# Respiratory potential of maize (*Zea mays* L.) roots exposed to hypoxia

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## ARTICLE INFO

## ABSTRACT

When hypoxia is not too severe, root aerobic metabolism can be partly supported by oxygen delivery via aerenchymateous tissues. In terms of supplying energy, this adaptation is of special importance in plants with a high metabolic demand, such as maize (*Zea mays* L.). The ability of maize to respond to hypoxia by morphological changes is well documented; however, little is known on the potential for oxidative metabolism in different types of maize roots. In our study, we assessed the root respiratory potential in seminal and adventious nodal roots of maize exposed to mild short-term hypoxia. Plants responded to the treatment with an increased portion of nodal roots per total root length, while there were no changes in the biomass of shoots and roots. Thick nodal roots had much higher respiratory potential (Electron Transport System Activity – ETS) than nodal roots of smaller diameter or seminal roots, irrespective of the aeration rate. The only change in ETS under oxygen deficiency was found for seminal roots where oxygen consumption increased by 25%. Increased root porosity was observed in all roots, the increase was higher in nodal roots. On the basis of ETS data and taking into account changes of root morphology, it can be concluded that large changes of root respiratory potential are not involved in the response of maize to hypoxia. Obviously, maize can cover the respiratory needs with shifts in the growth of different root types which inherently differ in their potential aerobic respiration.

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## 1. Introduction

In plants exposed to soil hypoxia, oxygen deficits can trigger functional and developmental responses that enable acclimation to adverse gaseous conditions. On the functional level, these responses are mainly related to the regulation of respiration. The root respiration rate is generally reduced under low oxygen concentrations (Lambers et al., 1978; Carpenter and Mitchell, 1980; McNamara and Mitchell, 1989; Huang and Johnson, 1995; Geigenberger, 2003; but see Moog and Brüggemann, 1998; Matsui and Tsuchiya, 2006.). When deprived of oxygen, plant cells switch from aerobic to anaerobic metabolism, which preserves the ability of limited ATP production. This metabolic adaptation is only short-termed, since the products of both fermentative pathways (lactate, ethanol) can disturb cellular metabolism and fermentation cannot maintain energy charge (Pearson and Havill, 1988). Only in highly tolerant species, can aerobic respiration, and consequently high ATP production, be preserved during hypoxia or it can be re-introduced quickly after its onset. These plants may possess some biochemi-

cal characteristics that could contribute to higher tolerance; they can keep the adequate supply of carbohydrates to the roots (Huang and Johnson, 1995), adjust waste of ATP in substrate (futile) cycles (Alonso et al., 2007), possess cytochrome oxidase with a high affinity for $O_2$ (Maricle et al., 2006), or increased haemoglobin levels, which could mitigate stress by maintaining levels of ATP and modulating NO levels (Sowa et al., 1998; Igamberdiev et al., 2005). Yet, many studies have revealed that anatomical and morphological adaptations enabling the transport of oxygen from the shoot to the roots and rhizosphere are the most crucial features for plant tolerance to hypoxia (Armstrong and Drew, 2002; Jackson, 2008). An efficient gas flow can be achieved when tissue porosity is increased by aerenchyma formation (Colmer, 2003). In roots, aerenchyma develops in the cortex in response to enhanced ethylene concentrations (Evans, 2003; Drew et al., 2000; Gunawardena et al., 2001). The latter also influence the development of impermeable barriers to radial $O_2$ loss (ROL) in more basal zones of roots (Colmer, 2003).

When a plant has to overcome stress, a sufficient amount of metabolic energy provided by respiration is needed. In plants that are not highly tolerant to oxygen deficiency (i.e. having high aerobic demand), the preservation of functional respiratory apparatus would be crucial, in addition to a sufficient supply of oxygen and carbohydrates to the roots, to overcome stress and also to recover aerobic metabolism after resumption of normoxia. However, lower aerobic demand may be advantageous for survival under hypoxic

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stress (Maricle et al., 2006; Huang and Johnson, 1995). Despite the fact that plants can decrease their oxygen consumption in response to low oxygen concentrations to avoid internal anoxia (Geigenberger, 2003), it is clear that tolerance will depend on a species' inherent specific respiratory demand (e.g. Maricle et al., 2006). Maize (*Zea mays* L.), which is the subject of our study, can endure oxygen deprivation in roots only for short time periods by switching to fermentative metabolism and by the formation of more porous adventious roots (Fukao and Bailey-Serres, 2004). The high respiratory demand of maize can be deduced from the high activity of root cytochrome *c* oxidase, an enzyme that catalyzes the terminal electron transport to oxygen and can be therefore regarded as a critical step in aerobic respiration. Maricle et al. (2006) showed that preserved or even increased cytochrome *c* oxidase activities can be measured when maize and some other species (salt marsh grasses) are exposed to hypoxia. The high activity of the enzyme may serve as an increased oxygen scavenging system in oxygen-deficient tissues (Pearson and Havill, 1988).

Measurements of radial and longitudinal aeration of primary maize roots indicate that cortical oxygen concentration in arenchymateous roots can support some aerobic metabolism (Darwent et al., 2003) when oxygen deprivation is not to severe. Since seminal and adventious nodal roots can differ in tissue porosity (Thomson et al., 1990), it is assumed that aerobic respiration contributes differently to respiratory energy production in both types of roots. The main purpose of our study was therefore to assess root respiratory potential, estimated as the efficiency of mitochondrial electron transport chain (ETS), in maize roots of different types. Respiratory potential was related to the growth response and morphological adaptations of maize grown under normoxia or short-term hypoxia.

