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 palustris 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 palustris 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 palustris 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 palustris, 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 palustris 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 palustris.

Key words – Auxin, ethylene, Ranunculus sceleratus, Rumex palustris, 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 palustris, 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 palustris 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 palustris 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 palustris. Therefore, the overall aim of this study was to determine whether auxin was also involved in submergence-induced petiole growth of

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Rumex palustris. In order to compare and validate our results, both Rumex palustris and Ranunculus sceleratus were used in similar experiments, since for the latter species auxin involvement has been clearly demonstrated (Musgrave and Walters 1973, Cookson and Osborne 1978, Horton and Samarakoon 1982, Smulders and Horton 1991). The main objectives were to determine whether (1) the submergence-induced petiole elongation of Rumex palustris can be arrested by application of an auxin transport inhibitor, ageing of petiole tissue or removal of the lamina, (2) submergence results in a change in petiole responsiveness of Rumex palustris towards auxin, and (3) the involvement of auxin in the submergence response of Rumex palustris and Ranunculus sceleratus is comparable.

Abbreviations – NAA, 1-naphthaleneacetic acid; NPA, N-1-naphthylphthalamic acid.

Materials and methods

Chemicals

Indoleacetic acid (IAA) and 1-naphthaleneacetic 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 few drops of ethanol (96%). Controls received an equal amount of ethanol. Ethylene (Hoekloos, mixture in medical air) was applied at a growth saturating concentration of 5 μl l⁻¹ (Voesenek and Blom 1989).

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, photosynthetic 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 submerged in 200-l tanks filled with tapwater (22°C) placed in the greenhouse (16-h photoperiod, day/night temperature of 22/16 ± 2°C) with additional illumination (150 μmol m⁻² s⁻¹ at water surface). The plants were approximately 50 cm below the water surface. Exogenous ethylene was applied in an air-tight growth cabinet, located in the greenhouse, at ambient temperature and with additional illumination (80 μmol m⁻² s⁻¹ from fluorescent lamps, Philips TL 8W/33, 16-h photoperiod, day/night temperature of 23/17 ± 2°C). Enough pure ethylene was initially injected into the cabinet to give a final concentration of 5 μl l⁻¹ ethylene in air. Subsequently, ethylene at a concentration of 5 μl l⁻¹ in air was then flushed through the system at a rate of 20 l h⁻¹. Drained plants were maintained in the greenhouse. Petiole elongation was measured after 48 h in leaves of three different developmental stages: (1) the youngest leaf with still furled lamina, (2) an intermediate stage with partly unurfled lamina, and (3) an old leaf with fully-expanded lamina.

Auxin and NPA dose response experiments

Intact Rumex palustris plants were submerged in 10 mM K-phosphate buffer (pH 6.0, tapwater) or in buffered NAA (10⁻⁵–10⁻⁴ M) or NPA (10⁻⁴–10⁻³ M) solutions in 20-l glass containers. Drained plants were sprayed until runoff once every 24 h with the same solutions used for submergence. Both submerged 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 submerged 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 remainders of the plantlets were put in latex serum caps. These serum caps were mounted in a PVC plate and placed in a 1-l
glass beaker. The beaker was filled to root level for drained experimental conditions (ca 200 ml) or with 1 l of test solution for submergence.

For Rumex palustris, ageing of detached petioles without lamina was performed by positioning the petioles upright with the root remainder in distilled water in 1-l glass containers covered with plastic foil or wrapped in aluminium foil for dark treatment. For Ranunculus sceleratus, petioles were floated on distilled water. In all experiments with detached petioles, with or without lamina attached, plant material was aged for 3 h.

Elongation of petiole segments of detached Rumex palustris and Ranunculus sceleratus petioles was measured by making marks with waterproof ink every 2 mm. Petioles were either kept drained or submerged in K-phosphate buffer (10 mM, pH 6.0) with and without lamina. Segment length was measured again after 48 h with a ruler to the nearest 0.5 mm.

For other experiments with detached petioles, test solutions were 10^{-7}–10^{-5} M IAA in 10 mM K-phosphate buffer (pH 6.0) and 10^{-4} M NAA, 10^{-5} M NPA or a combination of NAA/NPA in 10 mM K-phosphate buffer (pH 6.0). Ethylene (5 μl l^{-1}) was applied continuously in a flow-through system at 1 l h^{-1} in gas-tight 2-l containers with an in- and outlet port and a sealed port for gas analysis. Unless stated otherwise, all experiments lasted 48 h. Petiole length was measured with a ruler to the nearest 0.5 mm at the beginning and end of the experiments.

Statistics
For each experiment 10–12 replicates were used unless stated otherwise. Experiments were repeated at least once. Representative data are shown. Differences between the means of the treatments were assessed with Tukey and LSD multiple comparison procedures after ANOVA.

Results
Submergence and ethylene
Submergence and exposure to 5 μl l^{-1} ethylene significantly enhanced elongation for nearly all developmental stages of both species (Fig. 1). In Rumex palustris, treatment with ethylene partly mimicked the submergence response. In Ranunculus sceleratus petiole elongation of all three developmental stages was significantly higher upon ethylene treatment compared to submergence (Fig. 1). In both species petiole elongation upon submergence was most pronounced in leaves at the intermediate developmental stage. Therefore, this developmental stage was used in subsequent experiments.

