



Commentary

Differential responses in sympatric tree species exposed to waterlogging

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Climate change is projected to have a significant ecological impact on natural ecosystems, most notably through direct and indirect modifications of local precipitation regimes. In addition, anthropic activities such as the removal of vegetation, soil proofing due to building, the absence of storm drains and crop over-irrigation will all increase the occurrence of flooding. As a result, forest species, and more specifically trees, will increasingly be exposed to soil waterlogging. It is now well established that such flooding events can lead to changes in forest distribution and composition. For such reasons, it is becoming increasingly important to study forest ecosystems and more particularly the adaptive potential of tree species to better understand the ecological plasticity of forest communities to environmental modifications.

Oak is widespread in the northern hemisphere, where it represents the dominant vegetation of temperate forests. Two sympatric oak species predominate in temperate forest ecosystems, sessile oak (*Quercus petraea* Matt. Liebl.) and pedunculate oak (*Quercus robur* L.). The two species cohabit in most of Europe, but they have different local distributions and have different requirements towards various environmental factors such as light, soil, nutrition and humidity and thus generally do not occupy the same ecological niche (Epron and Dreyer, 1990). Sessile oak grows on dense, well-aerated and acidic soils while pedunculate oak prefers compact, calcareous and hydromorphic ground (Levy et al. 1992). As a result, the two species exhibit differential tolerance to flooding, with sessile oak considered more sensitive than pedunculate oak (Parelle et al. 2006). Many phenotypic differences distinguish the two species, but they are genetically close and, even if molecular differences exist, they are difficult to identify. However, these species offer a great opportunity to investigate inter-specific differentiation between sympatric species. Recent research has targeted specific genes differentially expressed in both oak species, such as those implicated in the response to flooding (Folzer et al. 2006, Parelle et al. 2006, Brendel et al.

2008, Parent et al. 2008, 2011). However, to date there has been no global approach to identify genes belonging to functional families or associated with the differential tolerance to flooding in these two species.

In their paper, Le Provost et al. (2012) have used a global approach to identify genes differentially regulated during flooding in two closely related tree species. This novel approach was used to evaluate if differentially expressed genes were associated with ecological preferences and thus underlie adaptive divergence between the two species. The authors found significant differences in gene expression in the roots of rooted cuttings of both oak species grown in hydroponics and subjected to gradual hypoxia. The most abundant functional categories were genes related to glycolysis, cellular communication, carbohydrate metabolism and protein synthesis. These data support the idea that some components of the response to waterlogging are broadly conserved in plants and that a universal core set of gene orthologs exists across kingdoms (Mustroph et al. 2010, Christianson et al. 2010a, 2010b). Early transcriptome differences in hypoxia-treated roots from cotton, *Arabidopsis* and gray poplar indicated that about 4–10% of the genes were differentially expressed in response to hypoxia

(Christianson et al. 2010a). This large set of genes contrasts with the unaffected transcript abundance reported in leaves of gray poplar exposed to root hypoxia, thus suggesting variable species and organ adaptations (Kreuzwieser et al. 2009). In their study, Le Provost et al. also found a small number of genes which exhibited a strong induction following root hypoxia. Furthermore, among 24 genes with a strong treatment response, 11 exhibited a differential expression between both species, thus suggesting that differences in tolerance in closely related species may be restricted to a small set of genes. These included genes implicated in the signaling pathways involving reactive oxygen species, calcium and ethylene (Ras-related leucine-rich repeat protein 3, calmodulin and 1-aminocyclopropane-1-carboxylate oxidase), genes assigned to glycolysis/gluconeogenesis (pyruvate kinase, fructose-biphosphate and β -amylase) and fermentation (alcohol dehydrogenase), others involved in protein synthesis and fate (translation initiator factor 5) and a gene involved in regulation of cellular water transport (intrinsic membrane aquaporin). All these genes were more rapidly and intensively induced in pedunculate oak, thus suggesting that they may be closely associated with the higher tolerance of pedunculate oak to waterlogging. It is interesting to note that hypoxia-induced adjustments involve sensing and signaling mechanisms that induce complex reconfiguration in the genome and that these signaling cascades

(signaling protein, transcription factors) are largely plant or species specific (Mustroph et al. 2010). The data presented here would tend to indicate that in the case of oak, it is the timing and the intensity of the signaling network which differs between both species and not its specificity.

Put into perspective, the results presented by Le Provost et al. support the idea that tolerance to waterlogging in oak may be linked to rapidity and efficiency of the response, followed by the induction of specific morphological adaptations which help alleviate the adverse effect of anaerobiosis. Indeed, as presented in Figure 1, it is generally accepted that the first step towards tolerance to waterlogging is the capacity to sense changes in the environment followed by the signaling network involved in adjusting metabolism to the adverse effect of O_2 decline. To this effect, the induction of genes involved in alternative metabolism (glycolysis, fermentation) is regulated by a complex array of signaling pathways including secondary messengers such as reactive oxygen species and calcium. The level of these messengers is tightly regulated through production/release (calcium stores, mitochondria, NADPH oxidase, etc.) or binding/detoxification (calmodulin, antioxidant enzymes). Following the induction of these early signaling molecules, genes coding enzymes involved in carbohydrate or alternative metabolism (glycolysis, fermentation) as well as other signaling pathways are induced (i.e., ethylene). Indeed,

