

REVIEW PAPER

Effects of genetic modification of roots for abiotic stress resistance and phytoremediation potential on rhizosphere processes and plant performance

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Abstract

Genetic modification of plants has become common practice. However, root-specific genetic modifications have only recently been advocated. Here, a review is presented regarding how root-specific modifications can have both plant internal and rhizosphere-mediated effects on aboveground plant properties and plant performance. Plant internal effects refer to pleiotropic processes such as transportation of the modified gene product. Rhizosphere-mediated effects refer to altered plant–rhizosphere interactions, which subsequently feed back to the plant. Such plant–soil feedback mechanisms have been demonstrated both in natural systems and in crops. Here how plant internal and rhizosphere-mediated effects could enhance or counteract improvements in plant properties for which the genetic modification was intended is discussed. A literature survey revealed that rice is the most commonly studied crop species in the context of root-specific transgenesis, predominantly in relation to stress tolerance. Phytoremediation, a process in which plants are used to clean up pollutants, is also often an objective when transforming roots. These two examples are used to review potential effects of root genetic modifications on shoots. There are several examples in which root-specific genetic modifications only lead to better plant performance if the genes are specifically expressed in roots. Constitutive expression can even result in modified plants that perform worse than non-modified plants. Rhizosphere effects have rarely been examined, but clearly genetic modification of roots can influence rhizosphere interactions, which in turn can affect shoot properties. Indeed, field studies with root-transformed plants frequently show negative effects on shoots that are not seen in laboratory studies. This might be due to the simplified environments that are used in laboratories which lack the full range of plant–rhizosphere interactions that are present in the field.

Key words: Hyperaccumulator, non-target soil, *Oryza sativa*, phytoremediation, plant–soil feedback, rice, transgenic root.

Introduction

Roots and shoots are distinctly different in both form and function. Both organs have different gene expression patterns and metabolic profiles, and respond differently to environmental factors (Barabasz *et al.*, 2010). Despite these differences, roots and shoots are intimately connected and mutually dependent on each other. Roots provide anchoring and supply nutrients and water; shoots in turn fix

carbon and supply energy for growth and reproduction. Shoots and roots can interact via the transportation of plant metabolites, nutrients, and water through phloem and xylem (Dodd, 2005). Changes in roots therefore can, but do not necessarily have to, affect shoot processes. For example, when roots are exposed to stress, the biomass of the shoots may increase, as observed in response to some root biotic

stresses (Wurst *et al.*, 2006), or decrease, as observed for a number of root abiotic stresses (Albacete *et al.*, 2008). A similar variation in shoot responses to root stress has been observed for other shoot characteristics such as the metabolite content of leaves (Kabouw *et al.*, 2011) or gene expression patterns (Jeong *et al.*, 2010). Potentially roots and shoots also respond differently to genetic modification; that is, the introduction, overexpression, or silencing of a gene (Nap *et al.*, 2003). A novel modification that is introduced into a plant can thus be beneficial for roots and processes mediated by roots, but detrimental for shoot properties, which may impede positive effects on plant production or yield.

The representation of genetically modified (GM) crops in agriculture is globally increasing. GM crops are now grown in 29 countries, mainly owing to their enhanced productivity (James, 2010). However, genetic modifications in commercially available crops so far target specific aboveground properties, either by constitutive expression throughout the plant or by tissue-specific expression in the shoot (e.g. herbicide tolerance or insect resistance) (James, 2010). Modifications of root properties have only recently been recognized as an option to improve plant properties (Ghanem *et al.*, 2011), and studies aiming at genetic modifications that target root properties are becoming increasingly popular (see Supplementary Fig. S1 available at *JXB* online). This review focuses on root genetic modifications in plants that have not been grafted; that is, in which the genetic origin of the rootstock is not different from the rest of the plant (root-specific transgenesis). Genetic modifications that target root properties might interfere either positively or negatively with aboveground processes, similar to the pleiotropic effects that have been demonstrated for aboveground-targeted modifications (Groot and Dicke, 2002). For example, the introduction of genes producing Bt proteins, one of the most commonly studied transformations, can either increase or decrease shoot lignin concentrations in maize (Escher *et al.*, 2001; Saxena and Stotzky, 2001), indicating how variable secondary effects of genetic modifications can be. Effects of genetic modifications on traits other than the targeted trait can arise either by plant internal or by rhizosphere-mediated mechanisms.

