Received: 1 October 2023 | Revised: 9 November 2023 | Accepted: 7 December 2023

DOI: 10.1002/pld3.558

RESEARCH ARTICLE

Characteristics of turion development in two aquatic carnivorous plants: Hormonal profiles, gas exchange and mineral nutrient content

Lubomír Adamec[1] | Lenka Plačková[2,3] | Karel Doležal[2,3,4]

[1]Institute of Botany, Czech Academy of Sciences, Třeboň, Czech Republic

[2]Laboratory of Growth Regulators, Faculty of Science, Palacký University, Olomouc, CR, Czech Republic

[3]Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic

[4]Department of Chemical Biology, Faculty of Science, Palacký University, Olomouc, Czech Republic

Correspondence
Karel Doležal, Department of Chemical Biology, Faculty of Science, Palacký University, Šlechtitelů 27, Olomouc 78371, Czech Republic.
Email: karel.dolezal@upol.cz

Funding information
Czech Academy of Sciences, Grant/Award Number: RVO 67985939

Abstract

Turions are vegetative, dormant, and storage overwintering organs formed in perennial aquatic plants in response to unfavorable ecological conditions and originate by extreme condensation of apical shoot segments. The contents of cytokinins, auxins, and abscisic acid were estimated in shoot apices of summer growing, rootless aquatic carnivorous plants, *Aldrovanda vesiculosa* and *Utricularia australis*, and in developing turions at three stages and full maturity to reveal hormonal patterns responsible for turion development. The hormones were analyzed in miniature turion samples using ultraperformance liquid chromatography coupled with triple quadrupole mass spectrometry. Photosynthetic measurements in young leaves also confirmed relatively high photosynthetic rates at later turion stages. The content of active cytokinin forms was almost stable in *A. vesiculosa* during turion development but markedly decreased in *U. australis*. In both species, auxin content culminated in the middle of turion development and then decreased again. The content of abscisic acid as the main inhibitory hormone was very low in growing plants in both species but rose greatly at first developmental stages and stayed very high in mature turions. The hormonal data indicate a great strength of developing turions within sink–source relationships and confirm the central role of abscisic acid in regulating the turion development.

KEYWORDS
abscisic acid, *Aldrovanda vesiculosa*, aquatic rootless plants, auxins, cytokinins, nutrient reutilization, photosynthesis, respiration, *Utricularia australis*, winter buds

1 | INTRODUCTION

Specific overwintering organs—turions or winter buds—are very common in subtropical to subarctic zones and are formed in at least 14 genera from nine plant families of aquatic vascular plants, in basal angiosperms, monocots, and eudicots (Adamec, 2018a; Adamec et al., 2020; Bartley & Spence, 1987; Sculthorpe, 1967). The evolution of turions is thus typically polyphyletic and represents functional convergency. Turions are green dormant storage vegetative organs formed in perennial aquatic plants in response to unfavorable ecological conditions (mainly temperature decrease, shortening day length, and/or reduction of daily light dosage) mostly at the beginning of autumn (Adamec, 2018a; Bartley & Spence, 1987; Sculthorpe, 1967). In Central Europe, the development of turions is usually initiated in relatively warm water (~14–22°C) at the turn of August–September, but much lower, long-term

Lubomír Adamec and Lenka Plačková contributed equally.

temperatures of ~5–8°C are required for full turion development and maturation (usually during October; Adamec, 2018a). Turions originate by an extreme condensation of apical shoot segments bearing modified shortened leaves or scales, which are dissimilar to summer leaves, while the mother shoots gradually senesce and decay and are sooner or later separated from the mature turions. Turions are tough organs and their shape can be spherical, oval, rhomboid, greatly enlarged, or flat circular (Adamec, 2018a; Bartley & Spence, 1987; Sculthorpe, 1967).

Two different ecological strategies of turion formation, germination, and sprouting have been described (Adamec, 2008, 2018a; Sculthorpe, 1967). Turions of bottom-rooted species (and the rootless *Ceratophyllum demersum*) are formed and maturate at depth and also sprout at the bottom in shade and colder water, whereas those of submerged rootless and free-floating species form, maturate, and also germinate and sprout in warmer water and light at the surface. Moreover, most species from the latter group (*Utricularia* spp.) form turions that are usually less dense than water, but their decaying mother shoots drag them to the bottom. By early spring, the turions separate and rise to the surface, while *Aldrovanda*, *Hydrocharis*, and *Spirodela* turions possess an active mechanism of sinking and rising.

Turions as storage organs exhibit low rates of metabolism. Their aerobic respiration rate (RD) is several times lower per unit fresh (FW) or dry weight (DW) than that in summer shoots/leaves of the same or similar aquatic plants (Adamec, 2008, 2011, 2018a). High contents of reserve substances in mature turions correlate with high dry matter content (DMC), being usually between 18–39%, that is, about 2.5–4 times higher than that in their summer shoots/leaves (Adamec, 2008, 2018a). Mature turions accumulate starch, free sugars, reserve proteins, lipids, and amino acids (see Adamec, 2018a, and the literature therein). Starch and free sugars are obviously the main reserve substances in many tested species, but their levels and ratios are very variable among species (Adamec et al., 2020). Moreover, turions are storage organs for some mineral nutrients (at least N, P, S, and Mg), but this trait is less marked than that for carbohydrates (Adamec, 2010, 2018a; Adamec et al., 2020). Although turions contain chlorophylls and carotenoids at sufficient levels (Adamec et al., 2020) their net photosynthetic rate (P_N) is around zero even under optimal conditions (Adamec, 2011, 2018a).

Two dormancy stages are attributable to true turions, while "non-dormant winter apices" in several species usually do not form morphologically distinct organs, are not detached from mother shoots, and are "quiescent." Their winter growth is only transiently inhibited by unfavorable ecological conditions (low temperature, short day, and low irradiance) and after these inhibitory conditions disappear their growth resumes again (Adamec, 2008, 2018a; Bartley & Spence, 1987; Winston & Gorham, 1979a). Developing turions enter a stage of innate dormancy lasting about 2 months, and their growth is blocked by endogenous factors in the turions. Innate dormancy can be broken only by low temperature with short days and is followed by imposed dormancy when turion germination depends only on higher temperature (Adamec, 2018a; Winston & Gorham, 1979a).

Phytohormones are endogenously occurring signaling molecules, controlling all aspects of plant growth and development, including responses to various endogenous and environmental factors. Among them, cytokinins and auxin have been known as the principal regulators of plant growth and development for almost 70 years (Skoog & Miller, 1957). Abscisic acid (ABA) has been found to be involved in the control of seed dormancy and organ abscission but plays important roles in many other physiological processes (Tarkowská et al., 2014). The function of these phytohormones has been extensively studied in land plants (Hussain et al., 2021; Tarkowská et al., 2014); however, their role in the morphogenesis and physiology of aquatic carnivorous plants still remains largely unexplored (Adamec et al., 2022; Šimura et al., 2016).

