Cytochromes P450 for engineering herbicide tolerance

Danièle Werck-Reichhart, Alain Hehn and Luc Didierjean

In recent years, genome sequencing has revealed that cytochromes P450 (P450s) constitute the largest family of enzymatic proteins in higher plants. P450s are mono-oxygenases that insert one atom of oxygen into inert hydrophobic molecules to make them more reactive and hydroxysoluble. Besides their physiological functions in the biosynthesis of hormones, lipids and secondary metabolites, P450s help plants to cope with harmful exogenous chemicals including pesticides and industrial pollutants, making them less phytotoxic. The recovery of an increasing number of plant P450 genes in recombinant form has enabled their use in experimentation, which has revealed their extraordinary potential for engineering herbicide tolerance, biosafening, bioremediation and green chemistry.

One of the striking features emerging from the various plant genome sequencing projects is the extraordinary diversity of the cytochrome P450 superfamily of oxygenases in higher plants: ~300 genes are expected in the diminutive genome of Arabidopsis (http://drnelson.utmem.edu/CytochromeP450.html; http://ag.arizona.edu/p450/). It is now clear that P450s form the largest class of plant enzymes, and that several hundreds of P450 proteins are probably encoded by most plant species. Even if the number of P450 genes is not proportional to the size of each genome, this number might be larger in the case of polyplloid crops. Some essential P450 functions are conserved among plant species1,2, including hormone, sterol and oxygenated fatty acid synthesis. Others, probably the majority, are involved in aspects of secondary metabolism that differ from plant to plant. Consequently, P450 number and substrate specificity also differs from plant to plant. This is one of the reasons for herbicide selectivity.

P450s are heme proteins that use electrons from NADPH to catalyse the activation of molecular oxygen. The catalysed reaction is usually a mono-oxygenation, with the formation of a molecule of water and an oxygenated product (Eqn 1), but other more atypical activities, such as dimerizations, isomerizations, dehydrations and reductions, have also been reported3,4.

$$\text{R-H} + \text{O}_2 + \text{NADPH} + \text{H}^+ \rightarrow \text{R-OH} + \text{H}_2\text{O} + \text{NADP}^+ \quad (1)$$

Electrons from NADPH are transferred one by one to P450s via FAD and FMN flavoproteins called cytochrome P450-reductases. Both plant P450s and their reductases are usually bound via their N-terminus to the cytoplasmic surface of the endoplasmic reticulum.

Catalysis is inhibited by carbon monoxide, which is a high-affinity ligand of the reduced-enzyme and competes with oxygen. The reduced-carbon monoxide complex absorbs light at 450 nm. This is a major characteristic of P450 proteins (P450 means ‘pigment absorbing at 450 nm’). Carbon monoxide can be displaced by light, with a maximum efficiency at 450 nm.

Common structural features

P450 proteins have molecular masses ranging from 45 to 62 kDa, and might have as little as 16% amino acid identity. However, their overall tridimensional structure is conserved5, as are a few residues on both sides of the heme. A Phe-x-Gly-x-Arg-x-Cys-x-Gly motif near the C-terminus is the most conserved sequence among P450s (Fig. 1). It includes the cysteine that serves as a fifth ligand to the heme iron. Another Ala/Gly-Gly-x-Asp/Glu-Thr-Thr/Ser consensus, located ~150 residues upstream, corresponds to the oxygen-binding and activation groove in the I helix on the distal side of the heme. A few other less conserved motifs, such as a Pro-x-Pro hinge near the N-terminus, or a Pro-Glu/Asp-Arg/His/Pho/Tip sequence between I helix and heme-binding cysteine are found in most P450 proteins.

Such conserved sequences, and their location, are considered to be signatures for the proteins deduced from P450 genes, as are the biochemical characteristics for P450-dependent activities, such as:

- O$_2$-dependence.
- NADPH-dependence.
- Inhibition by carbon monoxide and reversion by light.
- Endoplasmic reticulum location.
- Inhibition by antibodies directed against P450 reductases.