Plant internal mechanisms are pleiotropic effects of genetic modifications that are caused by processes occurring within the plant. Rhizosphere-mediated mechanisms refer to effects that alter the plant's biotic or abiotic interactions, which subsequently feed back to the plant. Plant internal effects can occur if the modified gene targets root processes without having a root tissue-specific expression. For example, a gene that is introduced to enhance resistance against soil pathogens may also be expressed in aerial parts (Fig. 1, pathway *a*). These effects can be positive, for instance if the expression in aerial parts also provides resistance against aboveground pathogens, or negative, for instance if the gene product interferes with primary metabolism in aerial parts (Ge *et al.*, 2004). Plant internal effects can also occur if expression of the gene is restricted to the roots, but the

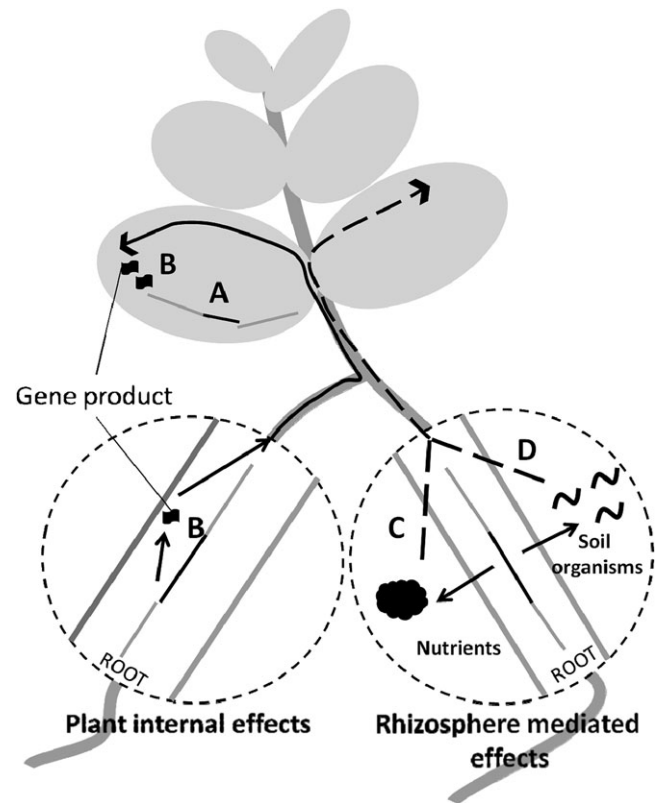


Fig. 1. Illustration of how genetic modifications targeted at roots could potentially affect shoot processes and performance. Effects can be plant internal (A) through expression of the modified gene in the shoot (pathway *a*) or (B) through translocation of the modified gene product (e.g. RNA or a protein) from the root to the shoot or through root-to-shoot signalling (pathway *b*). Effects can also be through altered rhizosphere interactions of the modified gene by (C) increasing or decreasing nutrient mobilization (pathway *c*) or (D) by altering interactions with soil beneficial and detrimental organisms (pathway *d*). Solid lines are plant internal effects and dotted lines are rhizosphere-mediated effects.

product(s) that are formed by the modified gene are transported from the roots into aerial parts (Fig. 1, pathway *b*). Like for pathway *a*, these effects may be either positive or negative.