Using bioassays, it was found in *Utricularia macrorhiza* that high endogenous levels of ABA induced turion formation and in the stage of innate dormancy, a high level of ABA was associated with low levels of free gibberellins, auxin, and cytokinins (Adamec, 2018a; Winston & Gorham, 1979b). Imposed dormancy was connected with a decreasing level of ABA but increasing levels of gibberellins, auxin, and cytokinins. Similar patterns of decreasing levels of auxin and cytokinins with the central role of ABA accumulation were also found in just-induced and developing turions of *Myriophyllum verticillatum* (bioassays, Weber & Noodén, 1976) and *C. demersum* (analyses, Best, 1979). The central role of ABA accumulation for induction of turion formation was confirmed in some species also as a result of exogenous ABA application to the growth medium (Li et al., 2022; Smart et al., 1995; Smart & Trewavas, 1983; Wang et al., 2014; Weber & Noodén, 1976), but not in summer growing *C. demersum* (Best, 1979). Šimura et al. (2016) analyzed different cytokinins and auxins in growing apical shoot segments and mature turions of *Aldrovanda vesiculosa* (AV) and *Utricularia australis* (UA). In AV turions, the proportion of molar contents of four cytokinin types (*trans*-zeatin, tZ; *cis*-zeatin, cZ; dihydrozeatin, DHZ; isopentenyladenine, iP) was very similar to that in growing shoot apices. In UA turions, however, the proportion was quite different: isopentenyladenine markedly dominated over the other three cytokinin types and the pattern mimicked that in medium-aged and old shoot segments. The molar content of indole-3-acetic acid (IAA) in AV turions was about two times lower than that in growing shoot apices but was the same in UA turions and shoot apices.

On review, knowledge of phytohormonal profiles in mature turions and namely in developing turions is rather fragmentary and mostly based on inaccurate and indirect bioassays. Thus, the aim of this study was to estimate contents of cytokinins, auxins, and ABA in growing shoot apices (controls) and gradually developing turions of two model aquatic carnivorous plant species, *A. vesiculosa* and *U. australis*, and to compare them with dark respiration and photosynthetic rates and contents of photosynthetic pigments and nitrogen (N) and phosphorus (P) in young trap-free leaves, estimated at the same time points and stages of turion development. In this way, to obtain more general ecophysiological knowledge on turion development and maturation, the role of photosynthesis for the production of

reserve substances and the extent of N and P reutilization from senescing leaves was an additional focus.

2 | MATERIALS AND METHODS

2.1 | Plant material and stages of turion development

Plants of *Aldrovanda vesiculosa* L. (AV, Droseraceae) and *Utricularia australis* R.Br. (UA, Lentibulariaceae) are submerged rootless, non-attached carnivorous plants forming distinct dormant turions in response to temperature decrease, which fully mature and detach in October–November (Adamec, 2003, 2008, 2018a). The selection of these two model species was based on the availability of dozens of individuals in a common outdoor culture and also on the many eco-physiological studies that have been conducted on these species and their turions (for review, see Adamec, 2018a, 2018b; Adamec et al., 2020, 2022). Adult plants of AV (origin from E Poland, voucher PL 0 HBT 2017.04079) and UA (from Třeboň Basin, S Bohemia, Czech Republic, voucher CZ 0 HBT 2017.04056) were grown outdoors in a 1.5 m^3 plastic container (~50 cm deep) together with *Trapa natans* and *Ceratophyllum submersum* within the Collection of aquatic and wetland plants of the Institute of Botany CAS at Třeboň, Czech Republic (Figure S1). A mixture of garden loam with acidic brown peat and milled limestone was used as substrate and tap water was used for compensation of water losses. The water in the container was considered humic (brownish), medium hard and oligotrophic. Between August 4 and September 13, 2022, the electrical conductivity of the water in the zone with AV and UA plants ranged between 21.9 and 24.1 mS m^{-1}, pH 7.3–8.7, total alkalinity 1.17–1.50 meq L^{-1}, free CO_2 concentration 5.5–172 μM, O_2 concentration (afternoon) 13.2–14.5 mg L^{-1}, NO_3^--N concentration below the detection limit, NH_4^+-N 0–4.0 μg L^{-1}, and PO_4–P 9.6–14.9 μg L^{-1}. During August and September, the container was supplied with fine zooplankton species to promote plant growth. A submersible temperature datalogger (Minikin T, EMS Brno, Czech Republic) placed ~10 cm below the surface monitored the water temperature in the container every 30 min from July 30 to October 10. The container was not shaded, and, thus, water temperature displayed marked daily oscillations mimicking those at shallow natural sites of both experimental species. The sampling of both species for turion phytohormonal profiles and simultaneously also for foliar ecophysiological traits was carried out on summer growing plants (as controls preceding turion development) on August 11 and then at three times for each species as dependent on the stages of turion development: on September 15 and 25 and October 10 in AV, and on September 4 and 13 and October 9 in UA. To characterize the temperature course, which preceded each sampling time and was attributable to development of each turion stage for each species, mean temperature and the range of daily maximum and minimum temperatures were estimated for 10 days preceding each sampling time (see Table S1). To determine when new leaf node formation had ceased, an internode below the shoot apex was labeled by a fine thread in five plants of both species (see Adamec, 2000).

Three discrete morphological stages of gradual turion development were chosen arbitrarily and defined in both species to characterize the morphological and developmental traits of turions and whole shoots (Figure 1), and the sampling times were selected accordingly. Growing experimental AV plants (Figure 1a) could be induced to form turions by temperature decrease around between September 5 and

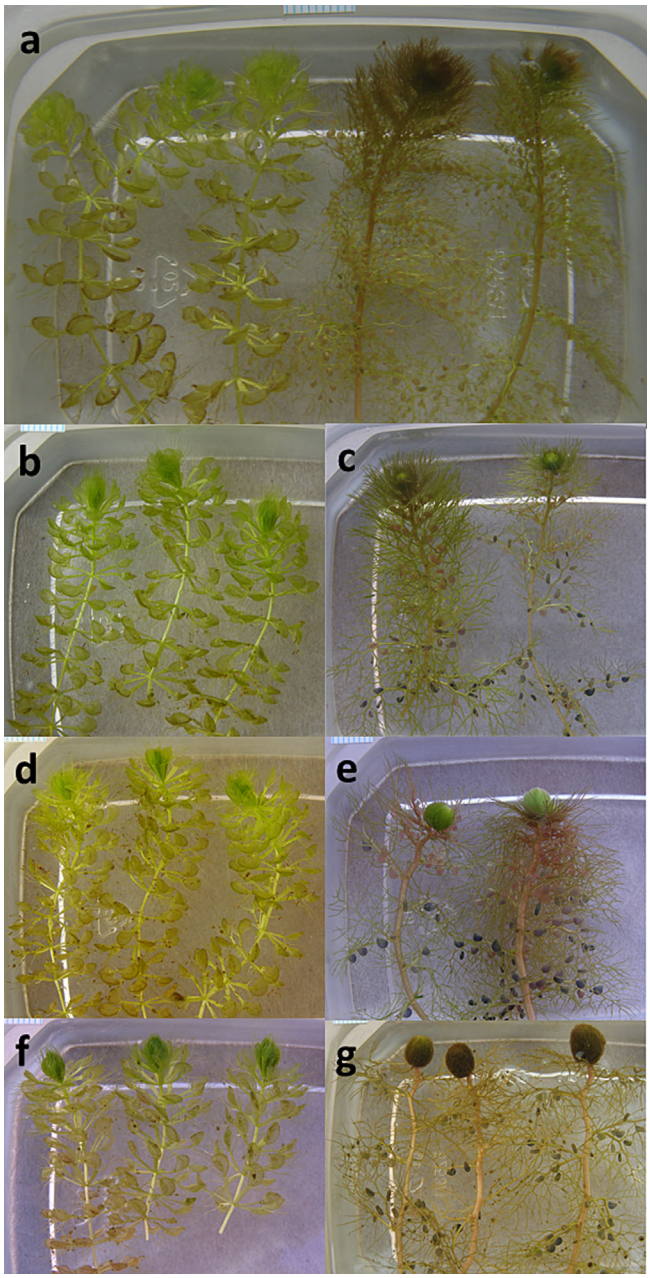

FIGURE 1 Photos of summer growing plants (a) of *Aldrovanda vesiculosa* (left) and *Utricularia australis* (right) and three arbitrary stages of turion development. (b, c) Stage I, first symptoms of turion development; (d, e) Stage II, turion ripening; (f, g) Stage III, morphological turion maturity. The ticks on the top of sections denote 1 mm. For details, see the text and Table S1.