First hints of P450-dependent herbicide metabolism

An indication that P450s might be involved in herbicide metabolism came from the analysis of herbicide residues formed in vivo. Among the major metabolites of most classes of herbicides are aryl- or alkyl-hydroxylated, and N-, S- or O-dealkylated products and their glucuronide conjugates. Dealkylation results from the oxygenation of the carbon to the heteroatom, followed by spontaneous hydrolytic cleavage, and the elimination of aldehyde. Most herbicides, for example prosulfuron, diclofop and chlorotoluron, can be converted by P450s into several metabolites (Figs 2 and 3). In the case of the phenylurea chlorotoluron, detoxification of the herbicide is achieved either via hydroxylation of the ring-methyl, or via di-N-demethylation. The mono-N-demethylated product remains phytotoxic. Chlorotoluron provides a good example of metabolism associated with herbicide selectivity in weeds and crops. In the tolerant winter wheat, the half-life of chlorotoluron is ~24 h, and the main metabolite is ring-methyl hydroxylated. In the susceptible weed Alopecurus myosuroides, the main metabolite is the mono-N-demethylated compound. Phytotoxicity of the metabolite is associated with a half-life of only 6 h, and is converted to the non-phytotoxic di-N-demethylated product6.
P450 inducers and inhibitors behave as herbicide safeners or synergists. Other clues to P450 involvement in vivo can be obtained using P450 inducers (Fig. 4), such as:

- Herbicide safeners (or antidotes).
- Ethanol.
- Drugs, such as phenobarbital, aminopyrine or clofibrate acid.
- Or by using inhibitors (Fig. 4), such as:
  - Compounds with a methylendioxo or acetylenic function.
  - Azoles.

The disadvantage of such effectors is that they are usually selective activators or inhibitors of some P450 isozymes. Their advantage is that they can be used to differentiate between P450 isoforms catalysing different reactions. Some other P450 inducers, such as ethanol, phenobarbital, herbicides or MgCl₂, have partially additive to synergistic effects when added together with safeners. Some of these appear to be active only during specific stages of plant development. One of the P450 inhibitors most commonly used as a synergist to characterize P450-dependent reactions in vivo is 1-aminoxytriazole (ABT). This commercially available molecule is a mechanism-dependent irreversible inactivator of P450 enzymes. It shows some other P450 inducers, such as ethanol, phenobarbital, herbicides or MgCl₂, have partially additive to synergistic effects when added together with safeners. Some of these appear to be active only during specific stages of plant development. One of the P450 inhibitors most commonly used as a synergist to characterize P450-dependent reactions in vivo is 1-aminoxytriazole (ABT). This commercially available molecule is a mechanism-dependent irreversible inactivator of P450 enzymes. It shows little selectivity towards P450 isoforms, especially when used at high concentration (>100 μM) because of its small size and the extreme reactivity of the benzene, which is the product of the oxygenation reaction. Synergism between organophosphate isomelites (e.g. malathion or terbutyl and its metabolites) and herbicides is also an indication of the involvement of P450 in herbicide tolerance, because such isomelites behave as mechanism-based inhibitors of some P450 enzymes.

Weed resistance
Resistance might result from increased metabolism
In the past ten years, an increasing number of herbicide-resistant weed populations have been reported, sometimes showing multiple or cross-resistance to herbicides with different chemistries and target sites. Weeds with several mechanisms of resistance are usually allelic species: resistance mutations accumulate in a single plant as a result of cross-pollination. Resistance mechanisms depend on the weed biotype. They are often related to mutations of target sites, but can also result from increased metabolism, or from both. An increase in P450-dependent metabolism was first demonstrated in resistant Phalaris minor biotypes from Australia and Alopecurus myosuroides biotypes that appeared in Europe. More recently, phenylurea-resistant populations of Phalaris minor in Asia have been described. Because of the isolation of active microsomal fractions from weeds is difficult, evidence for P450 involvement has been provided mainly by in vitro experiments using inhibitors. In most cases, resistance appears to result from an increase in metabolism that can also be detected in the susceptible biotype. Polar products formed by resistant weeds are the same as those isolated from the tolerant crops.
How do weeds become rapid metabolizers?

