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REVIEW

Root-targeted biotechnology to mediate hormonal signalling and improve crop stress tolerance

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Abstract Since plant root systems capture both water and nutrients essential for the formation of crop yield, there has been renewed biotechnological focus on root system improvement. Although water and nutrient uptake can be facilitated by membrane proteins known as aquaporins and nutrient transporters, respectively, there is a little evidence that root-localised overexpression of these proteins improves plant growth or stress tolerance. Recent work suggests that the major classes of phytohormones are involved not only in regulating aquaporin and nutrient transporter expression and activity, but also in sculpting root system architecture. Root-specific expression of plant and bacterial phytohormone-related genes, using either root-specific or root-inducible promoters or grafting non-transformed plants onto constitutive hormone producing rootstocks, has examined the role of root hormone production in mediating crop stress tolerance. Root-specific traits such as root system architecture, sensing of edaphic stress and root-to-shoot communication can be exploited to improve resource (water and nutrients) capture and plant development under resource-limited conditions. Thus, root system engineering provides new opportunities to maintain sustainable crop production under changing environmental conditions.

Keywords Abiotic stress · Root-to-shoot signalling · ABA · Cytokinins · ACC · Grafting · IPT · Plant growth promoting rhizobacterium

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Introduction

The Green Revolution has substantially improved world-wide food production, especially by breeding crop varieties

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with improved vigour and harvest index, and by the massive use of water (for irrigation), fertilizers and pesticides, consequently, depleting and contaminating natural resources. Since 1990, the Millenium Development Goals (MDG) have simultaneously targeted halving the proportion of people suffering from hunger by 2015 (Goal 1) and enhancing world environmental sustainability (Goal 7) (Millenium Development Goal Report 2010). So far, progress in this direction has been insufficient as it is difficult to produce extra food without using extra resources (land, water, fertilizers and pesticides), and since climate change has intensified environmental stresses. Producing more food with fewer inputs, by increasing the resource use efficiency of the world's major crops, is necessary to deliver a safe, secure supply of food to a rising global population, while minimising harmful impacts on cropping ecosystems (Royal Society 2009). Increasingly, these aspirations challenge plant scientists, since current crop improvement strategies may not meet the growing food needs of the world.

One major area of crop improvement that has hitherto been comparatively neglected is the role of the plant root system in maximising resource (water and nutrients) capture and sensing and adjusting to environmental stresses such as water deficit, nutrient imbalances, high temperature and soil compaction. Only recently has the scientific community recognised the importance of manipulating plant root systems to produce improved crops with significantly and sustainably elevated yields (Lynch 2007; Gewin 2010). It is also worth noting that the rhizosphere (adjacent to plant roots) is a biological hotspot where microorganisms can play major roles in plant resource capture and in ameliorating plant stress tolerance (Ryan et al. 2009; Dimkpa et al. 2009). This review aims to emphasise the relative importance of the root system in mediating shoot responses to environmental stress and discuss potential biotechnological approaches to improve crop resource use efficiency.

As soil resources are heterogeneously distributed both spatially and temporally, significant efforts have aimed to exploit natural genetic variation in root system architecture (RSA) to optimise resource capture. Nevertheless, additional biotechnological efforts have aimed to manipulate specific genes (notably those encoding aquaporins and nutrient transporters) to alter root system function in order to improve resource capture, essentially independently of effects on RSA (see "Physiological root-targeted approaches to increase crop resource capture"). While RSA is undoubtedly under constitutive genetic control, soil conditions induce adaptive developmental responses in RSA and there is increasing evidence that these are mediated by changes in the concentrations of, or sensitivity to, the major groups of phytohormones (see "Hormonal-regulation of root system architecture"). Given the importance of these hormones in regulating both RSA (Péret et al. 2009) and long-distance root-to-shoot signalling (Dodd 2005) thus influencing shoot development and adaptation to stress (Pérez-Alfocea et al. 2010), various techniques have aimed to alter root system hormone concentrations by exploiting root-specific plant promoters (see "Root-specific promoters to localise transgenic gene expression"), and/or bacterial genes that affect plant hormone status (see "Plant growth promoting rhizobacteria as a source of genes to manipulate plant hormone status"). Furthermore, the surgical technique of grafting (to combine different root and shoot genotypes) allows exploitation of both natural and biotechnological variation in hormone-related traits. While much of this work has considered the responses of such chimeric plants to abiotic stresses (see "Grafting, a horticultural tool to manipulate root-to-shoot hormonal signalling and abiotic stress responses"), such transformations may also provide opportunities to increase root resistance to biotic stresses (pests and diseases—see "Impacts of root hormone status and volatile emission on rhizosphere biotic interactions").

