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Comparative Shoot and Root Regeneration from Juvenile and Adult Aerial Leaf Explants of Variable-Leaf Milfoil[1]

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ABSTRACT

Adventitious shoot and root regeneration from aerial leaf explants from the juvenile and adult growth phases of the heterophyllous aquatic *Myriophyllum heterophyllum* were studied *in vitro*. Explants were cultured for 28 days on Gamborg B5 basal medium supplemented with specific cytokinin (N^6-benzylaminopurine [BA], isopentenyladenine [2iP], or zeatin [Z]) and auxin (α-naphthaleneacetic acid [NAA] or indole-3-acetic acid [IAA]) combinations in concentrations from 0 to 20 μM. Low numbers of adventitious shoots were produced on juvenile and adult leaf explants cultured on basal medium. All three cytokinins tested promoted shoot regeneration on both explant types with the order of effectiveness being Z > 2iP > BA. Maximum shoot proliferation occurred on adult leaf explants in the presence of 10 μM zeatin and 5 μM IAA. Shoots regenerated from both leaf explant types were always juvenile as judged by leaf morphology and absence of flowering. Medium supplementation with NAA significantly enhanced adventitious root formation. Adventitious shoot-buds originated *directly* from the abaxial/adaxial epidermal cells. Adventitious roots formed endogenously from the vascular tissue. Although morphologically distinct, aerial leaf explants derived from both growth phases exhibit similar competencies for organogenesis.

Key words: Myriophyllum heterophyllum, tissue culture, organogenesis, growth regulators, zeatin, BA, NAA.

INTRODUCTION

In vitro culture techniques have often been used to study the environmental effects on and physiological regulation of growth and development in aquatic angiosperms. For example, heterophylly (Anderson 1982; Kane and Albert 1987a; 1987b), turion formation and germination (Weber and Nooden 1976; Klaine and Ward 1986), floral induction (Doreswamy and Mohan Ram 1969; Uma and Mohan Ram 1972; Mohan Ram and Rao 1982; Pieterse 1982), and photosynthetic carbon assimilation (Francko 1986) have been examined using *in vitro* culture techniques. However, in these studies either seedlings, nodal segments, tubers or other tissues with pre-existing buds (meristems) were used as starting propagules. While it is clear that whole aquatic plants can be generated from any plant fragment containing a bud (Sculthorpe 1967), little is known of the capacity and cultural requirements for *in vitro* adventitious shoot and root regeneration (organogenesis) from non-meristematic aquatic plant tissues. Consequently, similar to progress made using terrestrial species (Thorpe 1980), cultural studies on *in vitro* organogenesis may provide further insight into the physiological mechanisms controlling aquatic plant growth and developmental processes including reproduction and cell differentiation.

In many terrestrial species the regenerative potential expressed by excised tissues cultured *in vitro* is dependent upon their specific origin from and the physiological state of the donor plant (Hicks 1980). For example, in certain plants exhibiting morphologically distinct juvenile (vegetative) and adult (flowering) growth phases, differences in competency for adventitious shoot, root, and floral bud formation have been observed in cultures established from tissues of the juvenile and adult growth phases (Miller and Goodin 1976; Tanimoto and Harada 1979). It has been suggested that the developmental responses of tissue explants *in vitro* may provide model systems for determining the physiological mechanisms regulating vegetative and reproductive growth (Hackett 1983). Although the feasibility of using this approach has been examined in a few terrestrial species (Miller and Goodin 1976), no such studies have been attempted using aquatic angiosperms.

Variable-leaf milfoil, *Myriophyllum heterophyllum* Michx. (Haloragaceae), is a perennial heterophyllous aquatic angiosperm which exists in morphologically distinct juvenile (Vegetative) and adult (flowering) growth phases (England and Tolbert 1964; Manuel 1973). Juvenile shoots produce elongate pinnately dissected astomatous leaves if growing submerged or smaller cutinized dissected stomatous leaves when growing above the water surface (Manuel 1973). In early spring, an abrupt switch to development of cutinized lanceolate spatulate leaves occurs at the apices of rapidly growing submerged juvenile shoots. Lanceolate spatulate leaf development is correlated with the production of axillary flowers and is considered indicative of the adult phase (England and Tolbert 1964). Adult shoots subsequently become emergent and continue to produce lanceolate spatulate leaves throughout the growing season.

Expression of the adult phase is apparently labile. We have observed that pond-collected apical shoot cuttings of adult variable-leaf milfoil root readily but stop growing