Rhizosphere-mediated effects of root-targeted genetic modifications can occur through feedback loops of the root modification with the rhizosphere. For instance, the modified gene or its product can positively or negatively affect the mobilization of plant nutrients (Fig. 1, pathway *c*). Positive effects on mobilization of nutrients may result in positive effects aboveground, whereas negative effects on mobilization may result in self-inhibition. This means that when the plant grows, more modified gene product will be released and fewer nutrients will be mobilized. Another rhizosphere-mediated pathway is that the modification can influence the abundance of soil organisms (Fig. 1, pathway *d*). This may result in positive effects if the modified gene unintentionally causes a reduction of belowground pathogens, for example if its product is toxic for plant pathogens, or by increasing the abundance of beneficial organisms, for example

plant growth-promoting rhizobacteria. However, this rhizosphere-mediated effect can also be negative, for instance if it reduces beneficial soil organisms that control plant pathogens or mobilize nutrients (Groot and Dicke, 2002).

Plant internal pleiotropic effects of root modifications on shoots have thus far received most attention, while rhizosphere-mediated effects have frequently been ignored. However, these rhizosphere-mediated effects, commonly called plant–soil feedback, can be just as important. Plant–soil feedback is thought to affect a range of biological processes in both wild and cultivated plants, including plant fitness, plant invasions, maintaining a diverse plant community, and succession of plants (for a review, see Ehrenfeld *et al.*, 2005). Variation in plant–soil feedbacks can occur due to variation in even a single trait (Ehrenfeld *et al.*, 2005) and, therefore, genetic modification of a single trait could also influence plant–soil feedback.

If rhizosphere-mediated effects of genetic modifications indeed enhance or reduce their intended benefit (e.g. an increase in productivity), this could explain the genotype by environment interactions that are often recorded when combining field and greenhouse trials with GM plants (Anand *et al.*, 2003; Birch *et al.*, 2007; Moon *et al.*, 2007). Greenhouse studies may involve less complex root–rhizosphere interactions than field studies (e.g. if horticultural substrates are used in which the diversity of soil organisms is lower than in the field). Therefore, modified genes might have a beneficial effect in the greenhouse that is not counteracted by negative effects through altered plant–soil feedback. In field studies with more complex rhizosphere interactions (e.g. a diverse soil community and, for example, the presence of larger soil biota), negative effects of the genetic modification through altered interactions can potentially arise. However, effects of genetic modifications on plant performance through plant–soil feedback have hardly been reported, as normally only the net effect of a modified gene on aboveground plant properties, such as productivity, is considered. Here, evidence is discussed showing that modifications targeted at root processes can affect the properties of aerial plant parts through both rhizosphere and plant internal mechanisms.

Most common root transformations and transformed plant species

As it has only recently been recognized that modification of root traits is a feasible option for improving plant properties (Ghanem *et al.*, 2011), no plants with modifications of root-specific processes have been released commercially yet (Macek *et al.*, 2008). Therefore, this discussion involves root-specific modifications that have been reported most frequently in the literature and this will be taken as an indicator of future commercial release. To identify the most commonly transformed species and the nature of envisaged root-specific modifications, the most common keywords in scientific studies when searching for the term ‘transgenic root’ were evaluated (see Supplementary Fig. S2 at *JXB* online for an explanation). The plant species that were most commonly recorded among these keywords are *Arabidopsis*

(in 30% of the studies), tobacco (14%), rice (11%), maize (8%), *Medicago* (5%), potato, and tomato (both 4%). *Arabidopsis* and tobacco are both mainly used as model species. As rice seems to be one of the most commonly reported crop plants in relation to transformed roots, this species is used here as a case study. The other commonly encountered keyword that will be discussed is ‘phytoremediation’ (5% of the studies), meaning the use of plants to remove toxic compounds from polluted sites. Based on these keywords, the possible plant internal and rhizosphere-mediated effects of modifying rice roots and transforming plant roots for phytoremediation will be discussed.

