The contrasting role of auxin in submergence-induced petiole elongation in two species from frequently flooded wetlands

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The involvement of auxin in the submergence-induced petiole elongation has been investigated in Rumex paliistris and Ranunculus sceleratus. Both wetland species are capable of enhanced petiole elongation upon submergence or treatment with exogenous ethylene (5 μl l⁻¹). Treatment of intact Rumex paliistris plants with 1-naphthalene acetic acid (NAA) at 10⁻⁴ M enhanced petiole elongation, while treatment with N-1-naphthylphthalamic acid (NPA) had no effect on petiole elongation. The elongation response after NAA or NPA treatment was comparable for plants in both submerged and drained conditions. Pre-ageing of detached petioles of Rumex paliistris for 3 h in light or in dark conditions had no effect on the submergence-induced elongation. In comparison to intact plants, detached petioles of Rumex paliistris, with or without lamina, did not show significant differences in responsiveness to IAA between drained or submerged conditions. This was in contrast to Ranunculus sceleratus where submergence caused a clear increase in responsiveness towards IAA. Removal of the lamina, the putative source of auxin, or treatment with NPA did not hinder the submergence-induced elongation of detached Rumex paliistris petioles, but severely inhibited elongation of detached Ranunculus sceleratus petioles. This inhibition could be restored by application of NAA, suggesting the specific involvement of auxin in the submergence response of Ranunculus sceleratus. It is concluded that, in contrast to Ranunculus sceleratus, auxin is probably not involved in the submergence-induced petiole elongation of Rumex paliistris.

Key words – Auxin, ethylene, Ranunculus sceleratus, Rumex paliistris, submergence.

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Introduction

One of the adaptative features of plants to submergence is the stimulated elongation of petioles, stems or internodes, enabling plant structures to emerge from the water. This elongation is causally related to increased levels of endogenous ethylene found under submerged conditions (Ku et al. 1970, Musgrave et al. 1972, Voesenek et al. 1993) and leads to a relief of oxygen deficits, thus ensuring survival and sexual reproduction (Van der Sman et al. 1991, Voesenek and Van der Veen 1994). Enhanced petiole elongation and its relation to ethylene has been well described for the flooding resistant rosette plant Rumex paliistris, occurring at low-elevation, frequently-flooded sites in river floodplains (Blom et al. 1994). However, the mechanism by which ethylene stimulates shoot elongation in Rumex paliistris is still largely unknown. For a number of aquatic and semi-aquatic species (Regnellidium diphylum, Walters and Osborne 1979; Ranunculus sceleratus, Horton and Samarakoon 1982; Nymphoides peltata, Malone and Ridge 1983) the presence of auxin has been shown to be prerequisite for optimal submergence-induced elongation. The similarity between Rumex paliistris and Ranunculus sceleratus in submergence response, growth form (both are dicotyledonous rosette species) and habitat (frequently flooded sites) led to the assumption that auxin would also play a role in Rumex paliistris. Therefore, the overall aim of this study was to determine whether auxin was also involved in submergence-induced petiole growth of
**Materials and methods**

**Chemicals**

Indoleacetic acid (IAA) and 1-naphthaleenacetic acid (NAA) were purchased from Sigma Chemical Company. N-1-naphthylphthalamic acid (NPA) was synthesized in the Dept of Organic Biochemistry at the Univ. of Nijmegen. IAA, NAA and NPA were first dissolved in a M aterials and methods.

**Plant material**

Seeds (achenes) of *Rumex palustris*, collected in river floodplains, were germinated on moistened filter paper in Petri dishes for 10 days in a germination cabinet (16-h photoperiod, day/night temperature of 25/10°C, photo-synthetic photon flux density, PPFD, of 30 μmol m⁻² s⁻¹ from fluorescent lamps, Philips TL 8W/33). Seedlings were planted in plastic pots (volume 60 ml) filled with a mixture of sand and potting compost (1:1, v/v). Seedlings of *Ranunculus sceleratus* were planted in plastic pots (volume 150 ml) filled with the same substrate. Both plant species were grown for 4–5 weeks in a growth chamber (16-h photoperiod, day/night temperature of 21/15°C, PPFD of 95 μmol m⁻² s⁻¹ from fluorescent lamps, Philips TLD 58W/84). Plants were selected for experiments on the basis of homogeneity of developmental stage of the 5th leaf.