Physiological root-targeted approaches to increase crop resource capture

Virtually, all living root cells contain membrane integrated proteins that facilitate capture of water and nutrients. In recent years, many genes have been cloned that code for nutrient and water transport proteins in plant roots (Amtmann and Blatt 2009; Gojon et al. 2009; Maurel et al. 2010). Although it is tempting to speculate that overexpression of these transporters could increase water and nutrient acquisition and plant performance, there are few supporting examples in the literature.

Aquaporins (AQPs) are channel proteins integrated in plasma and intracellular membranes that allow transport of water, small neutral solutes and gases such as CO_2 (Maurel et al. 2008). Several stresses such as drought and nutrient deficiency decrease root hydraulic conductivity (L_{p_r}) by decreasing AQP activity and gene expression (Javot and Maurel 2002), which can limit plant water uptake and consequently photosynthesis. Although ectopic *AQP* expression can inhibit endogenous gene expression (Jang et al. 2007; Tsuchihira et al. 2010), an increasing number of studies have attempted to overcome stress limitation of plant water status and biomass production by *AQP* overexpression. Some of these demonstrate that *AQP* overexpression is correlated with improved plant performance under optimal (Aharon et al. 2003; Peng et al. 2007) and/or stress conditions (Sade et al. 2009). However, we are aware of only one specific evaluation of the role of the root

system and one functional evaluation of hormonal regulation of these responses.

Constitutive expression of the tobacco aquaporin encoding gene *NtAQP1* in tomato increased leaf photosynthesis and transpiration rates by 25–40% under optimal conditions despite no change in L_{p_r} (root hydraulic conductance) compared with non-transformed plants. Yet *NtAQP1* overexpression had minimal effects on leaf gas exchange (<15% change) despite a doubling of L_{p_r} under 100 mM NaCl (Sade et al. 2010), suggesting that its effects were not root system mediated. In support of this suggestion, reciprocal grafting experiments demonstrated no effect of the rootstock on leaf photosynthesis rate, stomatal conductance and whole plant transpiration of non-transformed scions of salinised and non-salinised plants. However, a non-transformed rootstock decreased mid-day whole plant transpiration of *NtAQP1* scions in the absence of leaf-level responses (Sade et al. 2010). Thus, transgenically increasing L_{p_r} may only affect whole plant carbon gain during periods of maximum evaporative demand.

Hormonal regulation of *AQP* expression and protein activity was demonstrated in maize plants transformed with the *NCED* (*9-cis-epoxycarotenoid dioxygenase*) gene encoding the key enzyme involved in abscisic acid (ABA) synthesis (Parent et al. 2009). Transgenic maize lines expressing *ZmNCED* in sense and antisense orientation increased and decreased xylem sap ABA concentration and expression of *AQP PIP* (*Aquaporin plasma membrane intrinsic protein*) genes in the roots, respectively. These changes resulted in more than sixfold difference between lines in L_{p_r} under both hydrostatic and osmotic gradients of water potential, suggesting that ABA has long-lasting effects on plant hydraulic properties via AQP activity, which contributes to the maintenance of a favourable plant water status and stimulates leaf growth recovery after rewatering (Parent et al. 2009). Based on these studies, Maurel et al. (2010) concluded that the most convincing evidence for a role of AQP during water stress does not concern the primary response of the plant to drought but its growth recovery following rewetting.

While upregulation of nutrient transporters seems to be an attractive biotechnological target to improve crop nutrient status, nutrient absorption by plant roots depends on both influx and efflux and physiological studies have shown that the benefits of increased influx are usually undone by increased efflux (Britto and Kronzucker 2006). Clarkson and Hawkesford (1993) astutely pointed out that more detailed knowledge of the linkages between the processes that consume nutrients and those that provide them are required before more purposeful manipulation of nutrient uptake can be achieved. Many current studies are focused on identifying regulatory elements for nutrient uptake, which will not only increase our understanding of how plants adapt to conditions of nutrient shortage but also provide potential targets for future bioengineering efforts aimed at improving crop performance on marginal soils (Amtmann and Blatt 2009).