The mechanism of acquiring increased metabolism remains to be determined. Some ultra-rapid metabolizer phenotypes in humans have been shown to result from the duplication of drug-metabolizing P450 genes. A point-mutation in a structural gene is also possible. Another plausible explanation is the altered regulation of a stress-response pathway, in which one or more P450s are induced. In insects, constitutive overexpression regulated in trans is the only mechanism of P450-mediated resistance to an insecticide reported to date. Recent data on the regulation of glutathione transferases suggest that a similar mechanism might occur in resistant weed biotypes, which would explain some cases of cross-resistance.

In vitro assays

Direct proof of the involvement of plant P450s in herbicide metabolism was obtained as early as 1969 (Ref. 27), when it was shown that the phenylurea monuron was actively dealkylated in cotton microsomes. Together with kaurene hydroxylase, this was one of the first two P450-dependent reactions characterized in higher plants. This work was confirmed with other phenylurea 20 years later, and started a more systematic exploration of the importance of P450s in the metabolism of other classes of herbicides. This work, mostly performed on major crop plants, confirmed the main role of P450s in the oxidation of most classes of herbicides.

In vitro assays allowed more systematic enzymologic studies, confirming the mode of action of some inhibitors, and showed that P450s with catalytic parameters that were different from those of the constitutive forms were induced by chemical treatments. Several P450 isoforms thus appeared to participate in the metabolism of a single herbicide in the same plant. Assays performed with plant microsomes showed that herbicide-metabolizing activities were almost always induced in much larger proportions than the total P450 content. This indicated that the detoxification pathways involve P450 isozymes that are relatively minor, and hence difficult to purify compared with constitutively expressed enzymes. However, the question remained: whether a few specialized P450s were responsible for herbicide metabolism, or if agrochemicals are fortuitous substrates of iso- zymes involved in physiological pathways? Data suggesting that inducers and inhibitors have similar effects on diclofop and lauric acid hydroxylases in wheat microsomes, and that both substrates are mutual competitive inhibitors have provided support for the second hypothesis. In this case, the herbicide and the biological substrate appear to be metabolized by the same P450 protein.

Fig. 2. Examples of cytochrome P450-catalysed oxygenations of herbicides. (a) Different reactions can be catalysed by P450 on a single herbicide molecule. In wheat, prosulfuron is metabolized via phenyl-ring hydroxylation, alkyl hydroxylation and O-demethylation of the triazine ring. It is not yet known if these different reactions are catalysed by a single P450 or different isoforms, although metabolism in wheat, maize and sorghum appear to involve different P450s. (b) Metabolism of diclofop is an example of what is usually called an 'NIH shift'. The oxidative attack of halogenated phenyl rings by P450 enzymes often results in a hydroxylation with simultaneous migration of the halogen atom to an adjacent position. One explanation proposed for this migration is the formation of an intermediate arane oxide.
ferredoxin, to obtain reducing equivalent from NAD(P)H. A ble enzymes that need two electron transporters, a reductase and a bacterium urea metabolizing P450s, from bacteria and mammals. The genes of two inducible sulfonyl-herbicide-metabolizing P450s were not isolated from plants, but of minor plant P450s being difficult, the first genes encoding to help the optimization of new active compounds. The purification demonstrated, the isolation of their genes became a commercial Once the involvement of P450s in herbicide selectivity had been Bacterial genes P450 genes for engineering herbicide tolerance

