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Low-molecular-weight hydrocarbons produced by water-stressed *Larix leptolepis* seedlings

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Summary

Drought stress affected production of low-molecular-weight hydrocarbons in *Larix leptolepis* seedlings. Ethylene production in shoots was increased at a low level of water potential below turgor loss point after 5 days of water withholding. In contrast, water deficit significantly suppressed ethylene production in root segments. Shoot segments of water stressed seedlings also showed burst emanation of ethane at low level of water potential. However, such enhancement of ethane production was not detected in root segments. The effect of water deficit on propane production was not remarkable. The possibility of utilizing of hydrocarbon detection as a diagnostic tool of drought stress is discussed.

Introduction

For the diagnosis of degree of water stress and estimation of stress tolerance of plants, it is necessary to develop effective methods to monitor physiological changes in stressed plants. Production of low-molecular-weight hydrocarbons is a common phenomenon occurring in plants under environmental stresses. Ethylene and ethane are known to increase in plants as a result of various stresses including wounding, fumigation of air contaminants, freezing, and water deficits[2,3,7,8,9]. Ethylene, a kind of plant growth regulator, may be an important factor in physiological processes manifesting stress tolerance and adaptation. Ethane evolution, which was found to be correlated with leaf necrosis[3,7], can be a useful indicator of mortality of stressed plants. Further, we found propane was also produced by water stressed coniferous species (unpublished data). In the present study we tried to determine production of ethylene, ethane, and propane by water stressed *Larix leptolepis* seedlings and investigated whether hydrocarbon production can be a useful diagnostic tool for evaluation of water deficit.

Materials and Methods

Larix leptolepis seedlings were grown from seeds collected in Ibaragi Prefecture, Japan, in loam in a field of the Tottori University Forest. When these were 12-months old, well-grown seedlings were transplanted into 13.0 × 13.0 × 12.0 cm pots, then nursed in a

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greenhouse. On 19 October 1988, thirty two 16-month-old seedlings were selected for uniformity of size and development and were transferred to a growth chamber in the Aridtron of the Sand Dune Research Institute of Tottori University. Average values of plant height and stem diameter were 43.5 ± 1.5 cm and 5.7 ± 0.3 mm, respectively. Photosynthetic photon flux density (PPFD) at plant height in the chamber was about 490 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400−700 nm). Day length was 14 h beginning at 06.00 ; temperature was 25 ℃ ; and relative humidity about 45%.

To determine hydrocarbon production, these seedlings were subjected to three watering regimes : 1) non-stressed, 10 seedlings watered to saturation every day ; 2) drought stressed, 10 seedlings were not watered for 3 or 5 days and ; 3) rewatered, 10 seedlings were not watered for 3 or 4 days, then watered to saturation every day until harvest. The remaining 2 seedlings were used for estimation of turgor loss point of the experimental materials.

To determine xylem water potential and hydrocarbon production, five non-stressed and five stressed plants were harvested twice from one to two o'clock in the afternoon at 3 and 5 days after stress initiation. The remaining rewatered plants were also harvested at 5 days after initiation.

Xylem water potential was measured on one current shoot segment per sample seedling with Daiki pressure chamber DIK-PC40 (Daiki Rika Kogyo Co., LTD). Water potential of turgor loss point of the plant materials was preliminarily estimated using P-V curves obtained in two seedlings by the pressure-chamber-technique at the beginning of the experiment.

Release of low-molecular-weight hydrocarbons including ethylene, ethane, and propane was measured for three segments of 3-cm length each of the current shoot (terminal shoot with needles) and lateral root segments. Each shoot or root sample was placed in a 5-ml vial and sealed with a silicone rubber stopper. The samples were incubated in a water bath at 30 ℃ for 12 h under darkness. Aliquots (1 ml) of head space gas were sampled at intervals and analyzed for ethylene, ethane, and propane by Hitachi gas chromatograph 263-50 with a flame ionization detector (FID) and a spiral glass column (3 mm × 200 cm) packed with activated alumina (60/80 mesh). Chromatograph conditions were as follows : column, injector, and detector temperatures were 70 ℃, 80 ℃, and 100 ℃, respectively ; carrier gas (N_2), 40 ml/min. Measurements of hydrocarbons were calculated from standard curves calibrated from authentic gas samples. The amount of hydrocarbons released by shoot and root segments (nmol/gDW) was calculated using a method reported by Yamamoto et al. [11].