Case study one: root modifications in rice

Rice is the most important crop in the world as it provides food for roughly 2 billion people (Khush, 1997). However, it is also relatively prone to abiotic stresses such as drought and salt (Jeong *et al.*, 2010; Plett *et al.*, 2010). Therefore, introducing tolerance to these stresses is the focus of many studies. Indeed, when examining the keywords that are frequently encountered in combination with ‘rice’, it was observed that ‘tolerance’ and ‘resistance’ to ‘drought’ and ‘salt’ are common transformations. In 11% and 6% of the studies, the words ‘salt’ or ‘drought’ are encountered, respectively. Thus, transformations increasing tolerance to salt and drought will be focused on, although other modifications will also be mentioned. Tolerance to abiotic stress through genetic modification of roots can be achieved by several processes, for example, by physiological, cellular, and molecular adaptation (Nguyen *et al.*, 1997; Mitra, 2001; Shinozaki and Yamaguchi-Shinozaki, 2007).

Plant internal effects of introducing root-specific genes

One of the ways to achieve enhanced drought tolerance in rice varieties is by genetic modification that results in plants producing more or deeper growing roots. However, this increased allocation of energy and resources to the roots might impair shoot growth (Price *et al.*, 2002). Indeed, differences in root length between conventional rice varieties are correlated with shoot properties such as tiller number (Champoux *et al.*, 1995; Price *et al.*, 1997). Whether these correlations are negative or positive depends on the intensity of the drought (Babu *et al.*, 2003).

Overexpressing the *OsNAC10* gene in rice, a gene involved in auxin signalling, with either a constitutive or a root-specific promoter, resulted in increased drought tolerance under laboratory conditions. Compared with plants that had been transformed with a constitutive promoter, plants transformed with a root-specific promoter had a larger root diameter (Jeong *et al.*, 2010) and a more efficient photosynthesis under laboratory conditions (Redillas *et al.*, 2011), and higher grain yield under drought conditions in the field (Jeong *et al.*, 2010). The mechanism underlying the increased grain yield of root-transformed plants under drought conditions was not elucidated. This might have

been related to decreased whole plant transpiration rates. However, whatever the mechanism, it indicates that under drought conditions, alterations associated with root-specific expression of the gene had a net positive effect on yield, that was apparently counteracted if the gene was constitutively expressed (negative pathway *a* effect, Fig. 1).

In another study, rice plants were made more tolerant to salt stress by introducing an *Arabidopsis* gene (*AtHKT1*) that is responsible for maintaining a low Na^+ translocation to the shoot by retrieval of Na^+ from the transpiration stream and sequestering Na^+ in roots (Plett *et al.*, 2010). However, increased tolerance was only achieved when the modified gene was specifically expressed in the root cortex. If the gene was constitutively expressed, plant growth was negatively affected. The constitutive expression of the gene might have increased Na^+ influx in all plant cells, counteracting the reduction in root to shoot Na^+ translocation (Moller *et al.*, 2009; Plett *et al.*, 2010) (Fig. 1, pathway *a*). The plant internal pleiotropic negative effect of the modified gene when expressed in the shoots thus outweighs its benefit of the root process of increased sequestration.

Rhizosphere-mediated effects of introducing root-specific genes

Conventional varieties of rice are sensitive to changes in the rhizosphere. This can subsequently result in negative or positive effects on plant properties. For example, the addition of the soil protozoan *Acanthamoeba castellanii* induced changes in rhizosphere bacterial community composition and root architecture of rice (Kreuzer *et al.*, 2006). The effect on root architecture might have been due either to nutrient alteration by the protozoa or to their selective grazing on the rhizosphere bacterial community. For instance, plant growth-promoting rhizobacteria can affect plant hormone status, thereby altering both root architecture and plant root-to-shoot hormonal signalling regulating leaf growth (Dodd *et al.*, 2010). Effects of selective grazing by the protozoa on these rhizobacteria could thus alter both root architecture and shoot processes. In this example, the number of roots decreased, whereas the average length of the roots increased (Kreuzer *et al.*, 2006). This subsequently affected the shoot, which contained more nitrogen when exposed to protozoa (Fig. 1, pathway *d*), demonstrating that changes in the rhizosphere community composition can result in altered aboveground properties. Based on this, it is expected that if a genetic modification changes the abundance or diversity of soil organisms, as was mimicked by the addition of protozoa, this could give rise to either positive or negative plant–soil feedbacks. Several modifications that have been introduced in rice specifically target soil pathogens. These modifications could potentially affect non-target soil organisms and subsequently shoots, but, since they have mainly been studied in laboratory trials in the absence of non-target organisms, such effects have not yet been documented.