Possible hormonal-regulation of crop nutrient uptake aspects is addressed. Few studies have considered direct hormonal regulation of specific transporters to facilitate nutrient acquisition (Gojon et al. 2009; Rubio et al. 2009). Potassium (K) starvation enhances the expression of genes encoding enzymes involved in ethylene (Shin and Schachtman 2004) and jasmonic acid (Armengaud et al. 2004) biosynthesis, and concentrations of these hormones increase in roots and shoots of K-starved plants, respectively (Shin and Schachtman 2004; Cao et al. 2006). However, the exact role(s) of ethylene and jasmonate signals within the K starvation response is unknown. Interestingly, electrophysiological studies with excised barley roots demonstrated that exogenous kinetin (cytokinin) application increased root cell plasmalemma K uptake (Shabala et al. 2009), a possible mechanism for the 20% increase in foliar K concentration of transgenic tomato plants with increased root cytokinin production (Ghanem et al. 2011). Whether this improvement occurs because of increased expression of genes encoding K-transporters and/or increased K transporter activity is unknown, but these results open new root-targeted possibilities to improve K nutrition and crop stress tolerance. However, an opposite effect was reported for sulphate and phosphate transporters since cytokinin inhibits expression of the corresponding genes and suppresses their induction by S or P starvation (Martin et al. 2000; Maruyama-Nakashita et al. 2004; Hou et al. 2005; reviewed by Amtmann and Blatt 2009). Since agricultural soils may contain deficiencies of multiple nutrients, transgenic possibilities to alter root hormone status may only benefit plant nutrient uptake under certain conditions.

Although root-specific modulation of water and nutrient capture via overexpression of genes encoding specific proteins is now achievable, there is currently little evidence that this improves crop resource capture and performance. Furthermore, there is scant evidence that plant hormones are involved in the endogenous regulation of these proteins. Nevertheless, the involvement of plant hormones in root system architectural responses to nutrient and water availability offers an indirect but important strategy to increase resource capture by the plant and to adapt to soil-related constraints (Péret et al. 2009), as discussed below.

Hormonal-regulation of root system architecture

RSA results from both constitutive and adaptive traits that allow the plant to cope with an array of environmental

conditions. This adaptability is associated with phenotypic plasticity of the root system, by interacting with water and nutrient availability in the soil, and with the presence of specific rhizosphere microorganisms, allowing increased resource acquisition by the plant. Indeed, manipulation of RSA can increase plant tolerance to abiotic stresses, thus minimising their negative impact on crop yield (Beeckman 2004; Dorlodot et al. 2007; Coudert et al. 2010). Rhizosphere availability of K, nitrogen (N), phosphorus (P) and sulphur (S) and microelements such as iron (Fe) regulates root system branching as a major strategy to adjust nutrient uptake and soil availability (López-Bucio et al. 2003). Hence, understanding the physiological and genetic mechanisms that regulate RSA is required to manipulate this trait in breeding programmes or via management techniques (Péret et al. 2009).

Root morphology seems to be regulated by small-effect loci–environment interactions. For example, in maize, marker-assisted selection has produced near-isogenic backcross-derived lines for *root-ABA1*, a major QTL affecting foliar ABA concentration and other drought-related traits and grain yield (Landi et al. 2005, 2007). Fine mapping of this QTL has been pursued based on foliar ABA assays rather than RSA itself, assuming that *root-ABA1* effects on RSA (diameter, angle, branching and dry weight) moderate foliar ABA concentration (Giuliani et al. 2005; de Dorlodot et al. 2007). Indeed, ABA seems important among phytohormones in regulating root morphology, although responses to ABA can depend on medium water potential (Sharp et al. 1994—ABA inhibits root growth at high water potential, but is necessary to maintain root growth at low water potential). At high water potential, exogenous ABA application inhibits lateral root formation in peanut (*Arachis hypogaea* L.) by increasing endogenous ABA content and in *Arabidopsis*, *nced3* (*9-cis-epoxycarotenoid dioxygenase 3*) mutants deficient for ABA biosynthesis show more and longer lateral roots (Guo et al. 2009). Recently, a root-specific WNK kinase homolog, GmWNK1, which was identified in soybean (*Glycine max*) that apparently fine-tunes ABA-dependent ABA homeostasis (by interacting with a key ABA-hydroxylase), thereby mediating regulation of RSA by ABA and osmotic signals (Wang et al. 2010). This root-specific protein has been associated with lateral root formation, and expression of the corresponding gene is down-regulated by ABA and sucrose, as well as osmotic and saline stresses. The value of the positional candidate-gene approach for RSA has been demonstrated in *Arabidopsis* where a QTL for root elongation colocalise with a QTL for vacuolar invertase, and the role of this enzyme was confirmed by the phenotype of knock-out mutants for this gene (Sergeeva et al. 2006). The invertase encoding gene in turn seems to be controlled by ABA concentration in maize roots (Trouverie et al. 2004), thus indicating again that ABA levels seem to play a crucial role in regulating root morphology in dry soil.