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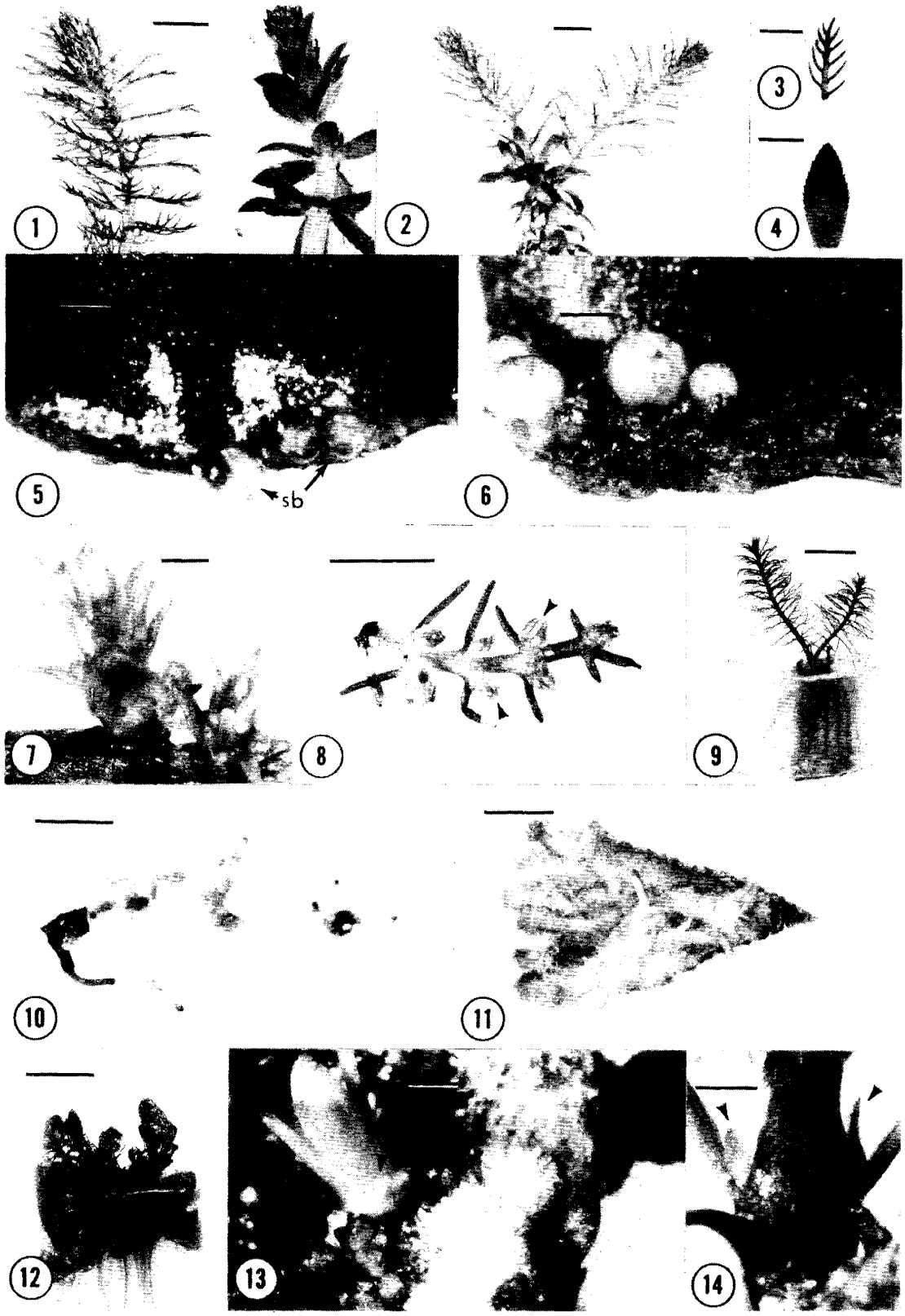

Figure 1-14. 1. Aerial juvenile (left) and aerial adult (right) shoots of *Myriophyllum heterophyllum*. Scale bar = 10 mm. 2. Juvenile reversion branches produced on rooted adult cuttings. Scale bar = 10 mm. 3, 4. Juvenile (JL) and adult leaf (AL) explants, respectively. Scale bars = 5 mm. 5. Shoot-bud (sb) formation on AL explant following 14 days culture on BM. Scale bar = 0.25 mm. 6. Swollen shoot-buds produced by day 11 in the presence of 1.0 μM BA. Scale bar = 0.25 mm. 7. Stubby adventitious shoot after 22 days in presence of 1.0 μM BA. Scale bar = 0.25 mm. 8. Shoot-bud development (arrows) in axils of juvenile leaf pinnae in presence of 1.0 μM BA. Scale bar = 5 mm. 9. Adventitious juvenile aerial shoots on AL explant after 24 days in presence of 1.0 μM NAA. Scale bar = 10 mm. 10. Callus development on JL explant by day 16 in the presence of 10 μM

from the apex and develop lateral branches that are morphologically juvenile. Expression of the juvenile phase is very stable once reversion occurs. However, the environmental and physiological factors required for the induction and expression of the adult phase are unknown.

The occurrence of phasic development in variable-leaf milfoil provides an opportunity to study the developmental responses of tissue explants derived from alternative growth phases of an aquatic plant. In the present paper an experimental system for the *in vitro* culture of leaf explants of *Myriophyllum heterophyllum* is defined with a dual purpose: 1) to examine the influence of plant growth regulators on organogenesis and 2) to determine whether leaf tissues from the juvenile and adult growth phase exist in differing determined states as might be reflected in their competency for organogenesis when cultured under identical and different conditions.