Genetic modifications to enhance drought or salt tolerance in rice can potentially affect non-target organisms or

nutrient mobilization. However, to date, no studies have addressed these issues, so their possible effects can only be speculated on. For instance, Jeong *et al.* (2010) showed that modification of drought tolerance in rice by overexpressing the *OsNAC10* gene in the roots resulted in pleiotropic effects on other genes that have a key function in nutrient mobilization. It was recorded that heavy metal transporter genes in the roots, and to a lesser extent in the shoots, were up-regulated as a result of the root-targeted modification. Consequently, modifying roots to enhance drought tolerance might increase metal uptake (Fig. 1, pathway *c*), which could result in higher metal concentrations in aboveground plant parts. This can lead to both positive and negative effects in aboveground plant parts, as metals represent essential nutrients but can also lead to oxidative stress.

Modifications in rice that do not target drought or salt tolerance but that target enhanced control of fungal pathogens, for example enhanced root expression of chitinase genes (Xu *et al.*, 1996), have also been found to affect non-target organisms, such as non-pathogenic soil bacteria and mycorrhizal fungi, negatively (Yang *et al.*, 2002). Mycorrhizal colonization of rice is an important determinant for yield (Solaiman and Hirata, 1997), and (partial) loss of mycorrhizal associations can lead to increased root growth but decreased shoot growth (Kothari *et al.*, 1990; Secilia and Bagyaraj, 1994). In another study, GM rice plants constitutively expressing a proteinase inhibitor to control nematodes (*Meloidogyne incognita*) experienced 50% lower abundances of this nematode in laboratory experiments (Vain *et al.*, 1998). In a field study, potato plants transformed with the same gene affected not only nematode abundances but also non-target microbes. In this field study, the modification had no or a negative net effect on biomass production (root, tuber, and shoot) compared with non-transformed potato plants, depending on the season (Cowgill *et al.*, 2002). However, it is difficult to attribute the reduced plant productivity to the effects of the modified gene on soil microbes (Fig. 1, pathway *d*). In both rice and potato, the modified gene was constitutively expressed (Fig. 1, pathway *a*), which could also have counteracted the initial beneficial result of lower plant pathogenic nematode numbers via pleiotropic effects on the shoot.

Rhizosphere-mediated effects may also arise from root-specific modifications due to altered root exudation patterns. Conventional rice cultivars differ in their exudation rate and in the composition of root exudates (Soejima *et al.*, 1992; Aulakh *et al.*, 2001). Root exudates in rice are known to influence plant growth-promoting rhizobacteria (Aulakh *et al.*, 2003). If modification of rice plants leads to pleiotropic effects on plant chemistry, this is likely to be reflected in changes in the rate or composition of the exudates, which then in turn can thus affect soil organisms and plant–rhizosphere interactions.

Case study two: phytoremediation

Many sites in the world are contaminated by pollutants, such as heavy metals, herbicides, and other chemicals.