Results

Water withholding strongly affected xylem water potential in *Larix leptolepis* seedlings (Table 1). Three days after watering ceased, xylem water potential was significantly lower in stressed than in non-stressed seedlings. At five days after stress initiation,

Table 1. Effect of water deficit on xylem water potential of *Larix leptolepis* seedlings.

Days after stress Initiation	Non-stressed	Stressed	Rewatered[a]	Rewatered[b]
	−MPa	−MPa	−MPa	−MPa
3	1.02 ± 0.04	2.39 ± 0.22	−	−
5	1.03 ± 0.05	4.00 <	1.25 ± 0.21	1.38 ± 0.17

a Stressed for 3 days, then watered to saturation for 2 days.
b Stressed for 4 days, then watered to saturation for 1 day.

the xylem water potential of stressed seedlings dropped below −4.0 MPa which was lower than turgor loss point (−2.27 MPa) of experimental materials estimated preliminarily using P−V curves. Rewatering of stressed seedlings efficiently arrested stress advancement. The xylem water potential of rewatered seedlings did not exceed turgor loss point at 5 days after stress initiation.

Water deficit also affected production of ethylene and ethane but not propane (Fig. 1, Table 2). Three days after stress initiation, ethylene production in shoot segments was not significant although xylem water potential was relatively low. In contrast, a fairly high amount of ethylene emanation was detected at a lower level of water potential below turgor loss point after 5 days water withholding. Ethylene production in shoots of rewatered seedlings was at the same level as that of non-stressed controls.

Water deficit significantly suppressed ethylene production in root segments at 5 days after stress initiation. However, reduction of ethylene emanation was not found in roots of rewatered seedlings. A large amount of ethylene was detected in roots of seedlings rewatered for 1 day in comparison with plants rewatered for 2 days.

Shoots of water-stressed seedlings also showed burst evolution of ethane when xylem water potential dropped below −4.0 MPa at 5 days after stress induction. Such enhancement of ethane production was detected neither in shoots of rewatered seedlings nor in roots of all other treatments.

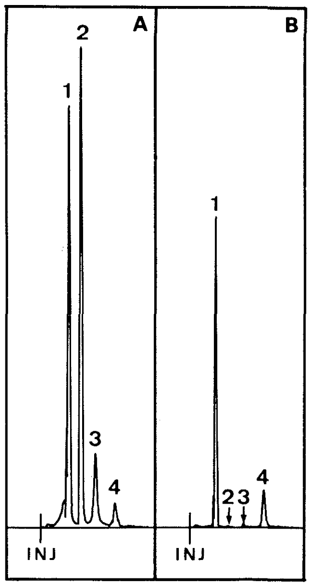

Fig. 1. Gas chromatograms of low-mol-wt hydrocarbons on activated alumina. A, Head space gas in a 5-ml vial from 12-h incubated shoot segments of a drought stressed *Larix leptolepis* seedling harvested at 5 days after stress initiation ; B, head space gas from 12-h incubated shoot segments of a non-stressed seedling. Peaks are : 1, air peak ; 2, ethane ; 3, ethylene, 4, propane.

Table 2. Effect of water deficit on production of low-mol-wt hydrocarbons by shoot and root segments of *Larix leptolepis* seedlings.