10, while growing UA plants as early as between August 25 and 30 when the minimum water temperatures could fall to ~14°C to 16°C (Table S1).

On September 15 in AV and on September 4 in UA, the first distinct, irreversible morphological changes initiated the turion development in each species (Stage I, Figure 1b,c). A rhomboid-shaped structure starts appearing in AV in shoot apex and is more condensed than that in summer growing plants. There is a sharp transition between the developed leaves and the developing turion and the formation (differentiation) of new leaf nodes stopped. AV plants at Stage I still possess their maximum shoot length but they start shortening afterwards. In UA on September 4, the formerly diffusive shoot apex formed relatively small spherical turion structure, which is light yellow-green at the apical end and contains unmature, compacted leaf nodes with miniature traps at its base; the young leaf nodes still give rise to new functional leaves with traps. The shoot length is not reduced.

On September 25 in AV, the chosen stage II exhibited more condensed rhomboid-shaped structures, but the developing turions were relatively narrow (Figure 1d). As the formation of young leaf nodes stopped and due to senescence and decay of basal shoot segments, the shoots shortened. On September 13 in UA, the analogous stage II was associated with the formation of immature, spherical, yellow-green turions of nearly final size (Figure 1e). Unlike Stage I, developing turions in Stage II are not partly surrounded by young leaves but are separated from them and are clearly gelatinous. The youngest leaf nodes with leaves bearing functional traps still partly grow and their internodes lengthen. However, due to the decay of basal shoot segments, shoot length decreases. Traps in older shoot segments are senescent and detach from the leaves.

At Stage III (AV on October 10 and UA on October 9), turions of both species were morphologically identical with quite mature turions but were physiologically immature (Figure 1f,g). In both species, they were still firmly attached to mother shoots, which exhibited clear symptoms of senescence and decay of basal shoot segments leading to marked shoot shortening; the symptoms of senescence were apparent also in younger shoot segments. In UA, the majority of mature traps were detached, and only the youngest ones remained. The color of UA turions changed to dark green-brown due to anthocyanin production. It can be assumed that a good deal of organic substances and some minerals (N, P) are reutilized (resorbed) from senescent shoots to maturating turions in both species at Stages II and III and afterwards (Adamec, 2000, 2018a).

2.2 | Gasometric measurements and processing of experimental material

To obtain information on the intensity of metabolism and photosynthetic capacity of young shoot segments bearing developing turions at the three stages, and in summer growing controls (see Figure 1 and Table S1), RD and P_N were measured. The standard process was to select two young shoot segments (with 3rd to 5th mature leaf nodes) in AV or young leaves (from 5th to 8th mature leaf nodes) of one plant in UA; the adult control AV plants were 10–14 cm long and those of UA plants 35–60 cm long. In both species, all traps were excised (Adamec, 2013). The freshly sampled plant material was thoroughly washed in tap water and cleaned by a pair of forceps of all sessile organisms. The FW of measured shoots/leaves was 25–40 mg.

Generally, oxygen-based RD and P_N were measured in a solution of .9 mM $NaHCO_3$ with .1 mM KCl (~80–90% O_2 saturation) in a 5.3-ml stirred, thermostatted chamber at 22.0 ± .1°C. We chose this temperature as it was commonly attained in the container by mid-September (Table S1). A Clark-type O_2 sensor and a chart recorder (for all details, see Adamec, 2003) were used. The initial pH was set to 6.92–6.93 using CO_2 and corresponded to free [CO_2] of .25 mM (Helder, 1988). It was chosen as a standard as it has been used frequently in similar studies (e.g., Adamec, 2011, 2013); it also commonly occurs at the natural sites of these species and approaches that for attaining the maximum P_N (Adamec, 2013). First, RD was measured in darkness for 15 min, then the chamber was irradiated at 400 µmol m^{-2} s^{-1} PAR (20 W halogen reflector; Adamec, 2011, 2013), and P_N was measured for ~15 min. FW was then estimated for all measured material, which was used for the determination of photosynthetic pigments (chlorophyll *a*, *b*, carotenoids) after Lichtenthaler (1987). All measurements were repeated six times with material from different plants. RD and P_N are expressed in mmol kg^{-1} h^{-1} per unit FW.

Moreover, freshly excised shoot apices (developing turions) from plants (~5–7 mm large, 1–2 apices per sample) were thoroughly washed in tap water, rinsed in distilled water, blotted dry on soft paper tissue, placed in 2 ml pre-weighed Eppendorf vials, weighed for FW, and stored in a freezer (approximately −26°C) for several weeks before lyophilization. After lyophilization, the vials were weighed again; DW ranged between 2 and 8 mg. Four parallel samples from different plants were always prepared for phytohormone analyses. At the same sampling times for both species (Table S1), DMC (i.e., DW/FW in %; 80°C) was estimated in one mixed sample of five shoot apices (turions) from other parallel plants and in trap-free shoot segments (from two plants in AV) or leaves (from one plant in UA), in the same positions as those for gasometric measurements (see above). First, the sampled material was thoroughly cleaned, washed in tap water, and rinsed with distilled water. After estimation of FW and DW (for DMC), the dry material (five parallels, each 2.5–3.5 mg) was stored in Eppendorf vials and used for estimation of tissue N and P content. Means ± SE intervals are shown where possible.

2.3 | Analytical procedures

Nutrients (NO_3^-–N, NH_4^+–N, PO_4–P) in filtered water samples from the cultivation container were analyzed colorimetrically by an automatic FIAstar 5010 Analyzer (Tecator, Sweden). Dry leaves/shoots were digested and mineralized by concentrated acids, and after dilution, tissue N and P content was estimated by the FIAstar 5010 Analyzer (for all analytical details, see Kučerová & Adamec, 2022).

2.4 | Phytohormone analyses

Lyophilized plant material from each sample was weighed into three technical replicates for both cytokinin and auxin analyses. Extraction and purification of cytokinins and auxins together with ABA were performed using the same methods as described in Adamec et al. (2022). Namely, an in-tip μSPE with three different layers of stationary phases (C18, SDB-RP and Cation AttractSPE Disks) was used to purify cytokinin samples, and auxin and ABA samples were purified using an HLB cartridge (Oasis®, HLB 1 cc [30 mg], Waters). Samples were analyzed by ultraperformance liquid chromatography (Aquity UPLC® I-class system; Waters, Milford, MA, United States) coupled to a triple quadrupole mass spectrometer equipped with an electrospray interface (Xevo TQ-S, Waters, Manchester, United Kingdom) by a method described in Svačinová et al. (2012) for the determination of cytokinins and in Pěnčík et al. (2009) for auxins and ABA. Quantification was obtained by multiple reaction monitoring of $[M + H]^+$ and the appropriate product ion. Optimal conditions, dwell time, cone voltage, and collision energy were optimized for each cytokinin (Novák et al., 2008; Svačinová et al., 2012) and auxin metabolite (Pěnčík et al., 2009). Quantification was performed by MassLynx software using a standard isotope dilution method (Tables S2 and S3).