A common procedure to clean these sites is to excavate them and treat or isolate the soil, which is costly and laborious. As an alternative, plants can be used to accumulate environmental pollutants. This method, called phytoremediation, is becoming increasingly popular (Eapen and D'Souza, 2005). Plants take up the pollutants via their roots, followed by translocation and subsequent sequestration in shoots. However, most plants that accumulate large quantities of pollutants are relatively small (Pomponi *et al.*, 2006; Kawahigashi, 2009). One of the ways to increase the overall efficiency and the rate at which contaminated sites can be cleaned is by modifying pollutant-accumulating plant species to increase their size. Alternatively, large plant species that are normally unable to accumulate pollutants can be genetically modified to increase their tolerance and ability to take up large quantities of pollutants. The popularity and expected future potential of these modifications is reflected in the large number of reviews published on this subject (Kramer and Chardonnens, 2001; Clemens *et al.*, 2002; Gisbert *et al.*, 2003; Eapen and D'Souza, 2005; Kramer, 2005, 2010; James and Strand, 2009; Kawahigashi, 2009; Kotrba *et al.*, 2009; Rascio and Navari-Izzo, 2011). However, from these reviews and the literature survey carried out it is clear that there is not one specific plant species that is frequently transformed, nor is there a specific pollutant at which genetic transformations are targeted. Modifications targeted at roots to increase phytoremediation potential primarily focus on enhancing root tolerance to stress caused by the accumulated pollutants (Arshad *et al.*, 2007), increasing root biomass to accumulate more pollutants (Eapen *et al.*, 2003), and excreting proteins that mobilize or degrade pollutants in the plants' rhizosphere (Wang *et al.*, 2004).

Plant internal effects of introducing root-specific genes

Modifying plants to tolerate higher levels of pollutants such as heavy metals is one of the mechanisms to increase phytoremediation potential. To increase the tolerance to nickel, a common environmental pollutant, a bacterial gene that produces 1-aminocyclopropane-1-carboxylic acid deaminase (ACCd) was introduced in tomato and canola, two crop species that are normally unable to accumulate pollutants and that were used as easily transformable model species. ACCd hydrolyses ACC, which is a precursor of ethylene. Normally exposure to nickel enhances ethylene production which initiates senescence and chlorosis in the shoots and decreases root growth (Arshad *et al.*, 2007). The production of ACCd by transgenic plants should result in decreased stress responses in their roots by decreasing ethylene levels. Tomato and canola plants with root-specific expression of ACCd were significantly better at maintaining their biomass compared with non-transformed plants and plants that constitutively expressed the gene (Grichko *et al.*, 2000; Stearns *et al.*, 2005). Actually, constitutive expression of the ACCd gene resulted in a negative effect on shoot biomass when exposed to nickel, similar to what was observed when non-transformed plants were exposed to

nickel (Grichko *et al.*, 2000; Stearns *et al.*, 2005). Therefore, the inadvertent expression of a root-targeted genetic modification in the shoot in this case resulted in a plant internal negative pleiotropic effect on the shoot (Fig. 1, pathway *a*). Contaminated sites generally contain multiple metals and therefore it is noteworthy that the observed increase in metal accumulation was dependent both on the plant species and on the metal that was accumulated. Canola plants with root-specific expression had higher nickel concentrations than non-transformed plants. In contrast, tomato plants with root-specific expression had lower nickel and higher zinc concentrations than non-transformed plants.

Field tests with the root-specific ACCd-expressing canola lines used by Stearns *et al.* (2005) revealed that these plants were indeed able to maintain a higher biomass than non-transformed plants, similar to the laboratory studies (Farwell *et al.*, 2006). The total nickel content of these field-tested plants was, however, similar to that of non-transformed plants (Farwell *et al.*, 2006). The reason why the intended benefit of the transformation, an overall higher nickel content, as was recorded in the laboratory, was not observed in the field is not entirely clear, but might have resulted from rhizosphere-mediated effects (Fig. 1, pathway *c* or *d*). As no plants under a constitutive promoter were used, the effects of the environment on plant internal pleiotropic effects could not be assessed in the field study by Farwell *et al.* (2006).

Rhizosphere-mediated effects of introducing root-specific genes

Currently root modifications for phytoremediation are mainly evaluated in laboratory studies. It is known that in field situations, transgenic plants that constitutively express genes involved in enhancement of phytoremediation can accumulate larger quantities of pollutants than non-transgenic plants, while maintaining their biomass (Banuelos *et al.*, 2005). For root-transformed plants, such effects have not yet been observed in the field (Farwell *et al.*, 2006). However given the potential of positive rhizosphere-mediated effects, root modifications might well have the desired effect on biomass and accumulation potential when grown on contaminated sites. For instance, field studies with conventional metal-accumulating plants have shown that when specific microorganism communities are present the phytoremediation of metals and biomass increases (Audet and Charest, 2007; Ma *et al.*, 2011). Interestingly, growing metal-accumulating plants specifically promotes these microorganism communities (Audet and Charest, 2007; Pongrac *et al.*, 2008). Thus, there is the potential for a positive feedback, as modifying plants for increased metal uptake can additionally result in favourable soil communities, which in turn increase the amount of accumulated metals (Fig. 1, pathway *d*).

