced swelling of potato tuber disks. Disks from young tubers responded to JA by considerable swelling while those from old tubers no longer responded to JA.

Analysis of soluble proteins from 2 month old gametophytes cultured on JA-media revealed some alterations in polypeptide patterns compared to the control (Fig. 4). The most

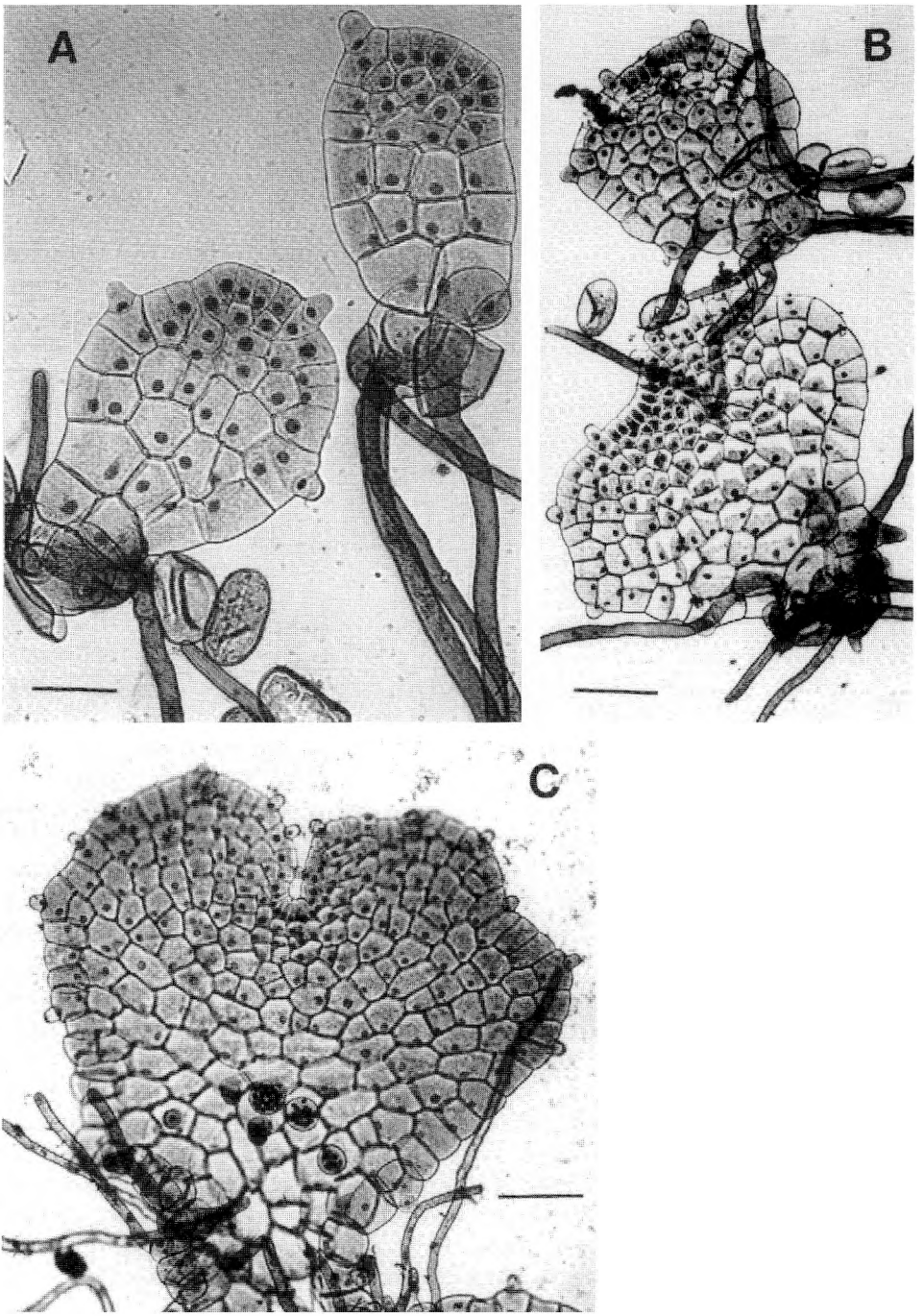

Fig. 1. Morphology of *P. bifurcatum* gametophytes at different developmental stages. A – gametophytes after 20 days of culture. Bar = 50 μm. B – gametophytes after 30 days of culture. Bar = 100 μm. C – gametophytes with antheridia after 45 days of culture. Bar = 100 μm.