### Bacterial genes

**Once the involvement of P450s in herbicide selectivity had been demonstrated, the isolation of their genes became a commercial goal for the control of herbicide tolerance in crops and weeds, and to help the optimization of new active compounds. The purification of minor plant P450s, being difficult, the first genes encoding herbicide-metabolizing P450s were not isolated from plants, but from bacteria and mammals. The genes of two inducible sulfonylurea metabolizing P450s, SU1 and SU2, were isolated from the soil bacterium Streptomyces griseolus. These bacterial P450s are soluble enzymes that need two electron transporters, a reductase and a ferredoxin, to obtain reducing equivalent from NAD(P)H. A chloroplast transit sequence was added to the SU1 (CYP105A1) gene to target the protein to the chloroplast stroma, a compartment providing an adequate redox partner (ferredoxin). It was then expressed in tobacco under the control of promoters directing the inhibition of photosynthetic electron transport by the herbicide is suppressed in the recombinant plants. Both CYP71A10 and CYP76B1 also increase resistance to linuron, which can be detoxified by a single dealkylation. The lower part of the figure shows control plants and CYP71A10-transformed plants growing on a medium containing 10 μM chlortoluron. CYP76B1 is a gene from Jerusalem artichoke. The protein expressed in yeast catalyses the double dealkylation of phenylureas. The second dealkylation is slower than the first, but the turnovers of both reactions are high, and comparable to those measured with physiological substrates. When over-expressed in tobacco, CYP71A10 increases its tolerance to chlortoluron. The upper part of the figure shows excised leaf pieces of control plants and CYP71A10-transformed plants that were aged for ten days under light on a medium containing 10 μM chlortoluron. Leaf bleaching caused by the inhibition of photosynthetic electron transport by the herbicide is suppressed in the recombinant plants. Both CYP71A10 and CYP76B1 also increase resistance to linuron, which can be detoxified by a single N-dealkylation. Figure reproduced, with permission, from Ref. 19.

![Fig. 3. Plant cytochromes P450 (P450s) metabolizing phenylurea. P450s metabolize the phenylurea chlortoluron either via ring-methyl hydroxylation or via N-dealkylation. The mono-N-dealkylated product remains phytotoxic, but ring-methyl hydroxylation and di-N-dealkylation lead to non-phytotoxic compounds. The genes of several plant P450s that catalyse these reactions were isolated recently. Their respective turnover rates are indicated next to each reaction in blue boxes. The mono-N-dealkylation of several phenylurea. When over-expressed in tobacco, CYP71A10 increases its tolerance to chlortoluron. The lower part of the figure shows control plants and CYP71A10-transformed plants growing on a medium containing 1 μM chlortoluron. CYP76B1 is a gene from Jerusalem artichoke. The protein expressed in yeast catalyses the double dealkylation of phenylureas. The second dealkylation is slower than the first, but the turnovers of both reactions are high, and comparable to those measured with physiological substrates. When over-expressed in tobacco, CYP71A10 also confers an increased tolerance to chlortoluron. The upper part of the figure shows excised leaf pieces of control plants and CYP71A10-transformed plants that were aged for ten days under light on a medium containing 10 μM chlortoluron. Leaf bleaching caused by the inhibition of photosynthetic electron transport by the herbicide is suppressed in the recombinant plants. Both CYP71A10 and CYP76B1 also increase resistance to linuron, which can be detoxified by a single N-dealkylation. Figure reproduced, with permission, from Ref. 19.