**Submergence and ethylene response**

Intact *Rumex palustris* and *Ranunculus sceleratus* plants were submergence in 200-L glass containers. Drained plants were sprayed until runoff once every 24 h with the same solutions used for submergence. Both submersed and drained plants were kept in the growth chamber mentioned in Plant material. Elongation of petioles of leaves in the intermediate stage was measured 48 h after the onset of experiments with a ruler to the nearest 0.5 mm.

**Experiments with detached petioles**

*Ranunculus sceleratus* petioles were detached according to Samarakoon and Horton (1983) by removing a leaf with fully expanded lamina and cutting off the petiole at 25 mm from the lamina. For drained conditions of detached petioles with lamina attached, petioles were inserted through Parafilm, spread over a 100-ml beaker, into the test solution, thus leaving the lamina in air (cf. Musgrave and Walters 1973). Petioles without lamina were floated as another drained control. For submerged conditions, petioles (with or without lamina) were weighted by gently clamping the petiole in a latex serum cap and submerged in 1 L of test solution in a 1-L glass beaker.

For *Rumex palustris* a different method was followed. Preliminary experiments showed that floating or partly submersed petioles also displayed enhanced elongation and that removal of the root system had no effect on the enhanced petiole elongation at submergence (results not shown). Detached *Rumex palustris* petioles were obtained by removal of the leaves 1–4 (petiole and lamina), leaf 6 and the apex. The root system was cut off approximately 10 mm below the rosette node and laterals on the remaining part of the tap root were removed. This left the 5th petiole and lamina attached to the rosette node and ca 10 mm tap root. The tap root remainers of the plantlets were put in latex serum caps. These serum caps were mounted in a PVC plate and placed in a 1-L
Effect of NAA and NPA on petiole elongation of intact plants

Intact *Rumex palustris* plants were submerged in buffered aqueous solutions of $10^{-6}$–$10^{-4}$ M NAA while drained plants were sprayed daily with an identical range of NAA concentrations. There was no significant petiole elongation response to auxin at $10^{-6}$ and $10^{-5}$ M NAA over a 48-h period (Fig. 2A). Elongation was significantly stimulated by $10^{-4}$ M NAA and this effect was comparable in both submerged and drained conditions.

In *Rumex palustris* plants, application of the auxin transport inhibitor NPA did not affect growth at concentrations of $10^{-7}$ to $10^{-4}$ M, whether sprayed on drained plants or for plants submerged in NPA solution (Fig. 2B). Extra pretreatment with NPA 3 h before submergence in a NPA solution of the same concentration produced comparable results as shown in Fig. 2B (data not shown). However, it can not be excluded that high endogenous auxin levels interfered with exogenous auxin application and concealed these results. Therefore, in subsequent experiments petioles were detached and aged for 3 h in an attempt to minimize endogenous auxin levels (cf. Malone and Ridge 1983).

Effect of IAA, NAA and NPA on elongation of detached petioles

Detachment of *Rumex palustris* petioles reduced the response towards ethylene and submergence when com-
Fig. 2. Effect of NAA (A) and NPA (B) on petiole elongation of submerged and drained intact Rumex palustris plants (n=10; ±SE) over a 48-h period. Plants were either sprayed with NAA or NPA in 10 mM P-buffer (pH 6.0) or submerged in the same solutions. All solutions, including controls contained 0.1% ethanol. Spraying was repeated after 24 h. Bars represent SE for each treatment (P<0.05).

pared to the elongation response in intact plants (Tab. 1). Nevertheless, a significant growth stimulation upon submergence and ethylene treatment was noticed, permitting the use of detached petioles as a model system.