MATERIALS AND METHODS

Leaf Explant Source. Adult leaf (AL) explants of *Myriophyllum heterophyllum* Michx. were obtained from 5.0 cm emergent apical shoot cuttings (Fig. 1) collected on the day of inoculation from a dense population growing in an impoundment pond of Moscow Brook, Moscow, Rhode Island. Because of the limited availability of aerial juvenile shoots at the collection site, juvenile leaf (JL) explants were obtained from rooted juvenile cuttings originally established from juvenile reversion shoots which developed as branches on rooted cuttings of the adult phase (Fig. 2). Juvenile plant stocks were rooted in peat pots (5.7 cm diameter) containing a 3:1 (v/v) mixture of potting soil and perlite. Pots were kept moist in glass covered aquaria place in a greenhouse under the prevailing natural photoperiod (14.2 to 15 hr) at 27 ± 5 °C. Whole leaf explants from both the juvenile and adult phases were excised 1.0 cm below the shoot apices, rinsed in tap water, surface sterilized in 1.05% (v/v) NaOCl containing 0.01% (v/v) Tween-20 for 15 min, and then rinsed in three changes of sterile deionized water. Intact leaf explants (Fig. 3, 4) were used because further subdivision of the leaf tissue resulted in severe browning and subsequent necrosis.

Culture Medium and Conditions. The basal culture medium (BM) consisted of Gamborg B5 inorganic salts and vitamins (Gamborg 1970) supplemented with 20 g/L sucrose and solidified with 0.8% /w/v) TC Agar™ (Hazleton Research Products, Inc., Lenexa, KS). The medium was adjusted to pH 5.5 with 0.1 N NaOH prior to autoclaving. Cytokinin (zeatin, BA, 2iP)[3] and auxins (NAA, IAA) solutions were filter-sterilized by Millipore filtration (0.22 μm pore size) and then added individually or in combination to molten autoclaved basal medium (40 °C). Media were dispensed as 10 mL aliquots into sterile 20 mL glass scintillation vials. Each vial was inoculated with one leaf explant with the abaxial surface in contact with medium and then loosely covered with polypropylene caps. Cultures were maintained in growth chambers at 25 ± 2 °C in continuous light provided by fluorescent lamps (Sylvania F4812/CW/VHO) at an average photosynthetic photon flux density (PPFD) of 350 μmol m^2/s. Each experiment was comprised of 25 treatments consisting of all combinations of a specific cytokinin and auxin at five concentrations (0, 1.0, 5, 10, and 20 μM). Each replicate consisted of one explant per vial and 10 replicates were used for each treatment. All experiments were initiated over a three week period from May 17, 1982 to June 7, 1982. For each experiment, JL and AL explants were inoculated on the same day. Treatment effects on the number of primary adventitious shoots and roots originating *directly* from each explant were evaluated on day 28. Except where indicated, shoots produced secondarily as lateral branches or adventitious buds on primary adventitious shoots were not scored.

Light and Scanning Electron Microscopy. For histological observations, JL and AL explants were inoculated onto BM supplemented with either 20 μM 2iP or 10 μM NAA. At daily intervals up until day 14, explants were fixed in formalin-acetic-alcohol (FAA), dehydrated in a tertiary butyl alcohol series, embedded in Paraplast (57 °C), and sectioned at 10 μm. Sections were stained with safranin and fast-green. For scanning electron microscopy (SEM), explants were fixed in FAA, dehydrated through an ethanol series, critical point dried, mounted on metal stubs, and sputter coated with gold/palladium. Samples were then examined with a Cambridge Stereoscan S-4 microscope at 20 kV.

RESULTS

Regeneration on basal medium. Leaf explants from both growth phases developed adventitious shoot-buds by day 14 along the adaxial cut surfaces on BM (Fig. 5). By day 28, 60% of the JL explants cultured on BM had produced adventitious shoots (mean: 1.0 primary shoots/explant) compared to 90% of the AL exxplants (mean: 4.0 primary shoots/explant). Adventitious shoots produced in the absence of plant growth regulators were small, rootless, and usually showed signs of senescence (tissue blackening) by day 28. Although adventitious roots never developed from AL explants on BM, 20% of the JL explants had developed at least one adventitious root by day 28.

Growth regulator effects on organogenesis. Comparative effects of the various cytokinin-auxin combinations on shoot and root regeneration on both explant types are shown in Fig. 15-26. The pattern of responses to specific cytokinin and auxin combinations was similar in both JL and AL explants with differences being primarily quantitative. Adult leaf explants consistently displayed a greater capacity for shoot and root organogenesis than JL explants.

[3]Z = zeatin (6-[4-hydroxy-3-methylbut-2-enyamino]purine) mixed isomers, 80% in trans isomer; BA = N^6-benzylaminopurine; 2iP = (2-isopentenyl)adenine; NAA = α-napthaleneacetic acid; IAA = indole-3-acetic acid.

NAA. Scale bar = 2.5 mm. 11. NAA-induced root formation on AL explant. Scale bar = 2.5 mm. 12. Regeneration of juvenile shoots from AL explant after 24 days in presence of 20 μM 2iP. Scale bar = 10 mm. 13. Development of epidermal protrusions by day 18 in the presence of 20 μM zeatin and IAA. Note strap-shaped leaves and development of axillary buds (arrow) on young shoot. Scale bar = 0.25 mm. 14. Axillary buds (arrows) on older adventitious shoot produced in the presence of 20 μM zeatin and IAA. Scale bar = 0.25 mm.