- **Phytotoxic Non-phytotoxic**

<table>
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<th>Reaction</th>
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<th>CYP105A1</th>
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**Fig. 3.** Plant cytochromes P450 (P450s) metabolizing phenylurea. P450s metabolize the phenylurea chlortoluron either via ring-methyl hydroxylation or via N-dealkylation. The mono-N-dealkylated product remains phytotoxic, but ring-methyl hydroxylation and di-N-dealkylation lead to non-phytotoxic compounds. The genes of several plant P450s that catalyse these reactions were isolated recently. Their respective turnover rates are indicated next to each reaction in blue boxes. The mono-N-dealkylation of several phenylurea. When over-expressed in tobacco, CYP71A10 increases its tolerance to chlortoluron. The lower part of the figure shows control plants and CYP71A10-transformed plants growing on a medium containing 1 μM chlortoluron. CYP76B1 is a gene from Jerusalem artichoke. The protein expressed in yeast catalyses the double dealkylation of phenylureas. The second dealkylation is slower than the first, but the turnovers of both reactions are high, and comparable to those measured with physiological substrates. When over-expressed in tobacco, CYP71A10 also confers an increased tolerance to chlortoluron. The upper part of the figure shows excised leaf pieces of control plants and CYP71A10-transformed plants that were aged for ten days under light on a medium containing 10 μM chlortoluron. Leaf bleaching caused by the inhibition of photosynthetic electron transport by the herbicide is suppressed in the recombinant plants. Both CYP71A10 and CYP76B1 also increase resistance to linuron, which can be detoxified by a single N-dealkylation. Figure reproduced, with permission, from Ref. 19.

**P450 genes for engineering herbicide tolerance**

**Bacterial genes**

Once the involvement of P450s in herbicide selectivity had been demonstrated, the isolation of their genes became a commercial goal for the control of herbicide tolerance in crops and weeds, and to help the optimization of new active compounds. The purification of minor plant P450s, being difficult, the first genes encoding herbicide-metabolizing P450s were not isolated from plants, but from bacteria and mammals. The genes of two inducible sulfonylurea metabolizing P450s, SU1 and SU2, were isolated from the soil bacterium Streptomyces griseolus. These bacterial P450s are soluble enzymes that need two electron transporters, a reductase and a ferredoxin, to obtain reducing equivalent from NAD(P)H. A chloroplast transit sequence was added to the SU1 (CYP105A1) gene to target the protein to the chloroplast stroma, a compartment providing an adequate redox partner (ferredoxin). It was then expressed in tobacco under the control of promoters directing ectopic expression, or a promoter specific for the tapetum. When the gene product is targeted to the chloroplast in this way, it activates the harmless sulfonylurea R7402, which becomes a highly phytotoxic herbicide. When SU1 is expressed in the whole plant, R7402 treatment results in plant death. When the gene is expressed from a tapetum-specific promoter, R7402 treatment of immature flowers results in male sterility. Currently, the SU1/R7402 system is widely used as a negative selection marker (Fig. 5).

Another bacterial P450 gene has been isolated from a *Rhodococcus* strain that is able to grow on thiocarbamates as sole carbon and nitrogen sources. This P450 confers on other bacteria the ability to degrade the herbicide 3-ethyl-dipropylcarbamothioate (EPTC), and to exert a biosafening activity on inoculated maize seedlings; bacteria accumulated in the crop rhizosphere detoxify EPTC present in the soil, thus protecting maize from herbicide injury.

**Mammalian genes**

Only a few mammalian P450s have been shown to play a significant role in the metabolism of drugs and other xenobiotics. Their herbicide-metabolizing ability has been explored to create transgenic plants. This has been achieved largely by exploiting rat CYP1A1 to generate herbicide-tolerant tobacco and potato plants. The enzyme, which was reported previously to metabolize various planar xenobiotics, shows a low substrate specificity, metabolizing herbicides of different chemistries, including atrazine, chlortoluron and pyriminobac-methyl. In addition, CYP1A1...
metabolizes herbicides with low regio-specificity, catalysing both chlortoluron ring-methyl hydroxylation and N-dealkylation, as well as N-dealkylation of atrazine on two different nitrogens. To improve the enzyme efficiency, and input of reducing equivalents, two strategies have been used. The first strategy involves expressing a fusion of CYP1A1 with the yeast P450-reductase in plants, mimicking bacterial P450s with high catalytic turnovers. The second strategy targets the fusion proteins to the chloroplast, the major site of NADPH production under light, to improve coupling of P450 reduction with photosynthetic electron transfer. The fusion protein located in the thylakoid membranes shows enhanced activity under light irradiation. However, the transfer of mammalian genes into plants poses an ethical problem, in particular when they encode a protein such as CYP1A1, which has been shown to activate polyaromatic hydrocarbons into procarcinogens.