Detached Rumex palustris petioles without lamina were aged for 3 h in light and in dark by standing petioles with the root in water. Ageing was not found to have a significant effect on petiole elongation of this species over a 48-h period at either submerged or drained conditions (Tab. 2). Floating of the petioles on distilled water or age-times of 1 or 6 h produced similar results (data not shown), which could indicate that the submergence response is not depending on metabolites (e.g. auxin) produced outside the petiole.

To further analyze petiole elongation in Rumex palustris and Ranunculus sceleratus, 6 segments were marked on detached petioles from top to base to establish which part of the petiole elongates most. In drained petioles, each segment elongated more or less to the same extent in both species (Fig. 3). Upon submergence, the elongation of Rumex palustris segments remained more or less equal, while Ranunculus sceleratus segments closest to the lamina elongated significantly more than basal segments. Removal of the lamina did not affect this pattern in petiole elongation for either species, but drastically reduced overall elongation in Ranunculus sceleratus. This in contrast to Rumex palustris, where petiole elongation response only was slightly reduced (Fig. 3).

Detached petioles of Rumex palustris were less responsive to applied IAA than those of Ranunculus sceleratus (Fig. 4). Submergence increased the responsive-

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**Fig. 3.** Distribution of petiole elongation in detached Rumex palustris and Ranunculus sceleratus petioles. Petioles were either kept drained or submerged in P-buffer (10 mM, pH 6.0) with and without lamina. Petioles were marked with ink every 2 mm at the start of the experiment. Bars represent the elongation of each zone during the 48-h treatment period (n=5; ±SE).

**Fig. 2.** Effect of NAA (A) and NPA (B) on petiole elongation of submerged and drained intact Rumex palustris plants (n=10; ±SE) over a 48-h period. Plants were either sprayed with NAA or NPA in 10 mM P-buffer (pH 6.0) or submerged in the same solutions. All solutions, including controls contained 0.1% ethanol. Spraying was repeated after 24 h. Bars represent SE for each treatment (P<0.05).

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**Tab. 2.** The effect of ageing under light and dark conditions on petiole elongation of detached Rumex palustris petioles (n=10). Ageing was performed by standing petioles upright for 3 h with root remainder in distilled water in 1-l glass containers covered with plastic foil or wrapped in aluminium foil. Petiole length was measured before and after 48 h submergence. Different letters indicate significantly different means (ANOVA with Tukey's post test, P<0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drained</th>
<th>Submerged</th>
</tr>
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<tbody>
<tr>
<td>Not aged</td>
<td>5.0±0.4a</td>
<td>9.4±0.5b</td>
</tr>
<tr>
<td>Aged in light</td>
<td>3.3±0.6a</td>
<td>9.2±0.9b</td>
</tr>
<tr>
<td>Aged in dark</td>
<td>2.6±0.6a</td>
<td>8.6±0.4b</td>
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</tbody>
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**Tab. 1.** The effect of submergence and ethylene (5 μl l⁻¹) on petiole elongation of intact and detached Rumex palustris petioles (n=12). Elongation was recorded after 48 h. Different letters indicate significantly different means (ANOVA with Tukey's post test, P<0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Petiole elongation (mm ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detached petiole</td>
</tr>
<tr>
<td>Drained</td>
<td>4.6±0.4a</td>
</tr>
<tr>
<td>Ethylene (5 μl l⁻¹)</td>
<td>11.5±0.6b</td>
</tr>
<tr>
<td>Submerged</td>
<td>16.3±1.2c</td>
</tr>
</tbody>
</table>
Rumex palustris and Ranunculus sceleratus contained 0.1% ethanol. IAA was applied as submergence medium and as spray for drained treatment with IAA only (±SE) for submergence and drained treatment with IAA only for Ranunculus sceleratus, either with or without lamina.