In a laboratory study by Stearns *et al.* (2005), a root-specific promoter was used to increase metal uptake by canola by introducing ACCd. Canola and many other

plants that are genetically transformed for enhanced phytoremediation of heavy metals belong to the Brassicaceae (Eapen and D'Souza, 2005), which are characterized by a class of secondary metabolites, the glucosinolates. Glucosinolates are involved in plant defence (Mithen, 2001) and are considered to be at least partly responsible for the lack of an association of Brassicaceae with mycorrhizal fungi (Vierheilig *et al.*, 2000; Pongrac *et al.*, 2008). Previously, it has been shown that increased metal concentrations lead to decreased glucosinolate concentrations (Poschenrieder *et al.*, 2006; Pongrac *et al.*, 2008). This trade-off enables Brassicaceous plants (e.g. *Thlaspi*) to form associations with mycorrhizal fungi when accumulating high metal concentrations (Pongrac *et al.*, 2008). Genetic modifications that increase metal uptake can therefore potentially have a positive rhizosphere-mediated effect on the plant's fitness through enhanced association with mycorrhizal fungi, provided that these are able to increase plant nutrient uptake (Fig. 1, pathway *d*).

Pollutants such as phenolic compounds can also be removed from contaminated sites *ex planta*. This is achieved by the release of metabolites from the roots that degrade the pollutants in the rhizosphere. For example, transformed *Arabidopsis* and tobacco that excrete laccase degrade and tolerate higher concentrations of phenolics (Wang *et al.*, 2004; Sonoki *et al.*, 2005), whereas wild-type *Arabidopsis* plants suffer from chlorosis under these conditions (Wang *et al.*, 2004). However, the excretion of laccase could result in increased susceptibility to plant pathogens. Several (fungal) plant pathogens naturally excrete laccase (Mayer and Staples, 2002; Strong and Claus, 2011) to enhance their ability to infect host plants, as laccase can play a role in the detoxification of plant metabolites involved in defence (Mayer and Staples, 2002). The excretion of laccase by GM plants could thus result in increased fungal infection by enhancing the detoxification of the plant's own defences. On the other hand, contradicting this idea, it has been observed that some plant species naturally produce laccase in response to fungal pathogens. The functional significance of this is not yet entirely clear (Mayer and Staples, 2002). Possibly, fungal signals responsible for host finding might be disrupted. Either way, the production of laccase is likely to influence the infection rate of plant pathogenic fungi (Fig. 1, pathway *d*). Such changes in the infection rate might result in feedback effects on aerial parts.

Examining rhizosphere-mediated effects of root-targeted modifications

Several methods are available to examine the plant internal pleiotropic effects due the modification of a gene. It can be assessed where and to what extent the modified gene is expressed [e.g. by quantitative PCR (Q-PCR) or green fluorescent protein (GFP) enhancer trap lines], where its gene product is located (only in the root or also in the shoot), and whether other genes are up- or down-regulated due to the modification (e.g. by using microarrays) (Jeong *et al.*, 2010; Plett *et al.*, 2010; Ghanem *et al.*, 2011). Although time

consuming and expensive, these analyses are widely applied and relatively easy compared with the evaluation of rhizosphere effects of modified genes and their mechanisms.