Although root differentiation is an early event initiated during embryogenesis by intrinsic factors and mainly controlled by a balance between auxin and cytokinins (Müller and Sheen 2008; Bishopp et al. 2010), lateral root formation is an important post-embryonic event that seems primarily auxin-regulated and represents a crucial process in developmental plasticity and plant adaptation to environmental stresses (De Smet et al. 2006a). Thus, understanding key genetic components of these auxin-controlled responses opens new strategies to manipulate RSA. Lateral root formation in *Arabidopsis* has been enhanced by overexpression of *LBD16* and *LBD29* genes and inhibited by dominant repression of *LBD16*. Furthermore, *LBD16* and *LBD29* are directly activated by the auxin response factors ARF7 and ARF19 (Okushima et al. 2007; Hochholdinger and Zimmermann 2008). Auxin-responsive elements also govern root development in cereals, such as *ARL1*/*CRL1* in rice and *RTCS* in maize which encodes a conserved lateral organ boundary domain transcription factor involved in crown root initiation and development (Coudert et al. 2010). In addition to auxins, cytokinins (CKs) also shape the root system by altering lateral root initiation and patterning in *Arabidopsis* (Péret et al. 2009). Likewise in rice, the WUSCHEL-Related Homeobox WOX11 is involved in the control of crown root initiation and development, and interferes with CK signalling elements (Zhao et al. 2009). However, the effectiveness of the candidate gene approach can be limited by a tight crosstalk between plant growth regulators (notably auxins and cytokinins) or hormonal signalling. Indeed, CKs down-regulate the expression of structural genes encoding proteins involved in auxin signalling and polar transport, notably the *PIN* genes, while auxin itself regulates its accumulation in a feedback loop, underlining complexity of the hormonal response (Laplaze et al. 2007; Yadav et al. 2010). Actually CKs induce the expression of *SHY2*, encoding an auxin repressor of the Aux/IAA gene family, which in turn down-regulates expression of the *PIN* genes, resulting in decreased levels of auxin at the root meristem and a decrease in the rate of cell division (Ioio et al. 2008). Cytokinin induces the expression of *SHY2* in the root, which in turn downregulates expression of the *PIN* genes whose corresponding proteins are involved in auxin transport, resulting in decreased levels of auxin at the root meristem and a decrease in the rate of cell division.

Additional phytohormones participate in root system formation, often in an auxin-dependent way (Pérez-Pérez 2007). Indeed, ethylene inhibits lateral root formation and enhances auxin polar transport in *Arabidopsis* and tomato (Negi et al. 2008, 2010). Likewise, gibberellins (GAs) affect lateral root density and elongation by suppressing

primordia initiation in *Populus*, and synergistically act with ABA by down-regulating its biosynthesis. Gibberellins also interact with auxins, as auxin levels increases in GA-deficient and insensitive transgenic roots. Similarly, genes involved in auxin response are affected, since *PtPIN9* expression is repressed after GA treatment (Gou et al. 2010). Some reports indicate a concentration-dependent effect of brassinosteroids on primary root growth similar to that of auxin, namely a promotion at low endogenous concentrations and inhibition at high concentration (Osmont et al. 2007). ABA is also likely to influence auxin polar transport during lateral root development (reviewed in De Smet et al. 2006a, b). In *Arabidopsis*, the MYB transcription factor MYB96 integrates ABA and auxin signals during drought stress response. Mutant plants overexpressing *MYB96* had increased tolerance to drought stress, with reduced lateral roots due to a suppression of meristem activation and lateral root elongation (Seo et al. 2009). In summary, manipulating either ABA or IAA concentrations via altering their metabolism and transport (Tian and Reed 1999; Tanimoto 2005; Ruzicka et al. 2007) or their signalling components or downstream targeted genes involved in sink activity and osmotic adjustment could alter RSA. However, since optimising RSA to improve resource capture does not seem to be easily achieved because of complex genetic, physiological and environmental interactions, root-targeted genetic transformation with hormone-related genes offers additional opportunities to evaluate the role of hormones in root architectural responses.