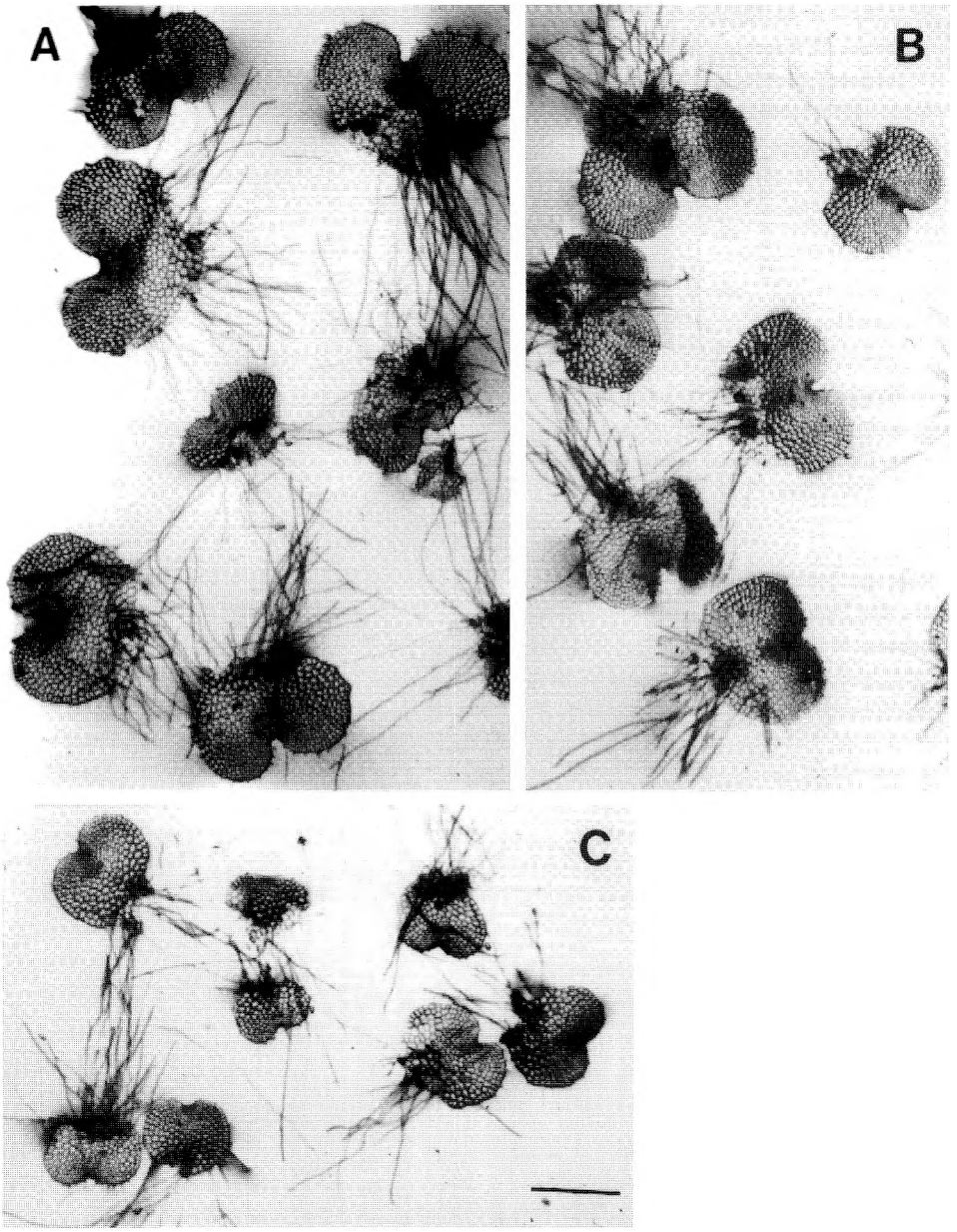

Fig. 2. Gametophytes of *P. bifurcatum* after 45 days of culture. A – control medium (without JA), B – 0.1 μM JA. C – 10 μM JA. Bar = 400 μm.