Rhizosphere effects of root-targeted modifications are difficult to establish and even harder to quantify. As a consequence, they are rarely evaluated, as also appears from our literature search. Nevertheless, there are tools available for accurate assessment of these processes. One commonly used approach in soil science when assessing treatment effects is to monitor sensitive indicator species (e.g. mycorrhizal fungi) and/or processes (e.g. nutrient turnover) closely in the rhizosphere. This same approach can be used to examine how the genetic modification of plants affects feedback mechanisms in their rhizosphere. A selection of sensitive indicator non-target species and/or processes can be made that have the potential to feed back to the plant when their abundances or performance are affected by the genetic modification (e.g. mycorrhizal fungi). These species and/or processes can be monitored subsequently (Bruinsma *et al.*, 2003). This approach is relatively straightforward, but carries the risk of missing feedback mechanisms when organisms and/or processes that cause them are initially quantified as trivial and thus not selected and monitored. Another approach is a large-scale evaluation of soil organisms and processes. For instance, functional diversity may be assessed using biochemical techniques such as Biolog plates. With the substantial improvements in the (molecular) toolboxes (Simon and Daniel, 2011), the use of these high-throughput methods is also becoming increasingly more feasible. There are several approaches available for the large-scale evaluation of soil organisms and processes. For instance, denaturing gradient gel electrophoresis (DGGE) can be used to assess changes in rhizosphere microbial community structure. Also next-generation sequencing techniques allow the screening of both the taxonomic (by considering species-specific genes) and functional diversity (by including information on functional genes) of soil organisms (Simon and Daniel, 2011).

Whatever approach is chosen, the evaluation of effects on rhizosphere processes and species composition can improve our understanding of the effects of modified genes. Additionally, the evaluation of these interactions and the use of modern molecular techniques are frequently a requirement for risk assessments (European Food Safety Authority, 2010) and should be initiated at some stage during application for approval of GM crops.

Additionally, greenhouse studies are not always indicative of field effects (see the greenhouse versus field tests of ACCd-expressing canola and potato transformed with a proteinase inhibitor). This can be due to the simplified horticultural soils that are used, which lack the full set of plant–rhizosphere interactions, resulting in overlooking potential effects of plant–soil feedbacks that could occur in the field. Therefore, field studies are always needed to deal with genotype by environment interactions. The use of more complex field soils in greenhouses, which have the full array of plant–rhizosphere interactions, could be helpful in elucidating potential negative or positive plant–soil feedbacks.

Conclusions

It has been argued here that besides potential plant internal pleiotropic effects of root-targeted modifications there might also be effects through altered plant–rhizosphere interactions that feed back to the plant, affecting its growth and reproduction. In a number of cases, genetic modifications that are intended to affect root processes result in enhanced plant performance when the modified gene is specifically expressed in the root compared with when it is expressed in both roots and shoots. Although examples are limited, this was highlighted by several studies (*AtHKT1* expression in rice and ACC expression in tomato and canola) where constitutive expression led to modified plants that actually performed worse than non-transformed plants. With respect to rhizosphere-mediated effects, studies in both natural systems and conventional crop systems have shown that single traits are able to affect plant–rhizosphere interactions and that these altered interactions can have a profound impact on plant performance. Although rarely examined, these effects can also be expected for root-targeted genetic modifications that have the potential to generate a plant–soil feedback effect. These effects could occur if the modification alters the uptake of valuable and/or harmful elements or via changed abundances of pathogenic or beneficial soil organisms. This review shows that for several modifications there could indeed be rhizosphere interactions reducing the beneficial effects intended by the modification.

Evidence for both plant internal and rhizosphere-mediated effects due to root-specific modifications is still limited, mainly due to the paucity of studies evaluating these processes. However, both could be evaluated using new molecular tools. With the advent of new molecular techniques, evaluation of plant internal pleiotropic effects and high-throughput assessment of soil processes is possible and more feasible. Therefore, it is recommended that both effects should be evaluated in early stages of greenhouse and field tests of plants with root-targeted genetic modifications. This can enhance the mechanistic understanding of the modification and increase the quality of the modified plant.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Number of studies encountered when searching for the term ‘transgenic root’.

Figure S2. Flow chart of how the list of keywords obtained from ISI Web of Science by searching for the term ‘transgenic root’ was processed.

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