Root-specific promoters to localise transgenic gene expression

Organ-specific genetic transformation offers the possibility of adjusting gene expression to plant requirements, thus avoiding undesirable pleiotropic effects or excessive energetic costs that could mask putative benefits of transgene expression. For example, hemizygous expression of *AsnA* (encoding a bacterial NH_4^+-dependent asparagine synthase that improves N-assimilation) specifically in roots by grafting increased tomato yield under a moderate saline stress more than its constitutive (whole plant) or homozygous expression (C. Martínez-Andújar 2006, unpublished results; Fig. 1). Similarly, expressing the stress-inducible rice regulator gene *OsNAC10* encoding a transcription factor under the *RCc3* root-specific promoter led to an enlarged root diameter and increased yield under water-limiting conditions compared with wild-type plants or those transformed with the constitutive *GOS2* promoter (Jeong et al. 2010). Although plant hormones involved in regulating shoot growth and development and adaptation to

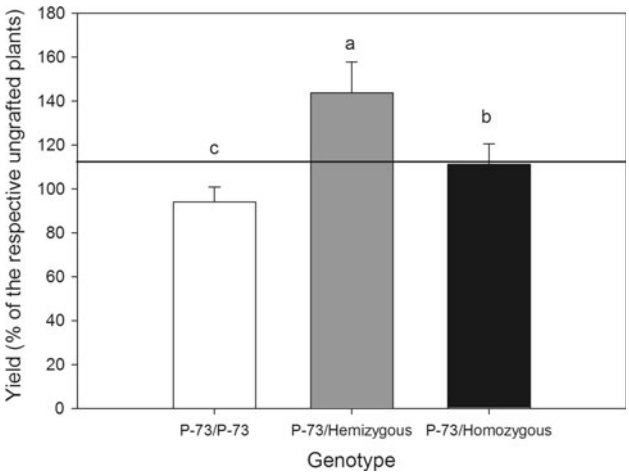

Fig. 1 Fruit yield (as a percentage of ungrafted plants) in grafted tomato plants cultivated in the presence of 75 mM NaCl for 3 months in a greenhouse in SE, Spain. Genotypes were the cultivar P-73 either self grafted (P-73/P-73) or the same genotype overexpressing the *AsnA* gene (Hemizygous and Homozygous) under control of the constitutive *pCPea* promoter (P-73/Hemizygous), (P-73/Homozygous). Data are means of 10 ± SE replicates. *Different letters* indicate significant differences between treatments for a given organ according to Student–Newman–Keuls test at $P < 0.05$ (C. Martinez-Andujar 2006, unpublished results)

biotic and abiotic stresses are often synthesised throughout the plant, there may be agronomic advantages in their selective expression in the root system.

Modifying root hormone production may be valuable since: (1) roots naturally produce hormones, (2) a more controlled production would minimise transgene expression and pleiotropic effects in the shoot and (3) transformed roots could be directly used as rootstocks for grafting compatible species to avoid transgenic events in harvested aerial organs. Therefore, promoters showing simultaneously strong but modulated activity in a strictly root-specific manner may have greater potential benefits than constitutive promoters in a wide range of applications (Bucher 2002). Moreover, since roots sense their environment, root-specific transformation offers possibilities to modulate transgene expression in response to rhizosphere conditions (e.g. salinity, drought, nutrients, temperature, fertilizers, and specific chemicals to induce gene expression).

Agrobacterium rhizogenes may be used to transfer T-DNAs into plant genomic DNA via a conserved T-DNA processing and type IV secretion system (Collier et al. 2005). *A. rhizogenes* infections, which differ from gall-forming infections of *A. tumefaciens*, cause neoplastic, plagiotropic transformed 'hairy' roots which develop from infected plant cells that have integrated a root inducing (Ri) plasmid-derived T-DNA in their genomic DNA (Limpens et al. 2004; Collier et al. 2005). This leads to the production of so-called composite plants comprising a transgenic hairy

root system attached to non-transformed shoots and leaves. This in vitro-induced composite plant decreased the time required to generate transgenic plant tissue in transformation-recalcitrant plants. This technique could also be used for silencing genes in the root via RNA interference reverse genetic tool (Limpens et al. 2004). However, the main disadvantage of the composite plants is that they require costly in vitro conditions for induction (Collier et al. 2005).