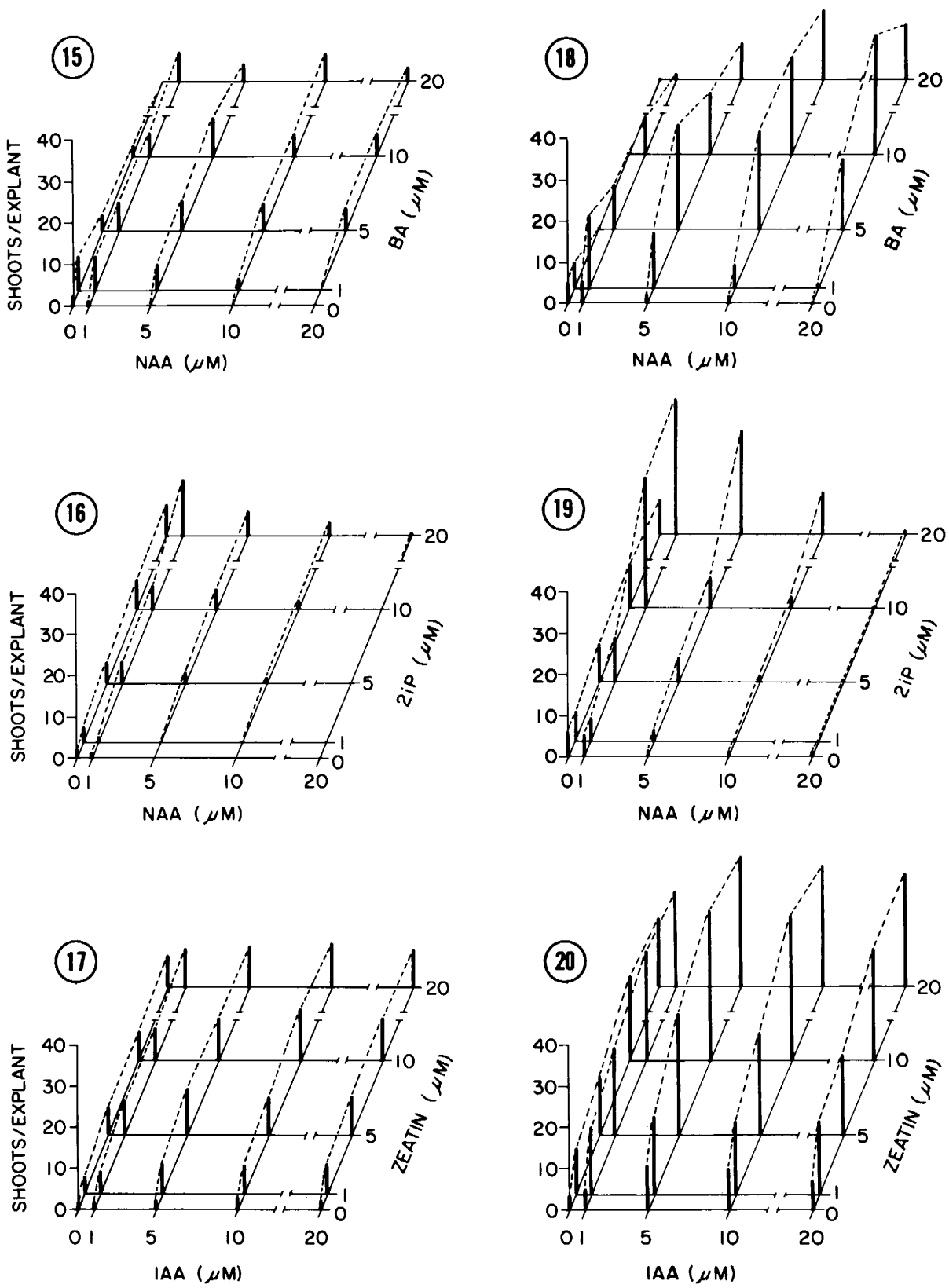

Figure 15-20. Influence of various cytokinin and auxin combinations on primary adventitious shoot formation on juvenile and adult leaf explants of *Myriophyllum heterophyllum* after 28 days. Each histobar represents the mean response of 10 explants.

Quantitative differences depended on the types of cytokinin and auxin used and their relative concentrations. Cytokinin-supplemented BM significantly promoted adventitious shoot formation while exogenous auxins promoted adventitious root production. Regardless of treatment, floral bud development was not observed on either JL or AL explants.

Of the cytokinins tested, BA was the weakest promoter of shoot regeneration. Adventitious shoot formation was stimulated in the presence of 1.0 μM BA alone but was completely inhibited on both explant types at 20 μM BA (Fig. 15, 18). Addition of NAA to the medium enhanced BA promoted bud formation and mitigated inhibition of bud formation at high BA concentrations. In the presence of 1.0 μM BA, numerous dark-red swollen shoot-buds were first macroscopically visible along the excised bases of both JL and AL explants (Fig. 6) by day 6. By day 22 these shoots were swollen and stubby (Fig. 7). Adventitious shoot-buds also consistently developed in the axils of the pinnae of JL explants (Fig. 8) but only on cytokinin-supplemented BM. Adventitious root development was promoted in the presence of NAA in JL (Fig. 21) and induced in AL explants (Fig. 11, 24) but was significantly inhibited on BM supplemented also with BA. Shoots produced in the presence of 1.0 μM NAA alone were rooted, displayed enhanced growth, and no signs of senescence (Fig. 9). Adventitious shoots which developed from either JL or AL explants were always juvenile as judged by leaf shape and the absence of flowering (Fig. 9). White non-morphogenic callus developed only on JL explants in the presence of 10 or 20 μM NAA alone (Fig. 10).