2.5 | Statistical analyses

The statistically significant differences in P_N, RD, pigment contents, DMC and in tissue N and P content in measured shoots or leaves among summer growing plants and the three developmental stages I–III (see Figure 1) were analyzed using one-way ANOVA, and the Tukey HSD test was used for multiple comparisons. The linear relationship between P_N, RD, chlorophyll $a + b$ (chl. $a + b$) content, and N and P content in shoots/leaves in each species in summer growing plants and the three developmental turion stages were tested by linear regression. Within each variant, sums of contents for the four CK types (*cis*-, *trans*-, dihydrozeatin, and isopentenyladenine types) in nmol kg^{-1} (DW) as well as total CK contents and active forms (sum of free bases and their ribosides; Adamec et al., 2022) were estimated for each sample. Active forms of CKs and auxins (as % of the total content) were also calculated, and the results are expressed as means ± S.E. interval for $n = 4$ independent samples. As there was no indication that the distribution of the data was non-normal, log-transformation of the data was not performed. The statistically significant differences in different CK or auxin forms between the developmental stages were evaluated by one-way ANOVA, and the Tukey HSD test was used for multiple comparisons.

3 | RESULTS

Induced shoot apices in both experimental species were maturating during the expected course of decreasing mean, minimum, and maximum temperatures in September and October (Table S1) but the turions fully maturated and detached as late as the end of October (UA) or at the beginning of November (AV), when the minimum water temperature reached to 4°C to 6°C.

The aim of the investigation of young trap-free leaves/shoots of both species was to find changes of some physiological markers during turion development. Gasometric measurements revealed some differences in young leaves/shoots between both species. In AV, the shoot RD decreased significantly from that in the controls as early as Stage I and then was constant, while in UA, the foliar RD decreased significantly as late as Stage II (Figure 2a). Consistently in both species, P_N values in the same young organs were maximal in the summer growing controls and at turion Stage I and were declining by 44–59% at further Stages II and III (Figure 2b). The shoot content of chl. $a + b$ was the highest or the same at Stage I during turion development in AV but was almost constant from the summer controls to Stage II in UA and declined afterwards (Figure 2c,d). In both species, the content of carotenoids was the same during turion development. The shoot N and P contents in AV were the highest in plants at turion Stage II (Figure 3). However, in UA, the foliar N and P content was significantly the highest in growing control plants and declined consistently during next turion development. Shoot DMC mildly increased at Stage I and then was constant in AV, while it decreased at Stage III in UA leaves (Figure 4). Taken together, all results indicate consistently that in UA, young leaves enter senescence during turion maturation earlier and their N and P stores are reutilized and allocated to maturating turions more effectively than in AV. In both species, the DMC increased gradually from ~11% in the apices of summer growing plants to ~26% in Stage III turions (Figure 4). In AV shoots, no significant linear correlation was found between P_N and chl. $a + b$ ($r = .32$, $p = .14$, $n = 23$) and between P_N and RD during turion development ($r = .15$, $p = .49$, $n = 23$); however, both correlations were significant in UA leaves ($r = .80$, $p = .0001$, $n = 23$; $r = .74$, $p = .0001$, $n = 23$, respectively). In both species, P_N correlated with neither N nor P contents in shoots/leaves.

Cytokinin profiles in shoot apices developing from summer growing plants to mature turions revealed considerable differences between both species (Tables 1, S2, and S3). In AV, the total content of CKs (tZ, cZ, DHZ, and iP) as well as of biologically active forms (free bases + ribosides) did not differ significantly between shoot apices of summer growing plants and developing or mature turions. The prevailing CK types in AV developing turions were tZ and DHZ. The content of tZ culminated at Stage II and was significantly the lowest in mature turions, while that of DHZ was gradually rising during the turion development; in contrast, the iP content was steeply decreasing. Surprisingly, in AV apices, the proportion of active CK forms was relatively constant (55–73%) during turion development. In UA, the content of both tZ and DHZ decreased significantly at Stage III and in mature turions, while the trends of the cZ and iP contents were ambiguous and non-significant (Table 1); iP was always the dominant CK type. The total CK content was relatively stable, but that of active forms was gradually decreasing from 148 ± 15 nmol kg_{dw}^{-1} in summer growing plants to 10 ± .1 nmol kg_{dw}^{-1} in mature turions. Therefore, the percentage of active CK forms was markedly decreasing

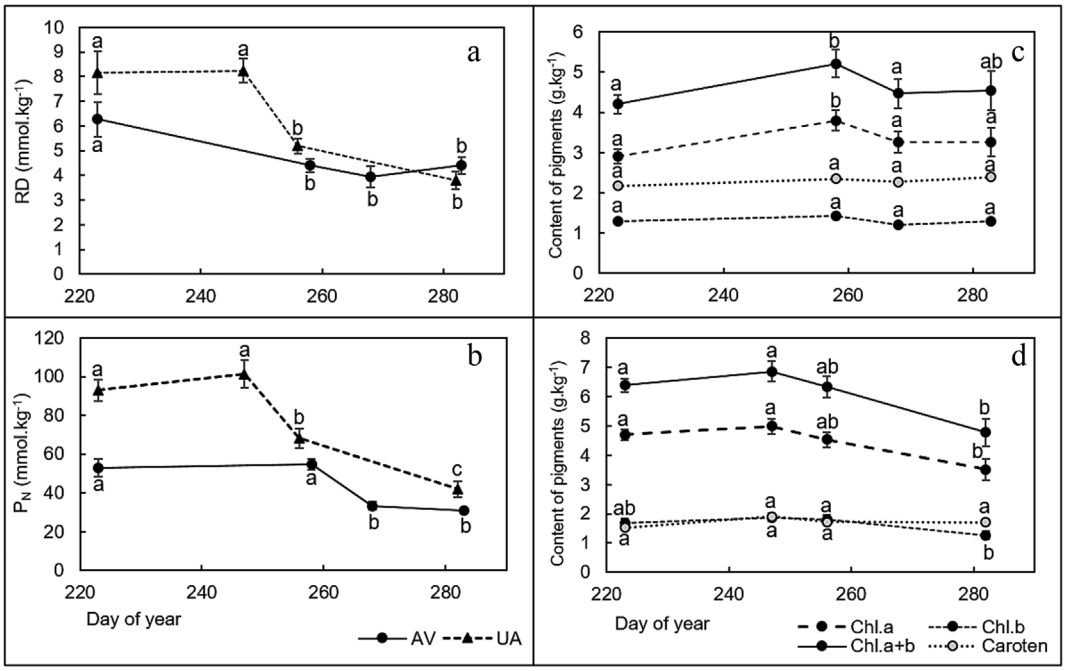

FIGURE 2 FW-based dark respiration (a) and net photosynthetic rate (b) of young leaves/shoots of *A. vesiculosa* and *U. australis* in summer growing plants and developing turions of the three stages. DW-based contents of photosynthetic pigments in young leaves/shoots of *A. vesiculosa* (c) and *U. australis* (d) in summer growing plants and developing turions. Means ± SE intervals are shown, $n = 6$. Different letters for one parameter and species denote statistically significant difference at $P < .05$. Day of year 223 is August 11 (start of the experiment).