The *Pyk10* promoter from *Arabidopsis thaliana* is root- and hypocotyl-specific (Nitz et al. 2001; von Schweinichen and Büttner 2005). Overexpressing a yeast cell wall invertase encoding gene in *Arabidopsis* roots under control of this promoter increased rates of phloem unloading and root development, while it decreased root invertase activity in tobacco plants overexpressing an invertase inhibitor (Schaarschmidt et al. 2007). Since *Pyk10* expression was not influenced by hormones, this gene promoter could be a good candidate for targeted expression of hormonal traits (Siemens et al. 2010). Similarly, a 2.1-kb fragment of a cryptic *CrypticT80* promoter directed expression of the GUS reporter gene mainly to the root of *A. thaliana* (Mollier et al. 2000). Siemens et al. (2010) have recently used both promoters (*Pyk10* and *CrypticT80*) to study the effect of an invertase inhibitor in *Arabidopsis*.

The *Arabidopsis alcohol dehydrogenase* (*Adh*) gene is expressed constitutively in immature seedlings and suspension cells, and may be induced by hypoxic stress only in roots of mature plants (McKendree and Ferl 1992). Using this root-targeted system to express the phytochelatin synthase gene (*TaPCS1*) in *Arabidopsis*, cadmium stress (20 μM $CdCl_2$ for 3 days) stimulated phytochelatin transport from roots to shoots to limit root Cd accumulation and enhance long-distance root to shoot Cd transport (Gong et al. 2003). While this expression system could be used to ameliorate plant stress tolerance when rootzone hypoxia is unavoidable, it is unlikely to have much impact in cropping systems with good soil aeration. Recently, a novel gene isolated from tomato (*SIREO*) displayed high expression in roots but very low expression in aerial plant organs, suggesting it could be useful for root targeted gene expression since this root-specificity was stable throughout plant development and maintained under a range of environmental conditions (Jones et al. 2008). Alternatively the partial *Pinus strobus* 796 bp promoter of the *PsPR10* gene also specifically drives *GUS* expression in tobacco roots, and positively responds to osmotic stresses and hormonal treatments, thus constituting an interesting inducible root-specific promoter (Xu et al. 2010).

Putatively root-specific induction of the CK biosynthetic gene *ipt,* regulated by a heat-shock promoter (by placing pots in a heated water bath for a few hours), transiently increased whole-plant transpiration and foliar CK concentrations in spite of enhanced foliar CKX (cytokinin oxidase) catabolic activity (Vysotskaya et al. 2010). A similar rootzone temperature induction in hydroponics localised *ipt* gene expression to the roots, enhanced root-to-shoot CK signalling, delayed leaf senescence and improved vegetative growth of tomato plants growing under salt (100 mM NaCl) stress (Ghanem et al. 2011) (Fig. 2). Improved CK status was correlated with increased shoot K^+ concentration (20%), and decreased leaf ABA concentrations and foliar Na^+ accumulation rate, at least partially due to the improved shoot growth rate (Ghanem et al. 2011), although a direct effect on ionic homeostasis cannot be ruled out. Thus, additional root-sourced CKs improved salt tolerance through regulating source–sink relations, not only by increasing sink activity (vegetative and fruit growth), but also by maintaining stomatal conductance, delaying leaf senescence and thus increasing source strength. Consequently, this maintenance of photosynthetic leaf area avoided or delayed the accumulation of toxic ions (Ghanem et al. 2008, 2011; Munns and Tester 2008; Albacete et al. 2009; Pérez-Alfocea et al. 2010).

Samalova et al. (2005) have developed the pOp/LhG4 and pOp/LhGR systems for spatial and temporal control of transgene expression in plants. These are based on a chimeric transcription factor, LhG4, comprising a high-affinity DNA-binding mutant of the *E. coli* lac repressor fused to a transcription activation domain from the yeast Gal4 protein. This molecule activates transcription from the pOp