Relative to BA treatments, 2iP more effectively promoted adventitious shoot formation (Fig. 16, 19). Unlike BA treatments, adventitious shoot-bud formation was not inhibited at higher 2iP levels. Addition ≥ 5 μM 2iP further promoted axillary branching of the primary adventitious shoots produced resulting in development of compact masses of multiple shoots bearing dissected leaves (Fig. 12). Maximum primary shoot formation on 2iP-supplemented BM occurred on both explant types cultured in the presence of both 20 μM 2iP and 1.0 μM NAA (mean ± SE: 13 ± 2 primary shoots/JL explant; 33 ± 2 primary shoots/AL explant). However, NAA concentrations exceeding 1.0 μM inhibited 2iP-promoted shoot formation and outgrowth on both JL and AL explants. Exogenous 2iP slightly antagonized NAA-promoted root formation (Fig. 22, 25).

Of the three cytokinins tested, zeatin most effectively stimulated bud formation. Maximum primary shoot regeneration occurred in the presence of 10 μM zeatin and 10 μM IAA on JL explants [mean: 13 ± 2 primary shoots/explant] (Fig. 17) and 10 μM zeatin and 5.0 μM IAA on AL explants [mean: 37 ± 5 primary shoots/explant] (Fig. 20). At these concentrations, yellow-red shoot-buds were macroscopically visible along the excised leaf bases of both explant types and in the axils of juvenile leaf pinnae by day 6. The first leaves produced were always strap-shaped (Fig. 13). However, subsequent leaves produced were highly dissected. Addition of both 20 μM zeatin and IAA to the medium also promoted the formation of epidermal cell protrusions (Fig. 13). In response to zeatin-IAA treatment, both JL and AL leaf explants formed large masses of adventitious shoots (Fig. 41, 42). These shoot masses consisted of not only of numerous primary adventitious shoots, but also greater numbers of small first and second order axillary branches and adventitious buds that were initiated during primary shoot growth (Fig. 13, 14). The *total* number of shoot meristems generated *de novo* from a single AL explant, at optimum zeatin and IAA concentrations described, exceeded 2600 by the end of the 28 day experimental period. The naturally-occurring auxin IAA did not inhibit zeatin enhanced shoots production (Fig. 17, 20). Compared to NAA, IAA was a weak promoter of adventitious root formation (Fig. 23, 26). Induction of root formation by IAA was completely inhibited in JL explants cultured on BM supplemented with zeatin ≥ 10 μM and significantly reduced by zeatin levels ≥ 1.0 μM in AL explants.

Early development of adventitious shoots and roots. The origin and pattern of adventitious shoot and root development was identical in both explant types. Shoot meristems arose directly from the epidermal layer of both explant types. On BM supplemented with 20 μM 2iP, meristematic centers first developed along the excised leaf bases by day 3-4 following localized anticlinal and periclinal divisions within the epidermal cell layer (fig. 27, 28). By day 6 these meristematic centers were domed-shaped (Fig. 29) and consisted of small densely cytoplasmic cells (Fig. 30). Procambial cell strand differentiation was evident within the rapidly dividing underlying mesophyll cells. By day 8 the shoot meristems were flanked by small leaf primordia (Fig. 31, 32) which were well developed by day 10) (Fig. 33).

The adventitious shoot-buds formed in the axils of the pinnae on JL explants differentiated more rapidly than those formed along the leaf bases and appeared to develop synchronously on individual explants. Pre-existing residual meristems were not observed within any of the pinna axils sectioned prior to culture. Development of meristematic centers and procambial strands was observed by day 3 (Fig. 34). By day 7 adventitious shoot buds with flanking leaf primordia were well developed with the vascular tissue continuous with the vascular elements of the leaf explant (Fig. 35, 36).

Adventitious root primordia differentiated endogenously from the vascular tissue of the leaf explants. In the presence of 10 μM NAA, root primordia formed internal to the bundle sheath cells by day 3 (Fig. 37). By day 4-5 these roots possessed well defined root apices (Fig. 38) and penetrated the abaxial epidermis by day 6. In response to contact with the root apex, the abaxial epidermal cells were induced to divide and elongated forming a prominent sheath around the outgrowing roots (Fig. 39, 40) prior to penetration of the root apex through the epidermis and growth into the medium.

DISCUSSION

Aerial leaf explants from both the juvenile and adult growth phase of variable-leaf milfoil exhibit extremely rapid and high capacities for adventitious shoot and root regeneration when cultured *in vitro*. Although the juvenile and adult growth phases are morphologically distinct,