Fig. 4. Effect of IAA on elongation growth of detached Rumex palustris and Ranunculus sceleratus petioles with and without lamina. Petioles were incubated for 48 h in 10 mM P-buffer (pH 6.0) with 0 to 10^{-5} M IAA. All solutions, including controls, contained 0.1% ethanol. IAA was applied as submergence medium and as spray for drained Rumex palustris. Initial petiole length was trimmed to 25 mm for Ranunculus sceleratus and 23 mm (±0.4) for Rumex palustris. Points represent the means (±SE) of 10–12 petioles. Bars represent LSD for each treatment (P<0.05). ANOVA showed significant interaction (P<0.05) for submergence and drained treatment with IAA only for Ranunculus sceleratus, either with or without lamina.

To investigate the importance of auxin transport, detached petioles of both species were submerged in either NPA, NAA or a combination of NPA and NAA (Fig. 5). Application of 10^{-5} M NPA, 10^{-5} NAA or a combination of NPA and NAA to detached Rumex palustris petioles led to a small but significant increase in elongation compared to controls. Removal of the lamina of detached Rumex palustris petioles reduced the overall elongation response but had no effect on the response to applied NPA and NAA. As shown before, removal of the lamina of Ranunculus sceleratus reduced the elongation of detached petioles. NPA applied to Ranunculus sceleratus petioles with lamina also suppressed petiole elongation significantly. This growth reduction could be overruled by application of 10^{-5} M NAA, thus showing the specific involvement of auxin in the submergence response of Ranunculus sceleratus.

**Discussion**

*Rumex palustris* and *Ranunculus sceleratus* are both dicotyledonous rosette plants occurring in river floodplains at frequently flooded sites. Both species are adapted to flooding by their ability to initiate rapid petiole elongation, especially those of the younger leaves. This elongation response can be mimicked to a large extent by exogenous application of ethylene and these results are in agreement with earlier reports (Musgrave and Walters 1973, Ridge 1985, Voesenek and Blom 1989).

While the morphological response upon submergence is similar for *Rumex palustris* and *Ranunculus sceleratus*, the results of the present study demonstrate that the involvement of auxin clearly differs in these two species. In *Rumex palustris* the responsiveness of petioles to exogenously applied auxin did not differ between drained or submerged conditions and the response was...
generally small (Figs 2 and 4). Petioles of Rumunculus sceleratus were found to be more responsive in general to exogenous auxin, either when drained or submerged (Figs 4 and 5 this report; Samaranakoon and Horton 1983, Smuldres and Horton 1991), and responsiveness was further enhanced by submerged conditions. The small elongation response towards auxin in Rumunculus palustris could be explained by the assumption that (1) the applied exogenous auxin does not reach auxin responsive cells, (2) sufficient endogenous auxin is present or (3) the tissue is not very sensitive in this respect for auxin. Floating of petioles on 10⁻⁴ M buffered NAA or IAA solution caused a 10- and 25-fold increase, respectively, in ethylene production compared to the control (results not shown). Furthermore, submergence of petioles in NAA or IAA caused a swelling of the petiole tissue that showed a strong linear relationship with the applied NAA or IAA concentration (results not shown). Both phenomena demonstrate that auxin is perceived by the petiole tissue even at low auxin concentrations. Since intact plants and detached petioles were completely submerged in NAA or IAA solution, assumption (1) can probably be neglected. For assumption (2) no definite conclusion could be made without determination of endogenous auxin. Although, in an effort to deleter petiole tissue of endogenous hormones, petioles were aged in both light and dark without significant effect on submergence response (Tab. 2), rejecting assumption (2) would be disputable. Consequently, regarding elongation, Rumunculus palustris petioles are either less susceptible towards auxin than Rumunculus sceleratus petioles or enough active auxin remains in the tissue after detachment and ageing.