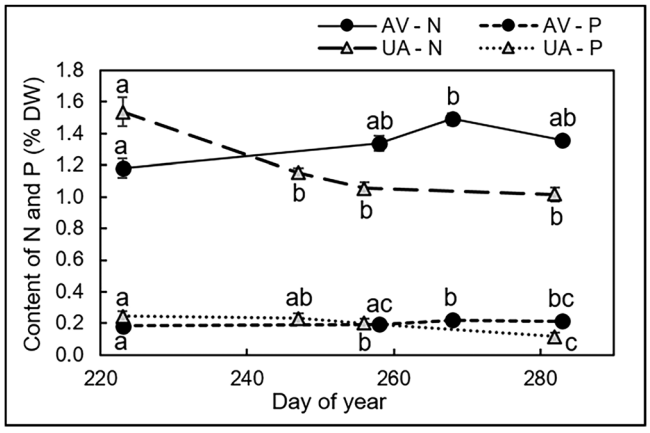

FIGURE 3 Content of N or P in young leaves/shoots of *A. vesiculosa* and *U. australis* in summer growing plants and developing turions of the three stages. Means ± SE intervals, $n = 5$. Different letters for one parameter and species denote statistically significant difference at $P < .05$. Day of year 2023 is August 11 (start of the experiment).

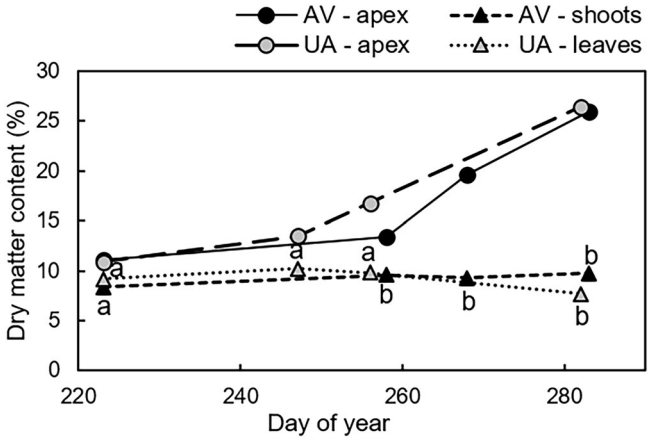

FIGURE 4 Dry matter content of shoot apices and young leaves/shoots in summer growing plants and developing turions of the three stages in *A. vesiculosa* and *U. australis*. Means ± SE intervals are shown for leaves/shoots, $n = 4$. Different letters for one parameter and species denote statistically significant difference at $P < .05$. Day of year 223 is August 11 (start of the experiment).

during turion development (from 7.0 to .48%). In UA, the content of active CK forms was thus 3.4–40 times lower than that in AV at the same developmental stages even though the total CK contents prevailed 2–3.6 times in UA.

Auxin profiles in shoot apices developing from summer growing plants to mature turions in both species markedly differed from CK profiles (Tables 2, S2, and S3). In AV, the contents of the four auxin forms—indole-3-acetic acid (IAA), 2-oxindole-3-acetic acid (oxIAA), indole-3-acetyl aspartic acid (IAAsp), and indole-3-acetyl-β-1-O-D-glucose (IAGlu)—and their total content were significantly the lowest (except for IAGlu) either in developing turions at stage III or in mature turions. The IAGlu content was always minimal and below the limit of determination. The proportion of IAA as the only active auxin form to the total auxin content was increasing from summer growing

TABLE 1 Cytokinin contents (in nmol kg_{dw}^{-1}) in summer growing shoot apices (controls), at three developmental stages of turions and in mature turions of *A. vesiculosa* and *U. australis*

	Aldrovanda vesiculosa					*Utricularia australis*				
Cytokinin	Controls	Stage I	Stage II	Stage III	Mature	Controls	Stage I	Stage II	Stage III	Mature
∑tZ	325^{ab}	435^{ab}	735^{a}	472^{ab}	209^{b}	558^{a}	361^{ab}	522^{ab}	195^{b}	211^{b}
SE	37	137	134	18	10	24	44	131	46	38
∑cZ	72	98	59	47	41	193^{ab}	140^{a}	165^{ab}	358^{b}	321^{ab}
SE	3	26	8.3	9.2	.8	34	16	9	79	37
∑DHZ	98^{a}	138^{ac}	273^{bc}	309^{b}	353^{b}	161^{ad}	271^{bd}	324^{b}	61^{ac}	29^{ac}
SE	12	42	43	4.7	7.1	13	26	64	8.7	9.8
∑iP	192^{a}	95^{b}	79^{b}	29^{bc}	2.4^{c}	$1,192^{ab}$	662^{a}	$1,339^{b}$	$1,529^{b}$	$1,666^{b}$
SE	19	14	20	2	.2	62	89	127	222	71
∑total	**686**	**766**	**1,146**	**856**	**605**	**2,104**	**1,435**	**2,350**	**2,143**	**2,227**
SE	60	214	197	21	14	102	154	281	355	123
∑active	**503**	**568**	**751**	**473**	**405**	**148^{a}**	**80^{b}**	**20^{c}**	**20^{c}**	**10^{c}**
SE	46	165	125	11	13	15	8.3	3.7	6.1	.08
Active (%)	73	73	66	55	67	7	5.7	.82	.85	.48

Note: The total cytokinin content and active forms are labeled in bold. Statistically significant difference at $p < .05$ between different developmental stages within each row for each cytokinin type within each species is labeled by different letters. The letters are absent at $P > .05$; $n = 4$.

TABLE 2 Auxin and ABA contents (in nmol kg_{dw}^{-1}) in summer growing shoot apices (controls), at three developmental stages of turions and in mature turions of *A. vesiculosa* and *U. australis*

	Aldrovanda vesiculosa					*Utricularia australis*				
Auxin	Controls	Stage I	Stage II	Stage III	Mature	Control	Stage I	Stage II	Stage III	Mature
IAA	$1,012^{a}$	687^{ac}	2067^{b}	632^{ac}	3.3^{c}	$3,315^{a}$	$9,891^{b}$	$6,783^{ab}$	$5,712^{ab}$	$2,531^{a}$
SE	51	88	268	313	0	400	2,055	819	703	400
oxIAA	$175,161^{a}$	$161,989^{ab}$	$100,603^{b}$	$14,835^{c}$	$20,457^{c}$	$3,596^{a}$	$3,718^{a}$	997^{b}	33^{b}	33^{b}
SE	7,079	28,883	6,202	2,024	2,173	223	427	279	0	0
IAAsp	$62,081^{a}$	$30,922^{b}$	$16,882^{bc}$	$2,175^{c}$	$3,617^{c}$	$16,474^{a}$	$6,844^{b}$	$2,555^{c}$	33^{c}	33^{c}
SE	10,529	2,001	1,666	160	479	1,776	534	293	0	0
IAGlu	33^{a}	33^{a}	33^{a}	33^{a}	33^{a}	$4,223^{a}$	$2,258^{b}$	$1,709^{b}$	33^{c}	33^{c}
SE	0	0	0	0	0	574	283	96	0	0
∑total	**$238,287^{a}$**	**$193,632^{ab}$**	**$119,585^{b}$**	**$17,676^{c}$**	**$24,111^{c}$**	**$27,608^{a}$**	**$22,710^{a}$**	**$12,043^{b}$**	**$5,812^{bc}$**	**$2,631^{c}$**
SE	13,031	29,726	4,897	2,258	1,999	2,953	2,380	808	703	400
IAA (%)	**.43**	**.37**	**1.7**	**3.2**	**.014**	**12**	**42**	**56**	**98**	**96**
ABA	33^{a}	$1,431^{a}$	$5,815^{b}$	$6,018^{b}$	$13,204^{c}$	33^{a}	33^{a}	$3,097^{b}$	$8,553^{c}$	$5,129^{d}$
SE	0	703	136	236	1,200	0	0	550	370	476

Note: The total auxin content and proportion of IAA as the only active form are labeled in bold. Statistically significant difference at $p < .05$ between different developmental stages within each row for each auxin type or ABA within each species is labeled by different letters. The letters are absent at $P > .05$; $n = 4$.

plants, culminated significantly at Stage III but was followed by a steep decline below the limit of determination in mature turions. Overall, in AV, the IAA proportion was only within .014–3.2%. The ABA content in AV shoot apices was below the limit of detection in summer growing plants but was gradually rising—the rise started at Stage I and culminated in mature turions (13,204 ± 1,200 nmol kg_{dw}^{-1}; Table 2).