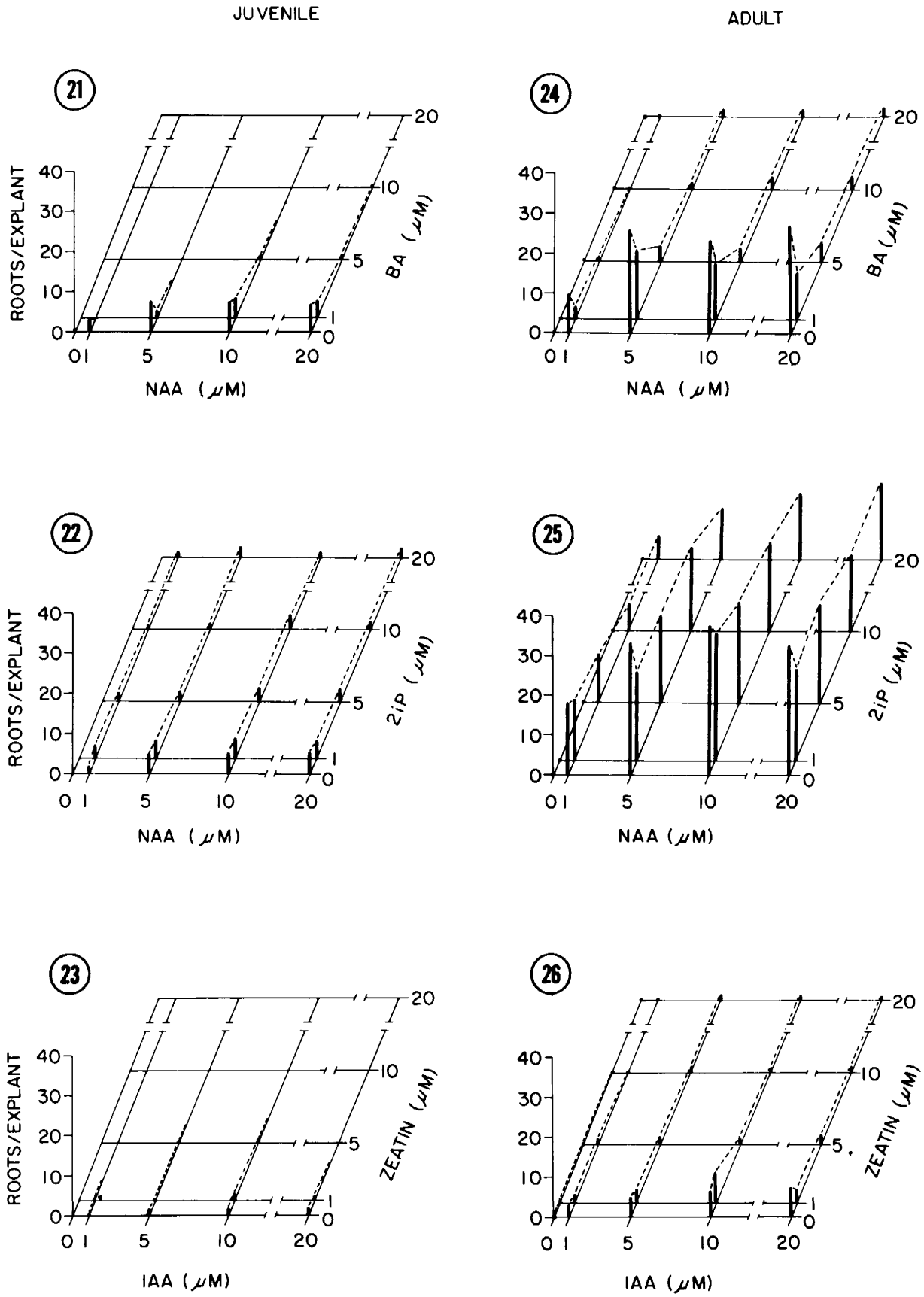

Figure 21-26. Influence of various cytokinin and auxin combinations on primary adventitious root formation on juvenile and adult leaf explants. of *Myriophyllum heterophyllum* after 28 days. Each histobar represents the mean response of 10 explants.

whole leaf explants derived from both growth phases exhibited similar competencies for organogenesis when cultured *in vitro*. Differences in organogenetic potential between explant types were quantitative except for the capacity of JL explants to form callus in the presence of NAA. In all cases, regenerated shoots were juvenile as judged by leaf shape and the absence of flowering. Consequently, these results support the contention that adventitious shoot meristems arising *de novo* in tissue culture are intrinsically juvenile irrespective of explant origin (Mullins et al. 1979; Hackett 1983).

On the basis of explant fresh weight, dry weight, and total surface area, AL explants of *M. heterophyllum* consistently developed significantly greater numbers of organs compared to juvenile leaf explants. Given the epidermal origin of adventitious shoots, the greater number of primary shoots produced on the relatively larger adult leaf explants likely reflected a greater number of potential meristematic loci (i.e., epidermal cells). Similarly, the extensive reticulate venation present in the adult leaf explants versus the single midvein in juvenile leaves, provided a greater number of potential sites for endogenous differentiation of adventitious root primordia.

Although adult leaf development in variable-leaf milfoil is closely associated with flowering and AL explants were excised from flowering shoots, the present results indicated no qualitative differences in morphogenetic expression between JL and AL explants. Regardless of explant origin, organogenesis was limited to adventitious shoot and root formation. Adventitious floral bud production was not observed on either explant type. Differing regenerative competencies to form shoot versus floral buds have been demonstrated for explants from vegetative and floral tissues in several herbaceous terrestrial species (Tanimoto and Harada 1979; 1981; Tran Thahn Van 1980). In one species, a hormonal basis for these differences has been demonstrated (Tanimoto et al. 1985). It is not clear what role inter-tissue correlative effects (Chlyah, 1974a; Tran Thanh Van 1980) may have played on masking possible differences in morphogenetic potential since the present study was limited to the use of whole leaf explants comprised of complex tissues. However, direct differentiation of floral buds *in vitro* have been reported from complex explants including detached whole leaves (Bajaj 1972), leaf sections (Allot-Deronne and Blondon 1985), and stem segments (Tanimoto and Harada 1979).