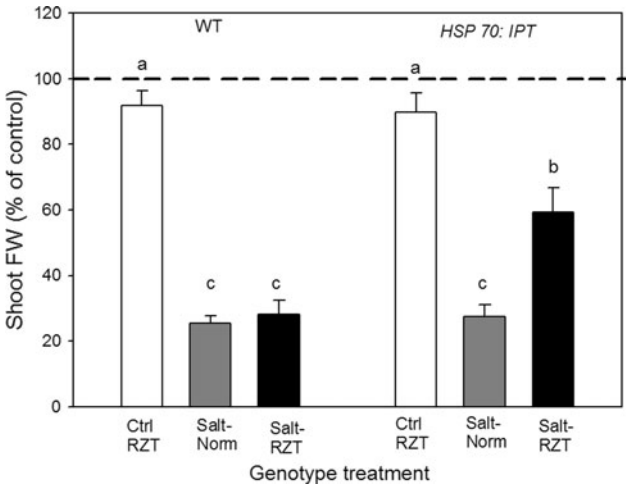

Fig. 2 Shoot fresh weight (as a percentage of unsalinised controls maintained at optimal rootzone temperature) of WT and *HSP70:IPT* tomato plants grown in half-strength Hoagland medium in the presence (salt) or the absence (ctrl) of 100 mM NaCl for 22 days and exposed transiently (2 h at 40°C every week) to elevated (RZT) or optimal (norm) root-zone-temperature. Data are means ± SE of ten replicates. Measurements were performed 48 h after the end of the third episode of elevated root-zone-temperature (22 days of salt treatment). *Different letters* indicate significant differences between treatments for a given organ according to Student–Newman–Keuls test at $P < 0.05$ (replotted from Ghanem et al. 2011)

promoter which is otherwise physiologically silent in transgenic plants. They have generated a collection of lines expressing LhG4 under a series of defined promoters and enhancer traps and these can be used in conjunction with the pOp promoter to express genes of interest in many tissue- and cell-specific patterns. This system is of particular value if a gene of interest needs to be studied in a variety of selected cell types and especially where the expression of the transgene is likely to compromise plant viability or fertility. They have also fused the ligand-binding domain of the rat glucocorticoid receptor to LhG4 to generate a steroid-inducible molecule, LhGR, which provides temporal control over pOp promoter expression (Craft et al. 2005). The pOp/LhGR system in *Arabidopsis* exhibits lower levels of uninduced expression and none of the inhibitory side-effects that affect other inducible expression systems in plants. Lines expressing LhGR under control of tissue-specific promoters might be of particular interest to activate transgenes at defined times in specific cell types like roots.

Transcriptomic analysis of the multiple root cell types and tissues in *Arabidopsis* has allowed the identification of candidate genes triggering root formation and development in no less than 15 discrete zones (Birnbaum et al. 2003). This expression map constitutes a valuable tool to identify root promoters driving expression within specific cell types or at a particular developmental stage. Transcript profiling of root-specific events such as early lateral root initiation has also been performed in *Arabidopsis* and is suitable for targeting a specific stage of development (Himanen et al. 2004). Also, numerous microarray studies of root gene expression responses to abiotic stresses such as high salinity in *Arabidopsis* (Jiang and Deyholos 2006) or tomato (Wei et al. 2000; Ouyang et al. 2007), exogenous nitrate stimulation (Liu et al. 2008) or water-stress in maize plants (Spollen et al. 2008) have been reported. Again, these data constitute a source of information to identify new root-specific promoters to allow gene expression to be modulated by external factors (Oltmanns et al. 2006; Puthoff and Smigocki 2007).

There are also promoters that regulate gene expression only within certain root tissues. Promoters that are root peel-specific and others that are constitutive, i.e. more highly expressed in root than in the leaf have been identified. A sugar beet root parenchyma cell-specific promoter has also been reported by Oltmanns et al. (2006). Three taproot expressed genes recently isolated include the *Mll*, a homologue of the major latex-like protein from *Mesembryanthemum crystallinum*, a thaumatin-like protein (*Tlp*), and a linker histone (*His1-r*) variant. Reporter gene expression analysis in transgenic sugar beet plants revealed that all three promoters are active in the storage root (Oltmanns et al. 2006). Expression in storage root tissues is either restricted to the vascular zone (*Tlp, His1-r*) or observed in the whole organ (*Mll*). The *Mll* gene is highly organ-specific throughout different developmental stages of the sugar beet root. In tobacco, the *Tlp* and *Mll* promoters drive reporter gene expression preferentially in hypocotyl and roots. Furthermore, studies to identify promoters that are expressed in specific root tissues or cells are ongoing (Ann C. Smigocki, unpublished results) and could be used to drive hormone-related genes.

In alfalfa (*Medicago sativa*), the root-specific promoter *MsPRP2* modulated *GFP* expression along the entire root length including the root cap and root hairs, with a high expression in epidermis and the central portion of the root (Winicov et al. 2004), while in tomato roots, *LeExtensin1* was exclusively active in trichoblast cells in the rhizodermis (Bucher et al. 2002). Expressing candidate genes in specific root tissues such as the outer cell layers also seems to be a promising technique to enhance abiotic stress tolerance. Expression of the *AtHKT1;1* gene encoding a sodium (Na^+) transporter in *Arabidopsis* but also in rice root cortex and epidermal cells improved salinity tolerance of transgenic plants. Indeed, rice transformants exhibited a decreased root-to-shoot Na^+ flux, attributed to a higher Na^+ vacuolar sequestration in root cortical cells. These results can also be explained by the enhanced expression of the endogenous gene *OsHKT1;5* encoding a transporter responsible of Na^+ retrieval from the transpiration stream (Plett et al. 2010).