In UA shoot apices during turion development, auxin profiles (both contents and trends) differed considerably from those in AV (Table 2). In UA, IAA and IAAsp were the dominant auxin forms, at least in the growing plants and at Stages I and II. The IAA content peaked significantly at Stage I and was then decreasing back to the level found in summer growing plants in mature turions. Both oxIAA, IAAsp, and IAGlu exhibited the highest contents in summer growing

plants, the contents were decreasing steeply during the next three developmental stages so that at Stage III, and in mature turions, their contents were below the limit of detection. The total auxin content decreased about 10 times between summer growing plants and mature turions. Due to the relatively high IAA content in all developmental stages (2,531–9,891 nmol kg_{dw}^{-1}) in UA shoot apices, and also due to simultaneous declining of the total auxin content, the IAA proportion to the total auxin content was gradually rising from 12% in summer growing plants to 96–98% at the two last stages of turion development. The ABA content in UA shoot apices was below the limit of detection in summer growing plants and also at Stage I but was greatly increased in all the following developmental stages (3,097–8,553 nmol kg_{dw}^{-1}) and culminated at stage III (Table 2).

4 | DISCUSSION

The main aim of the paper was to find changes in phytohormone profiles in shoot apices of two aquatic carnivorous plants during the gradual transition from the summer growth to mature, innately dormant turions. It should be noted that once a plant is induced by environmental factors to form turions, this developmental stage is irreversible by changing the environmental factors and has the character of the all-or-none rule (Adamec, 2018a; Winston & Gorham, 1979a, 1979b). As turions represent quite contrasting organs (both morphologically and physiologically) in comparison to summer growing shoot apices, it can be expected that the transition between both "stable" states shall include profound changes in the profiles of phytohormones such as CKs, auxins, and ABA in the developing shoot apices towards future turions, but also in the remaining, dying annual shoots (Adamec, 2018a; Šimura et al., 2016; Winston & Gorham, 1979b). The same conclusion can also be drawn from external applications of ABA or cytokinin kinetin to some aquatic plants (mainly *Spirodela polyrhiza*) to induce turion formation or to revert it (Chaloupková & Smart, 1994; Smart et al., 1995; Smart & Trewavas, 1983; Weber & Noodén, 1976) and also from first genomic or transcriptomic studies on turions (Li et al., 2022; Pasaribu et al., 2023; Wang et al., 2014).

We have chosen three discrete arbitrary stages of immature turion development (Figure 1 and Table S1) in order to subdivide the gradual turion development into three separate periods and/or processes. These stages include the gradual switch from the summer unterminated, continuous shoot growth to shoot growth cessation and are characterized by a marked and gradual rise in the DMC in the developing turions. The shortest period between two successive stages was 9 days; this might be long enough to expect marked changes both in young trap-free shoots/leaves (metabolic processes) and in phytohormone profiles in developing turions. Although the chosen three stages were comparable morphologically in both species, there was a great difference in the cessation of growth of new leaf nodes between both species: in *A. vesiculosa*, the growth ceased as early as at Stage I, while it continued partly even at Stage II in *U. australis*.

P_N and RD in young shoots/leaves were the highest in summer growing plants and at stage I in both species (Figure 2a,b), and the values of both parameters were comparable with those measured in both species in summer growing plants (cf. Adamec, 1997, 2013). Afterwards, P_N, RD, and also chl. $a + b$ content decreased more steeply in UA than in AV. A quite opposite pattern of tissue N and P content in senescing leaves/shoots between both species (Figure 3) indicates a very low N and P reutilization efficiency in AV as compared to that in UA during turion development, although Adamec (2000) suggested a very effective N and P reutilization in AV during turion development. The relatively high P_N and RD values in young leaves of both species during all stages of turion maturation may confirm that supply of carbohydrates as future reserve substances to maturating turions from photosynthesis of young leaves/shoots is much more important than reutilization of sugars from senescing shoots and that young leaves are still metabolically very active, even at the last stages of turion development without any new leaf growth (cf. Adamec, 1997, 2000, 2013). In AV, there is no apparent relationship between the time-course of P_N, RD, pigment, and N and P content in young shoots and the turion content of the inhibitory ABA. While in young UA leaves, the time-course of photosynthetic pigments and P_N and RD agreed with that of the turion ABA content.

Numerous reports describe the role of phytohormones in plant development (Davies, 2004); however, cytokinins and auxins in storage organs (except for roots) have been studied only a few times even in land plants; to our knowledge, no studies on these phytohormones in storage organs in aquatic plants have been published so far. Kara et al. (1997) studied the distribution of phytohormones in the roots of radish plants and concluded that both IAA and CKs were involved in the initiation and formation of the storage organ (regardless of light quality). Moreover, CKs appeared also to stimulate the assimilate flow to developing storage tissues (Kara et al., 1997). Similar results were obtained for changes in endogenous levels during the growth cycle of *Curcuma alismatifolia* (Hongpakdee et al., 2010). Off-season conditions induced a decrease in the photosynthetic rate and increases in ABA and tZR contents in various organs at different growth stages, leading to depressed shoot growth and an increase in rhizome numbers.

Our results confirmed a mild, transient increase of the tZ type and of total and active CKs in the middle of turion development in AV. In UA turions, the contents of all active CK forms gradually but markedly decreased though the total content was nearly constant (Table 1). It is evident that developing and maturating turions in aquatic plants represent organs possessing a very strong sink for both mineral nutrients and organic reserve substances, similar to seeds (e.g., Gonzalez-Lemes et al., 2023; Liang et al., 2023). In line with this, it is well known that CKs function in the regulation of sink–source relationships, both in source and sink organs, and in the activation of the genetic program of development (Ron'zhina, 2009). They stimulate the source function of leaves by stimulating leaf expansion, increasing net photosynthesis and by changing the balance between transportable and storage forms of photoassimilates (increase of sucrose and reduction of starch synthesis). Moreover, CKs also stimulate the sink strength and the activity and incorporation of soluble

organic substances into insoluble polymeric compounds (starch, structural polysaccharides, and proteins).

A number of studies have confirmed that IAA content is extremely important for plant development and is influenced by the rate of its biosynthesis, transport, and metabolic inactivation (Kondhare et al., 2021). In several tuber and storage-root crops, a common trend has been observed: IAA contents in tubers or developing storage roots are high during the early developmental stages of the belowground storage organs so that they could induce cell divisions. However, IAA contents drop during the later stages of development suggesting that high IAA content is essential only for the onset of storage organ formation, whereas its low content is required during the late processes of storage organ thickening (Kondhare et al., 2021). Our results confirmed a very similar trend in aquatic carnivorous plants: the content of free IAA as the only active auxin form is increasing gradually during turion development up to a certain stage, reaching the peak contents at Stage II in AV and at Stages I and II in UA, and then dropping in mature turions (in AV down to zero, Table 2). On the other hand, the contents of inactive auxin metabolites are decreasing consistently in both species during turion development. However, this is the first report on the role of auxins in turion development and should be confirmed by subsequent studies.