One important characteristic of most plants exhibiting phase change is that the adult phase, once attianed, is relatively stable and persists through asexual propagation (Hackett 1983). The consistent development of juvenile reversion shoots on rooted adult cuttings of variable-leaf milfoil indicates that the morphologic expression of the adult phase is unstable following fragmentation. Consequently, the underlying mechanism(s) regulating phasic development in this species probably differs from that in other plants. It could be argued that the leaf dimorphism evident between the juvenile and adult phase in *M. heterophyllum* may simply reflect alterations in the patterns of cell division and elongation during leaf development mediated by environmentally induced changes in hormone content as reported for other heterophyllous aquatics (Kane and Albert 1987b). However, neither photoinductive treatments nor exogenous application of cytokinins, auxins, gibberellins, or abscisic acid induce production of adult-type leaves on juvenile shoots or prevent juvenile reversion from occurring on rooted adult cuttings in *M. heterophyllum* (Kane unpublished). Conceivably, the expression of the adult growth phase is mediated and stabilized by factors produced *in situ* in the roots and then transported to the shoot apex. This is possible since removal of the original root stock from pond-collected adult plants *always* results in reversion to the juvenile state. Furthemore, since reversion still occurs on adult cuttings that have re-rooted, the possibility arises that roots produced *in situ* are in an induced state. Future studies should therefore address the efffects of photoperiod and thermoinduction (vernalization) on evocation and stabilization of the adult phase.

Skoog and Miller (1957) first demonstrated that the basic regulatory mechanism underlying shoot and root organogenesis *in vitro* involved a balance between cytokinin and auxin in the medium. In general, high cytokinin to auxin ratios induce adventitious shoot differentiation and suppress root development while lower ratios favor adventitious root formation. Since that report, the role of cytokinins and auxins in shoot and root organogenesis have been demonstrated in many terrestrial plants (Flick et al. 1983). *In vitro* organogenesis has been studied in very few aquatic angiosperms (Chang and Hsing 1978; Rao and Mohan Ram 1981; Kakkar and Mohan Ram 1986). However, it would appear that at least in *M. heterophyllum*, leaf explants differentiate organs *de novo* in response to exogenous cytokinins and auxins in a similar manner as reported for leaf explant systems in many terrestrial dicot species (Bajaj 1972; Davies and Dale 1979; Dunwell, 1981). For example, Chlyah (1974b) has similarly reported that bud formation occurs exclusively from epidermal cells and roots form endogenously from the vascular tissue in *Torenia* internode explants.

As noted in this study and others, the organogenic response is highly dependent on the level and type of cytokinin or auxin used (Flick et al. 1983). In variable-leaf milfoil, addition of the naturally produced cytokinins 2iP or zeatin were significantly more effective promoters of adventitious shoot formation than the synthetic cytokinin BA. These differences may reflect the inability of this species to metabolize BA, resulting in the endogenous buildup of supra-optimal BA levels (Blakesley and Lenton 1987). This response is common to other *Myriophyllum* species. We have observed that *in vitro* shoot organogenesis in parrot-feather (*Myriophyllum aquaticum* (Vell.) Verdcourt) is more effectively promoted on media supplemented with either 2iP or zeatin than BA (Kane and Sheehan 1988).

Adventitious shoot regeneration in the Haloragacceae is not limited to tissues cultured *in vitro*. Hagemann (1932) has reported that detached leaves of parrot-feather, Western milfoil (*Myriophyllum hippuroides* Nutt. ex Torr. & Gray) and mermaidweed (*Proserpinaca palustris* L.) produce plantlets when maintained under moist conditions. However, there is no evidence that adventitious shoot formation from fragmented tissues plays a role in the dispersal of these species. In other aquatic plant genera, *Utricularia*,

Figure 27-40. Histological origin and early development of adventitious shoot-buds and roots. With the exception of Fig. 34-36, all other figures depict responses of AL explants. On Fig. 27-38 scale bars= 50 μm. Scale bars= 100 μm and 2.5 mm on Fig. 39, 40, respectively. 27, 28. Shoot meristem (m) formation in the epidermal cell layer (ep) after 3 day culture in the presence of 20 μM 2iP. 29, 30. Dome-shaped meristematic center with procambial strand (pc) differentiation by day 6. 31, 32. Later stage of shoot-bud development showing a distinct apical meristem (am) flanked by the initial leaf primordia (lp) after 8 days. 33. Shoot-bud development after 10 days. 34. Shoot meristem development in the axil of a juvenile leaf pinna by day 3. Note early procambial strand development. 35. Adventitious shoot-bud with vascularization continuous with JL explant (arrow) by