evident was an increase in the intensity of a 92–93 kDa polypeptide band with increasing JA concentration. The large subunit of Rubisco and the polypeptides with molecular masses of 29 and 22 kDa decreased on JA media. Minor changes in the intensity were evident for some other bands.

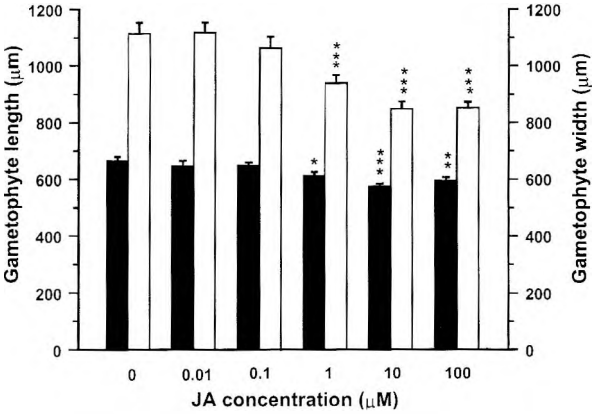

Fig. 3. The length (solid column) and the width (open column) of gametophytes cultured for 40 days on JA-media. Student's *t*-test was used to calculate the level of statistical significance. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Several alterations at the protein level were also observed in leaves of *P. bifurcatum* cultured for 2 months on JA-media (CAMLOH et al. 1999). However, with the exception of the decrease in the large subunit of Rubisco, no similarities were observed between gametophytes and sporophytes of *P. bifurcatum*. A reduced amount of the large subunit of Rubisco was reported also for different angiosperm species (REINBOTHE et al. 1994). Jasmonate-induced gene expression is, except for a few evolutionary conserved polypeptides, highly species- and even tissue-specific (REINBOTHE et al. 1994, HAUSE et al. 1996). Therefore, with the present experimental evidence, any comparison between JA-induced changes in protein synthesis in gametophytes and sporophytes of the same species as well as comparison between angiosperms and ferns would be speculative.

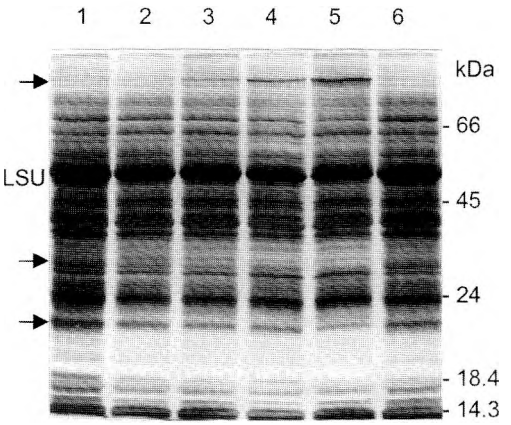

Fig. 4. Soluble proteins in gametophytes grown on the control medium (lanes 1,6) and on JA-media (lanes: 2 – 0.1μM JA; 3 – 1μM JA; 4 – 10μM JA; 5 – 100μM JA) for 2 months. Arrows indicate polypeptides affected by JA (their molecular masses are: 22, 29 and 92–93 kDa). LSU – the large subunit of Rubisco.

In conclusion, while JA promotes cell divisions in early gametophyte development (CAMLOH et al. 1996) we have shown in this study that prolonged treatment with JA results in inhibited growth of older gametophytes. In addition, the alternations in the LSU of Rubisco during JA-treatment similar in *P. bifurcatum* sporophyte and gametophyte correspond to changes observed in angiosperm species. These observation suggest that JA and/or other jasmonates may be an endogenous growth regulator in ferns, and that some jasmonate-related signal transduction pathways may be evolutionary conserved in ferns and angiosperms.

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