The crucial role of another phytohormone, ABA, in turion development mainly in *S. polyrhiza* has been known for a long time (e.g., Li et al., 2022; Smart et al., 1995; Smart & Trewavas, 1983; Wang et al., 2014; Weber & Noodén, 1976; Winston & Gorham, 1979b). Smart et al. (1995) quantified ABA contents in normally growing *S. polyrhiza* fronds and in those induced to form turions by an exogenous ABA supply. The comparison of these values allowed the authors to judge whether the frond ABA content associated with turion induction after the ABA supply could be attained by an endogenous synthesis and accumulation of ABA. However, the susceptibility of turion induction by an ABA supply may be rather low in more robust aquatic plants (cf. Best, 1979; Weber & Noodén, 1976). In a novel transcriptomic study on *S. polyrhiza* turions formed after ABA treatment, Wang et al. (2014) confirmed profound changes in gene expression in turions. In turions, a total of 208 genes exhibited four times more increased expression as compared to growing fronds, while 154 genes exhibited markedly reduced expression. In this species, turion formation thus represents very complex developmental changes on the genomic level comparable, e.g., with flowering (see also Pasaribu et al., 2023).

The formation of bulbils, considered another type of storage organ, is an important agronomic trait also found in yams. In a landrace of water yam (*Dioscorea alata*), which rarely forms bulbils, Hamaoka et al. (2023) investigated the effect of ABA on bulbil formation on the basis of changes in the sink–source relationships in response to a waterlogging stress. ABA treatment of leaf axils enhanced bulbil formation in unstressed plants, suggesting that increased ABA content is one of the factors that initiate bulbil formation. Also in our study, the ABA content increased from almost zero by 2 orders of magnitude during turion development and culminated in nearly mature or mature turions in both species (Table 2), thus confirming the central role of ABA in turion development and maintaining of the innate dormancy. In developing turions in UA at Stage I though, the ABA content was still nearly zero (Figure 1 and Table 2). It may indicate that increase of endogenous ABA content is not regulated by the turion induction alone but can occur at more advanced developmental stages (Stage II) before the growth of new leaves has ceased.

Overall, the data obtained in our study fully confirmed the importance of three phytohormone classes (ABA, auxins, and CKs) in the regulation of developmental stages of turions as overwintering and storage organs in two unrelated species of aquatic carnivorous plants. However, our results based on analyzing the endogenous contents of the phytohormones in developing turions should be confirmed by future studies based, for example, on exogenous treatment by phytohormones or comparing the hormone profiles in true dormant turions and non-dormant winter shoot apices in some relative species or elucidating the hormonal cross-talk between gradually developing turions and senescing leafy shoots. These further experiments should confirm, for example, the role of cytokinins and other plant hormones in regulation of sink-source relationships during turion development as well as their cross-talk, as these mechanisms were previously studied only in land but not yet aquatic plants. As *Aldrovanda* and *Utricularia* spp. shoots exhibit a marked physiological and growth polarity and their growing shoot apices represent a strong sink for N, P and organic substances (Adamec, 2018b), one can also ask whether the sink–source relationships in developing turions are the same as those in growing shoots.

AUTHOR CONTRIBUTIONS

Lubomír Adamec conceived the study, conducted the experiments, analyzed the data, and co-wrote the manuscript; Lenka Plačková analyzed the phytohormones, evaluated graphically the data, and co-wrote the manuscript; Karel Doležal discussed the design of all experiments and all data and co-wrote the manuscript.

ACKNOWLEDGMENTS

Thanks are due to the Hortus Botanicus Třeboň, Department of Experimental Garden and Collection of Aquatic and Wetland Plants, Institute of Botany of the Czech Academy of Sciences, Třeboň, Czech Republic, for providing the experimental plants. We thank Dr. Brian G. McMillan (Glasgow, Scotland) for English correction.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest in relation to this work.

DATA AVAILABILITY STATEMENT

The detailed data supporting the findings of this study are available from the corresponding author, Karel Doležal, upon request.

ORCID

Lubomír Adamec https://orcid.org/0000-0001-5544-2402
Karel Doležal https://orcid.org/0000-0003-4938-0350

REFERENCES

Adamec, L. (1997). Photosynthetic characteristics of the aquatic carnivorous plant *Aldrovanda vesiculosa*. *Aquatic Botany*, *59*, 297–306. https://doi.org/10.1016/S0304-3770(97)00054-5

Adamec, L. (2000). Rootless aquatic plant *Aldrovanda vesiculosa*: Physiological polarity, mineral nutrition, and importance of carnivory. *Biologia Plantarum*, *43*, 113–119. https://doi.org/10.1023/A:1026567300241

Adamec, L. (2003). Ecophysiological characterization of dormancy states in turions of the aquatic carnivorous plant *Aldrovanda vesiculosa*. *Biologia Plantarum*, *47*, 395–402.

Adamec, L. (2008). Respiration of turions and winter apices in aquatic carnivorous plants. *Biologia*, *63*, 515–520. https://doi.org/10.2478/s11756-008-0073-4

Adamec, L. (2010). Tissue mineral nutrient content in turions of aquatic plants: Does it represent a storage function? *Fundamental and Applied Limnology*, *176*, 145–151. https://doi.org/10.1127/1863-9135/2010/0176-0145

Adamec, L. (2011). Dark respiration and photosynthesis of dormant and sprouting turions of aquatic plants. *Fundamental and Applied Limnology*, *179*, 151–158. https://doi.org/10.1127/1863-9135/2011/0179-0151

Adamec, L. (2013). A comparison of photosynthetic and respiration rates in six aquatic carnivorous *Utricularia* species differing in morphology. *Aquatic Botany*, *111*, 89–94. https://doi.org/10.1016/j.aquabot.2013.06.004

Adamec, L. (2018a). Ecophysiological characteristics of turions of aquatic plants: A review. *Aquatic Botany*, *148*, 64–77. https://doi.org/10.1016/j.aquabot.2018.04.011

Adamec, L. (2018b). Ecophysiology of aquatic carnivorous plants. In A. M. Ellison & L. Adamec (Eds.), *Carnivorous plants: Physiology, ecology, and evolution* (pp. 256–269). Oxford University Press.

Adamec, L., Kučerová, A., & Janeček, Š. (2020). Mineral nutrients, photosynthetic pigments and storage carbohydrates in turions of 21 aquatic plant species. *Aquatic Botany*, *165*, 103238. https://doi.org/10.1016/j.aquabot.2020.103238

Adamec, L., Plačková, L., & Doležal, K. (2022). Cytokinins and auxins in organs of aquatic carnivorous plants: What do they reflect? *Annals of Botany*, *130*, 869–882. https://doi.org/10.1093/aob/mcac122

Bartley, M. R., & Spence, D. H. N. (1987). Dormancy and propagation in helophytes and hydrophytes. *Archiv für Hydrobiologie (Beiheft)*, *27*, 139–155.

Best, E. P. H. (1979). Growth substances and dormancy in *Ceratophyllum demersum*. *Physiologia Plantarum*, *45*, 399–406. https://doi.org/10.1111/j.1399-3054.1979.tb02603.x

Chaloupková, K., & Smart, C. C. (1994). The abscisic-acid induction of a novel peroxidase is antagonized by cytokinin in *Spirodela polyrhiza* L. *Plant Physiology*, *105*, 497–507. https://doi.org/10.1104/pp.105.2.497

Davies, P. J. (2004). *Plant hormones: Biosynthesis, signal transduction, action*. Springer.