More important, in Rumunculus palustris, the inhibition of auxin transport with NPA did not cause a reduction in elongation in either intact plants or detached petioles. NPA has been shown previously to be active in inhibiting polar auxin transport in Rumunculus palustris: next to petiole elongation, submergence or flooding also initiates the formation of adventitious roots, a process that is auxin mediated and arrested by application of NPA (Visser et al. 1995). Like NPA, removal of the lamina of detached petioles, the putative source of auxin, had no effect on petiole elongation under submerged conditions. In contrast, elongation in Rumunculus sceleratus during submergence was significantly inhibited by application of auxin transport inhibitors (Fig. 5; Horton and Samaranakoon 1982) or by removal of the lamina (Fig. 5; Samaranakoon and Horton 1983). This inhibiting effect on elongation could be counteracted by application of 10⁻⁶ M IAA or 10⁻⁵ M NAA, showing that the inhibition is auxin-specific and demonstrating the obligatory presence of auxin in submergence-elicited petiole elongation. Similar results were reported for Regnellidium diphyllum (Walters and Osborne 1979) and Nymphoides peltata (Malone and Ridge 1983).

Under drained conditions, the growth of petiole segments was found to be evenly distributed over the petiole for both Rumunculus palustris and Rumunculus sceleratus. If auxin is involved in this growth pattern, two explanations seem possible: (1) a classical longitudinal gradient in auxin content exists (high in tip, low at basal parts, Sánchez-Bravo et al. 1993) with an opposing gradient in responsiveness (low at tip, high at basal part, as mentioned in Hoson et al. 1992) resulting in a similar growth rate along the petiole, or (2) both auxin content and tissue responsiveness are evenly distributed over the petiole. Upon submergence the growth pattern did not change for Rumunculus palustris, unlike in Rumunculus sceleratus, where segmental growth declined along the apical-basal gradient. A comparable gradient was found for epidermal cell length in submerged or ethylene treated Rumunculus repens petioles (Ridge 1985). It is difficult to explain this discrepancy due to several, mutually influencing factors like variations in endogenous auxin levels between different organs (high in young leaves, low in older leaves or vice versa; Ueda et al. 1991 and references therein) or possible changes in auxin transport velocity or auxin responsiveness. For Rumunculus sceleratus it was shown that treatment with 50 μl l⁻¹ ethylene increased auxin transport velocity significantly (Musgrave and Walters 1973). This would mean that basal petiole parts receive more auxin and thus should show an enhanced growth compared to the apical petiole segments. However, our results show an opposing growth pattern even after removal of the lamina. Therefore, we hypothesise that in Rumunculus sceleratus submergence or ethylene causes an increase in responsiveness towards endogenous auxin of especially the apical segments and that a similar mechanism is lacking in Rumunculus palustris.

Together our results indicate that, in contrast to Rumunculus sceleratus, involvement of auxin in the submergence response of Rumunculus palustris is at least uncertain. This would be, as far as we know the first time an auxin independent ethylene-mediated elongation is reported for a terrestrial, dicotyledonous plant.

Noteworthy is the phenomenon of enhanced petiole elongation of Rumunculus palustris at floating or partially submerged conditions, contrasted to Rumunculus sceleratus. Rumunculus palustris shares this feature with deep water rice. Over the past ten years, Kende and co-workers showed that growth of deepwater rice under submerged conditions is elicited by gibberellin (GA) via an ethylene mediated change in responsiveness towards GA (Raskin and Kende 1984) and an enhanced endogenous GA concentration (Hoffmann-Benning and Kende 1992). Further experiments will show whether or not GA is involved in the submergence response of Rumunculus palustris.

In conclusion, it is shown that auxin does not play a crucial role in the submergence response of Rumunculus palustris, while in Rumunculus sceleratus previous findings have been confirmed and extended with respect to the elongation pattern down the petiole. Therefore, with respect to auxin, the hormonal mechanism of submergence-induced petiole elongation in Rumunculus palustris...
appears to be different from that in *Ranunculus sceleratus*.

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References