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 cannot 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 compared

Fig. 1. Elongation of petioles of three developmental stages in Rumex palustris and Ranunculus sceleratus under drained conditions, submergence and treatment with 5 μl l^{-1} ethylene. Young leaves had furled lamina, lamina of intermediate leaves were expanding and lamina of old leaves were fully expanded. Experiment lasted for 48 h. Different letters indicate significant difference of means (n=10; +SE) within leaf stages (ANOVA, P<0.05).
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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**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>
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</tbody>
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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).
Removal of the lamina of detached petioles. NPA applied to detached petioles. Removal of the lamina of detached petioles. NPA applied to detached petioles. NPA and NAA applied to detached petioles. With lamina (Fig. 4). Addition of 10^{-6} M IAA brought back the response to IAA, indicating a change in responsiveness to IAA upon submergence, was only found 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 petioles led to a small but significant increase in elongation compared to controls. Removal of the lamina of detached 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
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definite conclusion could be made without determina-

tion of auxin transport inhibitors (Fig. 5; Horton and Samara-

coon 1983, Smulders and Horton 1991), and responsiveness was

Further enhanced by submerged conditions. The small

eelongation response towards auxin in Rumex palustris could be explained by the assumption that (1) the ap-
plied 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 so-
lution 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 exogenous 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 determina-
tion of endogenous auxin. Although, in an effort to de-

plete petiole tissue of endogenous hormones, petioles were aged in both light and dark without significant ef-

fect on submergence response (Tab. 2), rejecting as-
sume (2) would be disputable. Consequently, regard-
ing elongation, Rumex palustris petioles are either less

susceptible towards auxin than Ranunculus sceleratus petioles or enough active auxin remains in the tissue af-

ter detachment and ageing.

More important, in Rumex palustris, the inhibition of

auxin transport with NPA did not cause a reduction in

eelongation in either intact plants or detached petioles.

NPA has been shown previously to be active in inhibiting

department of adventitious roots, a process that is

inhibited by exogenous auxin (Vis-

ser et al. 1995). Like NPA, removal of the lamina of de-

tached petioles, the putative source of auxin, had no ef-

fect on petiole elongation under submerged conditions.

In contrast, elongation in Ranunculus sceleratus during

submergence was significantly inhibited by application of

auxin transport inhibitors (Fig. 5; Horton and Samara-

coon 1982) or by removal of the lamina (Fig. 5; Samara-

coon and Horton 1983). This inhibiting effect on elonga-

tion could be counteracted by application of $10^{-4} M$ 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. Simi-

lar results were reported for Regnellidium diphyllum

(Walters and Osborne 1979) and Nymphoides peltata

(Malone and Ridge 1983).

Under drained conditions, the growth of petiole seg-

ments was found to be evenly distributed over the peti-

ole for both Rumex palustris and Ranunculus sceleratus.

If auxin is involved in this growth pattern, two explana-
tions 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 men-
tioned in Hoson et al. 1992) resulting in a similar growth

rate along the petiole, or (2) both auxin content and tis-

sue responsiveness are evenly distributed over the peti-

ole. Upon submergence the growth pattern did not

change for Rumex palustris, unlike in Ranunculus scel-

eratus, where segmental growth declined along the api-
cal-basal gradient. A comparable gradient was found for

epidermal cell length in submerged or ethylene treated

Ranunculus repens petioles (Ridge 1985). It is difficult
to explain this discrepancy due to several, mutually in-

fluencing 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
tions therein) or possible changes in auxin trans-

port velocity or auxin responsiveness. For Ranunculus sceleratus it was shown that treatment with 50 µl l$^{-1}$ ethyl-

ene 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. There-

fore, we hypothesise that in Ranunculus sceleratus sub-

mergence or ethylene causes an increase in responsive-

ness towards endogenous auxin of especially the apical

segments and that a similar mechanism is lacking in

Rumex palustris.

Together our results indicate that, in contrast to Ra-

nunculus sceleratus, influence of auxin in the sub-

mergence response of Rumex palustris is at least uncer-

tain. This would be, as far as we know the first time an

auxin independent ethylene-mediated elongation is re-

ported for a terrestrial, dicotyledonous plant.

Noteworthy is the phenomenon of enhanced petiole

elongation of Rumex palustris at floating or partially

submerged conditions, contrasted to Ranunculus sceler-

atus. Rumex 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

circle is elicited by gibberellin (GA) via an ethylene

mediated change in responsiveness towards GA (Raskin

and Kende 1984) and an enhanced endogenous GA con-

centration (Hoffmann-Benning and Kende 1992). Fur-

ther experiments will show whether or not GA is in-

volved in the submergence response of Rumex palustris.

In conclusion, it is shown that auxin does not play a

crucial role in the submergence response of Rumex

palustris, while in Ranunculus sceleratus previous find-

ings have been confirmed and extended with respect to

the elongation pattern down the petiole. Therefore, with

respect to auxin, the hormonal mechanism of submer-

gence-induced petiole elongation in Rumex palustris

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appears to be different from that in *Ranunculus sceleratus*.

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References


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