

ENHANCED ETHYLENE PRODUCTION BY PRIMARY ROOTS OF *ZEA MAYS* L. IN RESPONSE TO SUB-AMBIENT PARTIAL PRESSURES OF OXYGEN.

R. BRAILSFORD, L.A.C.J. VOESENEK¹, C.W.P.M. BLOM¹, A.R. SMITH², M.A. HALL² AND M.B. JACKSON.

Department of Agricultural Sciences, University of Bristol, AFRC Institute of Arable Crops Research, Long Ashton Research Station, Bristol, BS18 9AF, UK.

¹*Department of Ecology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands.*

²*Department of Botany and Microbiology, University College of Wales Aberystwyth, Penglais, Aberystwyth, Dyfed, SY21 3DA, UK.*

Flooding or soil waterlogging inhibits gaseous exchange between the soil and atmosphere. Flooded soils rapidly become oxygen deficient, whilst the products of plant and microbial respiration such as CO₂ accumulate. Plants require metabolic and/or morphological adaptations to survive such conditions. Many of the latter are mediated by plant hormones, most notably ethylene. There is evidence of a causal relationship between the rate of ethylene biosynthesis, oxygen supply and physiological adaptations such as aerenchyma development. When maize roots are exposed to hypoxia or to exogenous ethylene, aerenchyma develops in the cortex. Jackson *et al.* (1982; 1985) reported enhanced ethylene production in nodal roots of maize and barley under low oxygen partial pressures (3 - 12.5 kPa), whilst a similar response was observed in the stems of deep water rice by Metraux and Kende (1983). This enhancement of biosynthesis is controversial, since ethylene biosynthesis requires molecular oxygen. The techniques used by Jackson and co-workers and Metraux and Kende (i.e. head space analysis of excised tissue enclosed in small incubation vials) may have resulted in artifacts, e.g. as a result of wound-ethylene production after excision. To overcome this, we employed a sensitive laser-driven photoacoustic technique (sensitivity limit: 0.041 pmol m⁻³) to measure ethylene production from individual roots of intact seedlings, thus minimising physical perturbations.

Single primary roots (20 - 25 mm long) of intact, three day-old maize seedlings (*Zea mays* L. cv LG11) were sealed into glass cuvettes with plaster of Paris. The root was isolated in the darkened lower chamber of the cuvette, through which a humidified gas stream passed (flow rate: 1 x 10⁻³ m³ h⁻¹). Roots were exposed to 6 h of air before treatment with 21 kPa (control), 12.5 kPa, 5 kPa, 3 kPa, 1 kPa or 0 kPa O₂ (pure nitrogen). After 16 h, the gas was switched back to air for a further 6 h to monitor post-stress production. Before gas from the cuvettes entered the photoacoustic detector, it was scrubbed of CO₂, water and ethanol. All experiments took place at 22°C, with continuous light (200 μmol m⁻³ s⁻¹). Root length and morphology were recorded at the start, finish and before each gas change. Estimated fresh weight based on root

length was used during the experiment to express ethylene production as $\text{nl g.fw}^{-1} \text{h}^{-1}$. Experiments were replicated 2 - 5 times. Representative data are presented.

Primary root extension was inhibited by partial pressures of oxygen ($p\text{O}_2$) below 21 kPa compared to aerobic controls; the level of inhibition increasing with decreasing $p\text{O}_2$. Almost no growth was observed in the absence of O_2 . Post-treatment root extension rates were similar to those during treatment, except after 16 h of 5 kPa O_2 where extension recovered rapidly to approach that of air. Roots exposed to 3 or 5 kPa O_2 were thickened, plagiotropic and possessed dense root hairs whilst, those treated with 12.5 and 1 kPa O_2 resembled those of air-grown controls. The morphology of tissue produced after returning to air was similar to air-grown roots in all cases except 0 kPa O_2 . Returning roots to air after anoxia caused death of the meristem. There were clear trends in ethylene production ($\text{nl g.fw}^{-1}\text{h}^{-1}$) in response to different partial pressures of oxygen. Ethylene production during the preliminary 6 h acclimatization in air declined as the root recovered from the mild stress imposed during the transfer of plants. In aerobic controls and during and after treatment with 12.5 kPa O_2 , production continued to decline at a rate of approximately 0.15 nl h^{-1} . In contrast, 5 kPa, 3 kPa and 1 kPa O_2 caused ethylene production to increase. The most marked stimulation occurred in 3 kPa O_2 . Here the rate increased within the first hour of treatment and remained at a high level ($8 - 10 \text{ nl g}^{-1}\text{h}^{-1}$) for 16 h. Production rates returned rapidly to those of air controls when air was returned. Anoxia completely eliminated ethylene synthesis during and after treatment.

These results confirm that a stimulation of ethylene production occurs in roots of intact maize seedlings in response to hypoxia. Little is known about the mechanisms behind this stimulation or the effect of hypoxia on maize root ethylene forming enzyme. We are currently investigating whether 1-aminocyclopropane-1-carboxylic acid is produced in an anoxic root core and diffuses to better oxygenated cortical regions where it is converted.

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