Plant P450 genes

The first plant P450s available in recombinant systems that were assayed for herbicide metabolism were enzymes involved in phenoic or lipid pathways. They catalyse extremely low, but detectable, chlortoluron hydroxylation. However, the kinetic constants of the reaction indicate that these P450s do not play a significant role in herbicide detoxification in vivo. Two plant P450s that metabolize herbicides more efficiently have been reported in the past year. Both metabolize phenylurea, but none of the other classes of herbicides tested. One gene, CYP76B1, was isolated from Jerusalem artichoke (Helianthus tuberosus) on the basis of its inducibility by Mn\(^{2+}\) ions or drugs such as amitryptiline and phenobarbital, which also induce mammalian P450 enzymes. When expressed in yeast, together with an Arabidopsis P450 reductase, the CYP76B1 protein catalyses the dealkylation of several planar...
xenobiotics, including phenylurea, with a high turnover comparable to that of physiological substrates. Phenylurea are converted to non-phytotoxic di-N-dealkylated products. A second gene, CYP71A10, was isolated from soybean on the basis of P450-conserved sequences. The CYP71A10 protein expressed in yeast converts chlortoluron both to ring-methyl hydroxylated and, to a lesser extent, mono-N-demethylated compounds, as such behaving like rat CYP1A1. Other phenylureas were exclusively mono-N-demethylated. Catalytic turnover of CYP71A10 with phenylurea is more than ten times lower than that of CYP76B1, which is not so surprising considering its looser regio-specificity. Both genes confer increased tolerance to phenylurea when over-expressed in tobacco (Fig. 3).

Concluding remarks

It is clear that P450 differences in substrate specificity, redundancy or regulation from plant to plant are responsible for herbicide tolerance and selectivity. They are also one of the keys to herbicide cross-resistance, which is becoming more and more frequent in weeds. So far, only a few recombinant plant P450s have been examined for their ability to metabolize exogenous molecules, and those assayed have been tested for a few herbicides, and even fewer pesticides and pollutants. Recent data show that several P450 families can metabolize the same class of herbicides, some being poor metabolizers, and others being much more efficient.
This is a situation reminiscent of that observed for drug metabolism in humans – P450s showing sometimes broad, sometimes narrower, and sometimes no substrate specificity at all. It is not clear if the poor and non-metabolizers are enzymes with essential physiological functions. It is not clear either, if there are P450s specialized in stress response, metabolizing a broad range of exogenous molecules, and if these P450s have a physiological function. The information needed for efficient engineering of herbicide tolerance includes the relative efficiencies of the different isoforms that are able to catalyse detoxification of a given molecule, their substrate specificities, and their physiological impacts.

In the case of human P450s, it is usually considered that 20 out of ~50 P450 genes play a role in the metabolism of drugs and other xenobiotics. In crops, the similar proportion of plant genes participate in herbicide metabolism. Considering the huge number and diversity of P450s in the different plant species, the P450 family might provide an amazing source of catalysts for engineering pesticide tolerance by plant transformation, but also for bioinactivation using soil bacteria. Other possible applications, which have not been exploited to date, include positive markers for plant transformation, soil and waste-water bioremediation and green chemistry. Because P450s are active in the chloroplasts, it is possible to envisage their integration in the chloroplast genome. This would, at the same time, increase the number of copies of relevant genes per plant cell, improve the coupling of electron transfer with photosynthesis, and provide gene containment because plastid genes usually cannot be transmitted by pollen to crop-related weeds.