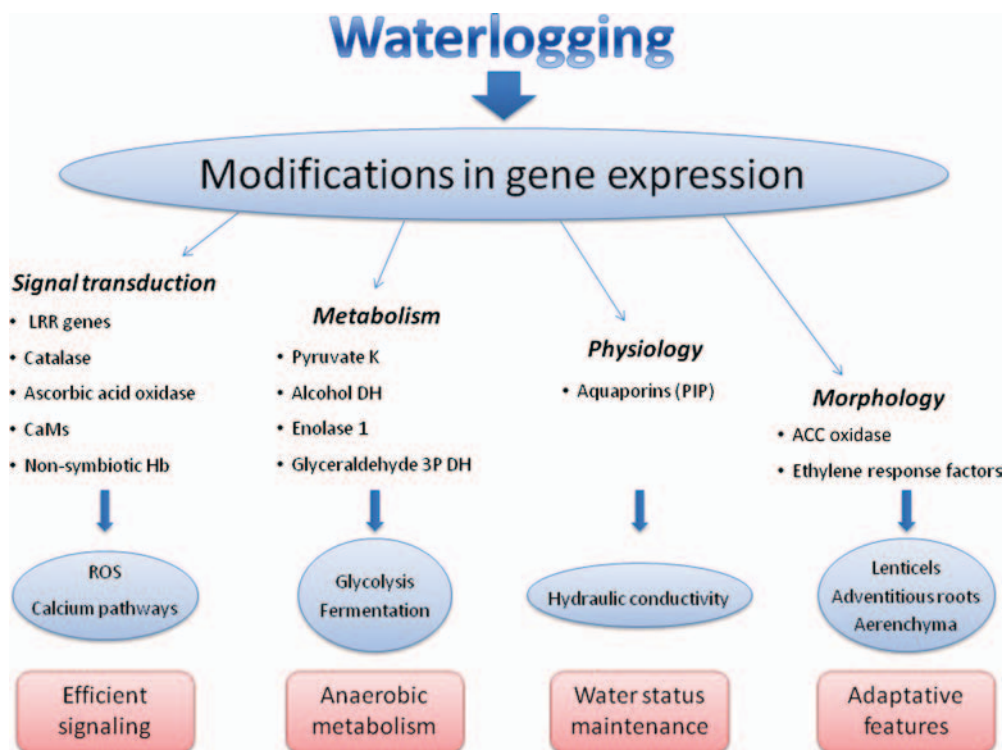


Figure 1. Changes in gene expression and subsequent adaptive features during the response of oak to waterlogging. Genes known to be involved in the response of oak to waterlogging are indicated, with their associated function (blue boxes) and modifications linked to tolerance (red boxes).

as anoxia conditions develop in the roots, respiration will decline as the electron flow through the respiratory pathway is reduced, thus diminishing ATP production. Consequently, chemical oxidizing power (i.e., nicotinamide adenine dinucleotide, NAD⁺) must be generated via alternative pathways that do not use O₂ as a reductant acceptor (Drew 1997, Summers et al. 2000). As limitation of adenosine diphosphate (ADP) oxidative phosphorylation occurs, plants shift their metabolism. This switch from aerobic respiration to anaerobic fermentation involves the induction of glycolytic and fermentative genes (Peng et al. 2001) and occurs in both tolerant and intolerant species (Fukao and Bailey-Serres 2004). However, it is generally observed that the kinetics of induction of the various enzymes involved in glycolysis and fermentation is very much dependent on the tolerance level (Umeda and Uchimiya 1994), as observed for pedunculate oak. As alternative metabolism steps in, other signaling cascades (ethylene, nitric oxide, non-symbiotic hemoglobin) will also be initiated to trigger long-term adaptive features. One of the best characterized molecules involved in these events is ethylene. Indeed, ethylene is one of the prime signals controlling apoplast acidification, transcription of expansin (Vreeburg et al. 2005), adventitious root growth (Mergemann and Sauter 2000, Bragina et al. 2001) and aerenchyma formation (Gunawardena et al. 2001, Evans 2003, Colmer et al. 2006). All these events are directly or indirectly linked to the development of adaptive features (i.e., aerenchyma, adventitious roots) which will help cope directly with oxygen shortages and maintain plant water homeostasis. The induction of some of these adaptive features has already been described in the more waterlogging-tolerant pedunculate oak (Parent et al. 2011), and this supports the results of Le Provost et al. that early differential changes in gene expression could be key elements of tolerance to waterlogging.

In conclusion, the paper by Le Provost et al. is an interesting insight into our broad understanding of the differential response of two closely related oak species to waterlogging. One main observation is that the more flooding-tolerant pedunculate oak is generally less affected by hypoxia than sessile oak, and differences in gene expression during the early response lead to rapid modifications in metabolism which preclude long-term morphological adaptations such as lenticels, aerenchyma and adventitious roots. The results presented in the study also confirm that some of the key metabolic factors responsible for enhanced tolerance to hypoxia are conserved across kingdoms and that they may serve as markers of tolerance in closely related species. For instance, signaling messengers such as ethylene may be promising candidates for manipulating waterlogging tolerance in tree species. The data coupled to further analysis of the relationship between gene expression and metabolic and/or morphological adaptations may also help predict tree species survival

under climate change. Further studies will need to confirm these findings under more 'natural' conditions and expand the analysis to other plant organs and tissues. In addition, the temporal pattern of the marker genes identified and consequent metabolic or physiological changes during recovery may also help assess the relative importance of the selected genes for waterlogging tolerance. Finally, future studies will need to assess the relationship between physiological traits and post-stress recovery in order to help direct strategies to manipulate waterlogging tolerance in oak.

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