## 2. Materials and methods

### 2.1. Plant material and hypoxia treatment

Seeds of *Z. mays* L. were surface sterilized in 0.6% Na-hypochlorite, rinsed in water and germinated on wet (dH$_2$O soaked) filter paper at room temperature. After 9 days, the seedlings were transferred to a nutrient solution modified according to Wiengweera et al. (1997), the composition was the following (mol m$^{-3}$): K$^+$, 5; Ca$^{2+}$, 1.5; Mg$^{2+}$, 0.4; NH$_4^+$, 0.6; NO$_3^-$, 4.5; SO$_4^{2-}$, 1.9; H$_2$PO$_4^-$, 0.2; micronutrients Cl$^-$, 0.05, B, 0.025, Mn, 0.002, Zn, 0.002; Cu, 0.005; Mo, 0.005; NaFeEDTA, 0.005 and MES, 2.5. The pH of the solution was adjusted to 6.5 by using KOH which contributed roughly 1 mol m$^{-3}$ to the final concentration of potassium. Plants were grown in aerated 1 l pots (four plants per pot, 12 pots in total) in the growth chamber with a 14 h light period, a photon flux density of 200 μmol m$^{-2}$ s$^{-1}$, 25/20 °C day/night temperature and a relative humidity of 70%. Oxygen concentration in the nutrient solution was measured by using a Profline Oxi 197 oxymeter (WTW, Weilheim, Germany).

The exposure to hypoxia started after 5 days of growing under normoxic conditions. In half of the pots (6), a stagnant non-aerated solution with 0.1% agar (Wiengweera et al., 1997) was used to simulate hypoxia. The oxygen concentration in the nutrient solution was measured daily. During the measurements, the roots were lifted out of the agar. The agar solution in each pot (with the lid on) was then mixed with a magnetic stirrer for 30 s before the O$_2$ concentration was measured.

### 2.2. Harvest and measurements

Seven days from the start of the treatment, the plants were harvested. One plant (of four) from each pot was used to mea-

sure growth parameters (plant height, shoot and root weight, root measurements) ($N = 5$–6, one pot of 'non-aerated' treatment was omitted from sampling). The lengths of roots of different types (nodal, seminal roots) and two diameter classes (<1 mm and 1 > mm; only for nodal roots) were measured by WinRhizo (Regent, Canada). Another plant from each pot was used to measure root porosity which was evaluated by measuring buoyancy with a balance using Archimedes' principle (Raskin, 1983). Intact and infil-trated roots (vacuum infiltration with 0.05% Triton X-100 solution) were measured. Porosity was calculated by using equations adapted by Thomson et al. (1990).

Root respiratory potential was measured according to Kenner and Ahmed (1975). The two plants left from each pot were used for ETS measurements ($N = 9$–12). Root samples were taken from seminal and nodal roots (two diameter classes) in a region 1–2 cm from the root tip. Root samples of 50 mg were weighed. Each sample was ground milled in a mortar with 2 ml of ice-cold buffer (75 μM MgSO$_4$, PVP 0.15% (w/v), Triton X-100 0.2% (v/v) in 0.1 M phosphate buffer, pH 8.4; Sigma) and then washed into polypropylene centrifuge tubes to the final volume of 5 ml. Samples were treated for 2 min in a ultrasonic bath and centrifuged for 3 min at 10,000 rpm and 0 °C. Triplicates of supernatant (analytical replicates; 0.3 ml) were incubated in 1.5 ml of substrate mixture (NADH 0.17 mM, NADPH 0.25 mM, Triton X-100 0.2% (v/v) in 0.1 M phosphate buffer, pH 8.4; Sigma) and 0.5 ml of the mixture of INT – iodonitrotetrazolium-chloride (2 mg ml$^{-1}$, Fluka) at 20 °C for 40 min in the dark. Thereafter, the reaction was stopped by using 0.5 ml of the formaldehyde:ortho phosphoric acid mixture (1:1, v/v). The formazan production was measured at 490 nm by using a PerkinElmer spectrophotometer. Averages of the triplicate measurements were calculated and used for further statistical analysis. ETS activity was measured as the rate of INT reduction, which was converted to equivalent oxygen utilized per dry mass over time (ml O$_2$ g$^{-1}$ DW h$^{-1}$) as described by Kenner and Ahmed (1975).

### 2.3. Statistical analysis

Data were subjected to two-way ANOVA followed by a Duncan's test. All calculations were made by using Statgraphics Plus (Manugistic, USA).

## 3. Results

Oxygen concentration in the hydroponic solution was relatively uniform during pre-treatment. The average for pots later used in normoxic treatment was $10.06 \pm 0.18$ mg O$_2$ l$^{-1}$ and in those later used in hypoxic treatment it was $10.24 \pm 0.11$ mg O$_2$ l$^{-1}$. A day after the start of hypoxic treatment, a 10-fold decrease in O$_2$ concentration was achieved in all non-aerated pots (Fig. 1). Low oxygen levels in this treatment ($1.41 \pm 0.07$ mg O$_2$ l$^{-1}$) corresponded to ca. 20% of oxygen saturation and persisted throughout the entire experiment. There was little variation in oxygen concentration between the pots within a single treatment.

The measurements of the growth parameters revealed no significant effect of oxygen deprivation on the shoot length or on fresh weights of shoots and roots (Table 1). There were however significant changes in root morphology and porosity. While the thicker nodal roots represent approximately 1.5% of the total root length (TRL) in aerated plants, they contributed to roughly 10% of TRL in non-aerated ones. Analysis revealed no significant differences in the total number of root tips and forks when two treatments were compared.

Air-filled porosity was similar in seminal and nodal roots when maize was grown under normoxia (Table 2). Under hypoxia, poros-