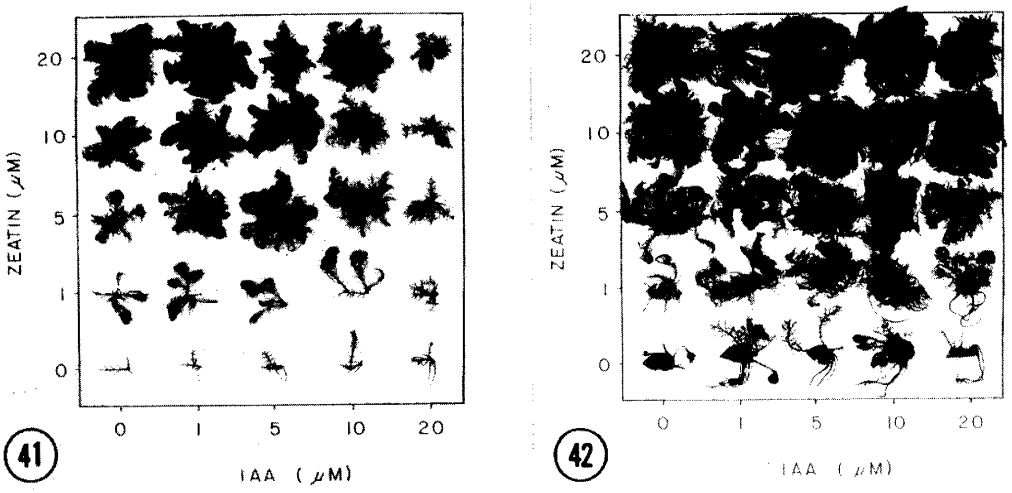

Figure 41, 42. 41. Combined effects of zeatin and IAA on adventitious shoot proliferation and root development from juvenile leaf explants of *Myriophyllum heterophyllum* after 28 days culture. 42. Combined effects of zeatin and IAA on adventitious shoot proliferation and root development from adult leaf explants after 28 days culture.

Rorippa, *Ceratopteris*, and *Podostemum* production of plantlets on detached or injured leaves can be an effective means of regeneration *in situ* (Sculthorpe 1967).

Throughout its range variable-leaf milfoil exhibits aggressive growth which hinders recreational activities in many lakes and streams (Crow and Hellquist 1983). This weed potential is clearly reflected in its capacity for regeneration in culture. The potential to produce more than 2600 *total* shoot meristems in one month from a single adult leaf explant indicates that this species possesses the greatest *in vitro* regenerative capacity of any plant reported to date. While it is well documented that aquatic growth *in situ* is influenced by many abiotic and biotic factors acting in concert, ultimately it is plant genotype which determines maximum proliferation under non-limiting growth conditions. Consequently, it may be feasible to evaluate the relative weed potential of aquatic species by comparing their growth and regeneration performance under non-limiting culture conditions. However, additional comparative studies of *in vitro* growth and regeneration using both prohibited (known weedy) and non-prohibited (putative non-weedy) species are required to determine the relationship between nuisance aquatic plant growth and its expression *in vitro*.

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day 7. 36. Axillary shoot-bud at day 7 development stage. 37. Origin of an adventitious root primordium (r) within the vascular bundle (vb) by day 3 in the presence of 10 μM NAA. 38. Adventitious root with well-defined cap after 4 days. 39. Stimulation of the abaxial epidermal cell growth by emerging root. 40. Abaxial epidermal sheath surrounding the adventitious roots.

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Isoenzymic Variability in Monoecious Hydrilla in the United States

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ABSTRACT

Monoecious hydrilla, from sites in North Carolina and Washington, D.C., was monitored for heterogeneity by polyacrylamide gel electrophoresis under non-denaturing conditions, followed by staining for isoenzymes. Extracts of subterranean turions from four sites in North Carolina showed a variable distribution of two biotypes. One biotype had patterns of activity for alcohol dehydrogenase, NADP-malic enzyme, aspartate aminotransferase and phosphoglucomutase identical to those for the turions from Washington. The second was different in the first three enzymes. Protein profiles of the two types were slightly different, although the major proteins were identical. Distribution ranged from nearly 100% of one biotype in Lake Anne to nearly 100% of the other in Lake Wheeler, and suggests two separate introductions of the plant in North Carolina. Isoenzymic profiles of turions from three sites near Washington were identical and are in accord with a single introduction.

Key words: electrophoresis isoenzymes, subterranean turions.

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INTRODUCTION

The aquatic plant hydrilla (*Hydrilla verticillata* (L.f.) Royle) may have its center of origin in southeast Asia (Swarbrick et al. 1981), although it is now distributed worldwide. Both monoecious and dioecious strains exist. Additionally, there are a number of biotypes which can be distinguished on the basis of isoenzymic banding patterns after electrophoresis. The electrophoretic and morphological characterization of biotypes has been described in a number of papers (Verkleij et al. 1983; Pieterse et al. 1984, 1985; Verkleij and Pieterse 1986). Two populations have been characterized at present in the United States. The original introduction into Florida (Haller 1978) was dioecious female, and this has now spread throughout the southeast and as far west as California, where it has become established in the Imperial Valley. More recently, a monoecious plant has been noted in the Potomac River and in other bodies of water in the vicinity of Washington, D.C. (Steward et al. 1984). Both of these strains have been characterized by enzymic analysis of the vegetative tissue (Verkleij et al. 1983) or the subterranean turions (Ryan 1988a) and are readily distinguishable by comparison of phosphoglucomutase (PGM) or aspartate aminotransferase (AAT).