	Days after stress Initiation	Non-stressed	Stressed	Rewatered[a]	Rewatered[b]
(ETHYLENE)		nmol/gDW	nmol/gDW	nmol/gDW	nmol/gDW
Shoots	3	0.4 ± 0.0	0.6 ± 0.1	—	—
	5	0.3 ± 0.1	3.6 ± 1.8	0.3 ± 0.1	0.4 ± 0.0
Roots	3	4.2 ± 1.2	3.5 ± 1.1	—	—
	5	8.8 ± 2.8	2.0 ± 0.8	4.8 ± 0.4	8.9 ± 2.4
(ETHANE)		nmol/gDW	nmol/gDW	nmol/gDW	nmol/gDW
Shoots	3	0.2 ± 0.1	0.4 ± 0.2	—	—
	5	0.1 ± 0.0	24.9 ± 12.0	0.3 ± 0.2	0.2 ± 0.0
Roots	3	0.1 ± 0.0	0.2 ± 0.0	—	—
	5	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
(PROPANE)		nmol/gDW	nmol/gDW	nmol/gDW	nmol/gDW
Shoots	3	3.0 ± 0.8	1.8 ± 0.7	—	—
	5	2.3 ± 0.5	2.8 ± 0.4	1.7 ± 0.5	1.2 ± 0.3
Roots	3	0.5 ± 0.1	0.3 ± 0.1	—	—
	5	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1

a Stressed for 3 days, then watered to saturation for 2 days.
b Stressed for 4 days, then watered to saturation for 1 day.

Production of propane was slightly higher in shoots of non-stressed and stressed seedlings than in their roots. The effect of water deficit on propane production was not remarkable.

Discussion

Production of both ethylene and ethane by plants increases as a result of various stresses including wounding, SO_2 fumigation, freezing, and water deficits[2,3,7,8,9]. Ethylene production following drought stress has been well documented for woody plants[6,10] and herbaceous plants[1,4]. Although it is possible to use ethylene detection as an indicator for monitoring water stress, the correlation between the advance of water deficit and ethylene production may not be linear[4,7,10]. Stumpff and Johnson[10] reported that ethylene production rates in needles of water-stressed *Pinus taeda* L. increased slightly with initial stress, followed by a decrease, and then re-increased sharply in proportion to the advance of water deficit. Such a second increase in pine may be related to the high content of ethylene in shoots of *Larix* at low water potential below turgor loss point. Acceleration of ethylene production under intense drought stress may be correlated with plasmolysis caused by turgor loss in stressed plants.

Root ethylene production of water-stressed *Larix* seedlings was notably suppressed after 5-day water withholding. Reirrigated plants, however, recovered from reduction of ethylene evolution soon after watering. Burst ethylene production just after rewatering was also detected in roots of *Pinus taeda* L.[6,10]. In our data, a large amount of ethylene was detected in roots of the seedlings rewatered for 1 day in comparison with seedlings rewatered for 2 days.

Production of ethane in shoots of stressed *Larix* seedlings was more drastic than that of ethylene. Ethane emanation is highly correlated with mortality of plant tissue compared to ethylene[3,7]. Kimmerer and Kozlowski[7] reported that production of ethane in SO_2 fumigated *Betula papyrifera* Marsh seedlings was linearly related to the percentage of necrosis. Ethane production by plants is known to be the result of peroxidation of membrane linolenic acid, which is the precursor of stress ethane[5,7]. In the present data, accelerated production of ethane at the greatest stress caused by water withholding for 5 days is considered to be a result of mortal injury of cells. Measurement of ethane in shoots with needles may be a useful diagnostic tool for monitoring water stress, especially for evaluating water deficit over turgor loss point.

There was no relation between propane emanation and water deficit. However, considerable production of propane was detected in water-stressed *Cryptomeria japonica* and *Chamaecyparis obtusa* seedlings (Yamamoto, unpublished data). Further experiments in propane production are needed.

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和 文 摘 要

樹木の水ストレス耐性の検索やストレス状況の生理的な診断などを目的としてカラマツを用い，乾燥ストレスがエチレンなどの低分子炭化水素生成に及ぼす影響を検討した。

乾燥ストレスは，葉条のエチレン及びエタン生成を促したが，根のエチレン生成は抑制された。エチレンおよびエタンの生成は原形質分離点を越える強いストレス下で大きくなり、細胞の損傷と関連するようであった。プロパン生成は、葉条においてやや多いようであったが、ストレスとの関連は明らかではなかった。