Gonzalez-Lemes, I., Acosta-Maspons, A., Cetz-Chel, J. E., Polania, J. A., Acosta-Gallegos, J. A., Herrera-Estrella, A., & Covarrubias, A. A. (2023). Carbon-concentrating mechanisms in pods are key elements for terminal drought resistance in *Phaseolus vulgaris*. *Journal of Experimental Botany*, *74*, 1642–1658. https://doi.org/10.1093/jxb/erac504

Hamaoka, N., Moriyama, T., Taniguchi, T., Suriyasak, C., & Ishibashi, Y. (2023). Bulbil formation on water yam (*Dioscorea alata* L.) is promoted by waterlogged soil. *Agronomy-Basel*, *13*, 484. https://doi.org/10.3390/agronomy13020484

Helder, R. J. (1988). A quantitative approach to the inorganic carbon system in aqueous media used in biological research: Dilute solutions isolated from the atmosphere. *Plant, Cell & Environment*, *11*, 211–230. https://doi.org/10.1111/j.1365-3040.1988.tb01140.x

Hongpakdee, P., Siritrakulsak, P., Ohtake, N., Sueyoshi, K., Ohyama, T., & Ruamrungsri, S. (2010). Changes in endogenous abscisic acid, transzeatin riboside, indole-3-acetic acid levels and the photosynthetic rate during the growth cycle of curcuma alismatifolia gagnep. in different production seasons. *European Journal of Agricultural Sciences*, *75*, 204–213.

Hussain, S., Nanda, S., Zhang, J. H., Rehmani, M. I. A., Suleman, M., Li, G. J., & Hou, H. W. (2021). Auxin and cytokinin interplay during leaf morphogenesis and phyllotaxy. *Plants*, *10*, 1732. https://doi.org/10.3390/plants10081732

Kara, A. N., Kotov, A. A., & Bukhov, N. G. (1997). Specific distribution of gibberellins, cytokinins, indole-3-acetic acid, and abscisic acid in radish plants closely correlates with photomorphogenetic responses to blue or red light. *Journal of Plant Physiology*, *151*, 51–59. https://doi.org/10.1016/S0176-1617(97)80035-1

Kondhare, K. R., Patil, A. B., & Giri, A. P. (2021). Auxin: An emerging regulator of tuber and storage root development. *Plant Science*, *306*, 110854. https://doi.org/10.1016/j.plantsci.2021.110854

Kučerová, A., & Adamec, L. (2022). Foliar resorption efficiency does not change along an elevational gradient in two dominant peatbog plant species. *Folia Geobotanica*, *57*, 247–257. https://doi.org/10.1007/s12224-023-09427-4

Li, G., Chen, Y., Zhao, X., Yang, J., Wang, X., Li, X., Hu, S., & Hou, H. (2022). Genome-wide analysis of the growth-regulating factor (GRF) family in aquatic plants and their roles in the ABA-induced turion formation of *Spirodela polyrhiza*. *International Journal of Molecular Sciences*, *23*, 10485. https://doi.org/10.3390/ijms231810485

Liang, G. H., Hua, Y. P., Chen, H. F., Luo, J. S., Xiang, H. K., Song, H. X., & Zhang, Z. H. (2023). Increased nitrogen use efficiency via amino acid remobilization from source to sink organs in *Brassica napus*. *Crop Journal*, *11*, 119–131. https://doi.org/10.1016/j.cj.2022.05.011

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids. Pigments of photosynthetic biomembranes. *Methods in Enzymology*, *148*, 350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Novák, O., Hauserová, E., Amakorová, P., Doležal, K., & Strnad, M. (2008). Cytokinin profiling in plant tissues using ultra-performance liquid chromatography-electrospray tandem mass spectrometry. *Phytochemistry*, *69*, 2214–2224. https://doi.org/10.1016/j.phytochem.2008.04.022

Pasaribu, B., Acosta, K., Aylward, A., Liang, Y. X., Abramson, B. W., Colt, K., Hartwick, N. T., Shanklin, J., Michael, T. P., & Lam, E. (2023). Genomics of turions from the greater duckweed reveal its pathways for dormancy and re-emergence strategy. *New Phytologist*, *239*, 116–131. https://doi.org/10.1111/nph.18941

Pěnčík, A., Rolčík, J., Novák, O., Magnus, V., Barták, P., Buchtík, R., Salopek-Sondi, B., & Strnad, M. (2009). Isolation of novel indole-3-acetic acid conjugates by immunoaffinity extraction. *Talanta*, *80*, 651–655. https://doi.org/10.1016/j.talanta.2009.07.043

Ron'zhina, E. S. (2009). Source and sink effects of cytokinins in potato (*Solanum tuberosum* L.) plants. *Acta Horticulturae*, *835*, 39–48.

Sculthorpe, C. D. (1967). *The biology of aquatic vascular plants*. Edward Arnold.

Šimura, J., Spíchal, L., Adamec, L., Pěnčík, A., Rolčík, J., Novák, O., & Strnad, M. (2016). Cytokinin, auxin and physiological polarity in the aquatic carnivorous plants *Aldrovanda vesiculosa* and *Utricularia australis*. *Annals of Botany*, *117*, 1037–1044. https://doi.org/10.1093/aob/mcw020

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposia of the Society for Experimental Biology*, *54*, 118–130.

Smart, C. C., Fleming, A. J., Chaloupková, K., & Hanke, D. E. (1995). The physiological role of abscisic acid in eliciting turion morphogenesis. *Plant Physiology*, *108*, 623–632. https://doi.org/10.1104/pp.108.2.623

Smart, C. C., & Trewavas, A. J. (1983). Abscisic-acid-induced turion germination in *Spirodela polyrhiza* L. I. Production and development of the turion. *Plant, Cell & Environment*, *6*, 507–514.

Svačinová, J., Novák, O., Plačková, L., Lenobel, R., Holík, J., Strnad, M., & Doležal, K. (2012). A new approach for cytokinin isolation from *Arabidopsis* tissues using miniaturized purification: Pipette tip solid-phase extraction. *Plant Methods*, *8*, 17. https://doi.org/10.1186/1746-4811-8-17

Tarkowská, D., Novák, O., Floková, K., Tarkowski, P., Turečková, V., Gruz, J., Rolčík, J., & Strnad, M. (2014). Quo vadis plant hormone analysis? *Planta*, *240*, 55–76. https://doi.org/10.1007/s00425-014-2063-9

Wang, W. Q., Wu, Y. R., & Messing, J. (2014). RNA-Seq transcriptome analysis of *Spirodela* dormancy without reproduction. *BMC Genomics*, *15*, 60. https://doi.org/10.1186/1471-2164-15-60

Weber, J. A., & Noodén, L. D. (1976). Environmental and hormonal control of turion formation in *Myriophyllum verticillatum*. *Plant & Cell Physiology*, *17*, 721–731. https://doi.org/10.1093/oxfordjournals.pcp.a075328

Winston, R. D., & Gorham, P. R. (1979a). Turions and dormancy states in *Utricularia vulgaris*. *Canadian Journal of Botany*, *57*, 2740–2749. https://doi.org/10.1139/b79-324

Winston, R. D., & Gorham, P. R. (1979b). Roles of endogenous and exogenous growth regulators in dormancy of *Utricularia vulgaris*. *Canadian Journal of Botany*, *57*, 2750–2759. https://doi.org/10.1139/b79-325

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Adamec, L., Plačková, L., & Doležal, K. (2024). Characteristics of turion development in two aquatic carnivorous plants: Hormonal profiles, gas exchange and mineral nutrient content. *Plant Direct*, *8*(1), e558. https://doi.org/10.1002/pld3.558