To date, little is known about the regulation of plant P450 genes or about the mechanisms of acquisition of herbicide tolerance. These mechanisms might be similar in tolerant crops and weeds. It is interesting to note that tolerance appears more frequent in dicotyledonous weeds than in dicot plants. This might be related to the higher GC content of their genome, which would favour recombinational events and gene duplication.

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References

Programmed cell death and aerenchyma formation in roots
Malcolm C. Drew, Chuan-Jiu He and Page W. Morgan

Lyssigenous aerenchyma contributes to the ability of plants to tolerate low-oxygen soil environments, by providing an internal aeration system for the transfer of oxygen from the shoot. However, aerenchyma formation requires the death of cells in the root cortex. In maize, hypoxia stimulates ethylene production, which in turn activates a signal transduction pathway involving phosphoinositides and Ca²⁺. Death occurs in a predictable pattern, regulated by a hormone (ethylene) and provides an example of programmed cell death.

R
oots, rhizomes and other plant organs in the soil usually obtain O₂ for respiration directly from their immediate environment – the soil gaseous atmosphere. But when the soil becomes excessively wet, transfer of O₂ from the air into the soil becomes excessively difficult because the larger soil pores, which are usually air-filled, are water-filled instead. Without replenishment from the air, any dissolved O₂ remaining in the soil is quickly consumed by microorganisms and plants, and the soil is then no longer able to supply O₂. Aerenchyma – tissue comprising a high proportion of gas-filled spaces or lacunae – provides the plant with an alternative strategy for obtaining O₂. The interconnected lacunae, extending from below the ground up into the stems and leaves, make up an internal aeration system, enabling parts of the plant to survive or grow for a time in environments that are O₂ deficient or even completely devoid of O₂.

Spaces form within aerenchymatous organs, either by cell separation at the middle lamella during development (shizogeny), or by cell death and dissolution (lyssgeny). Sometimes, as in Sagittaria lancifolia, both processes occur, with lyssigenous aerenchyma in the roots and shizogenous aerenchyma in the leaf petiole[1]. In lyssigenous aerenchyma formation in the root cortex of maize, rice and Sagittaria, cell death is first detected at a distance of a cm or less from the root apical meristem, in the zone where cell elongation has just been completed[2,3]. The space created by the death of cells becomes increasingly prominent in older zones behind the tip. The system of gas-filled lacunae therefore develops acropetally, extending into the root towards the tip, simultaneous with the root’s extension into the soil.

Aerenchyma function
Aerenchyma provides not only an internal pathway for O₂ transfer, but also simultaneously reduces the number of O₂-consuming cells, a feature that might assist in low O₂ environments. It has been suggested that aerenchymatous organs are often water- or fluid-filled[4], a feature that would prevent them from functioning in O₂ transfer. However, evidence from the measurement of gas-filled porosity, microscopic observation (Fig. 1) and O₂ transport[5] is overwhelming – the lacunae are indeed usually gas-filled and therefore are able to transfer gases by convection and diffusion.

Aerenchyma formation is inducible by flooding in maize[6], in the coastal grass Spartina patens[7] and in many other native species, both monocot and dicot, that occupy wetland habitats[8]. By contrast, in the maize relatives Tripsacum dactyloides (eastern gamagrass) and Zea luxurians (teosinte)[9], as well as in wetland species, such as rice[10] and S. lancifolia[11], aerenchyma forms constitutively, apparently without any requirement for an external stimulus. Eastern gamagrass possesses notable tolerance to drought and flooding, which is thought to be because of its deep rooting pattern and constitutive aerenchyma[12]. The roots have been found to exceed 1.8 m in depth, penetrating a resistant clay pan at or below 0.90 m, apparently when the soil was wet and mechanically least resistant. Therefore aerenchyma was likely to have assisted oxygenation of the root at that time. In general, soil compaction can lower the oxygen concentration of the soil (fewer large, gas-filled pores for gas exchange with the atmosphere). Therefore, aerenchyma could be potentially beneficial for growth or function of roots in a densely packed soil layer.


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