Hence, identification of root-specific promoters may not only allow manipulation of root-specific traits directly or indirectly involved in resource capture, but also allow shoot development through root-to-shoot (hormonal) communication. This potential could be further extended via environmental (e.g. low nutrient inducible) or developmental (e.g. senescence associated expression) self-regulated promoters to avoid pleiotropic effects of constitutively and/or continuously expressing plant hormone biosynthesis genes. Furthermore, root-specific expression of interesting hormone-related genes isolated from rhizosphere microorganisms may allow more reproducible effects on root architecture and root-to-shoot signalling than relying on natural soil-root biotic interactions to ameliorate crop yield penalties (Dodd 2009).

Plant growth promoting rhizobacteria as a source of genes to manipulate plant hormone status

Although both rhizobia and mycorrhizae can synthesise plant hormones and/or regulate their *in planta* concentrations (Costacurta and Vanderleyden 1995; Barker and Tagu 2000; Strack et al. 2003; Tsavkelova et al. 2006), and have already been exploited to improve abiotic stress tolerance,

there is increasing interest in free-living plant growth promoting rhizobacteria (PGPR) as a source of genes to manipulate plant hormone status. These organisms are commonly found in the rhizosphere (adjacent to the root surface) and may promote plant growth via several diverse mechanisms, including the production or degradation of the major phytohormones that regulate plant growth and development (reviewed in Dodd et al. 2010), which can improve plant stress tolerance (Dimkpa et al. 2009). Although there has been intense interest in isolating and applying (as commercial inoculants) beneficial PGPR to crops, rhizobacterial inoculation of field soils can produce inconsistent results due to competition between introduced and indigenous microbes causing poor inoculum persistence in the rhizosphere (Strigul and Kravchenko 2006). Rather than selecting beneficial PGPR based on their putative growth promoting properties observed in vitro (e.g. auxin production, ACC deaminase activity), an alternative strategy may select bacteria for their ability to compete effectively in the rhizosphere, and re-engineer these bacteria with phytohormone-related genes. An added advantage of this approach is that successful PGPR may have multiple growth promoting properties, not all of which may regulate phytohormone concentrations *in planta* (e.g. phosphate solubilisation, associative nitrogen fixation). Nevertheless, there are many examples where up- or down-regulation of the expression of a specific bacterial gene (e.g. those encoding an ACC deaminase that breaks down the ethylene precursor ACC, or iaaM and iaaH that allow bacterial IAA production) has altered the physiological impacts of a specific rhizobacterium (reviewed in Dodd et al. 2010). Consequently, expression of these genes *in planta* may be expected to have physiological impacts, although most of this work has relied on constitutive promoters.

One of the earliest biotechnological efforts at limiting plant ethylene synthesis was constitutive expression of a bacterial ACC deaminase (*ACCd*, which metabolises ACC to alpha-ketobutyrate and ammonia)-encoding gene from *Pseudomonas* sp. strain 6G5 in tomato (Klee et al. 1989), which delayed fruit ripening. Subsequently, *ACCd* from the PGPR *P. putida* strain UW4 has been expressed in tomato (Grichko et al. 2001; Grichko and Glick 2001) and canola (Stearns et al. 2005; Sergeeva et al. 2006) under the *35S* (constitutive), PRB-1*b* (pathogenesis related) and *rolD* (preferentially root-expressed—Elmayan and Tepfer 1995) promoters. In the tomato lines, a single copy of the *ACCd* gene was inserted in each transformant (Grichko et al. 2001). Since the *rolD*:*ACCd* canola plants had two *ACCd* copies (Sergeeva et al. 2006), additional work may be required to separate effects of gene dosage from site of expression. Nevertheless, across a range of experiments in different species and with different abiotic stresses (Fig. 3b), enhanced root *ACCd* expression gave a more consistent growth promotion than non-transformed plants (Fig. 3). While this suggests that *rolD*:*ACCd* transformation may be a viable strategy to allow plants to cope with multiple stressors, further field testing is needed in realistic multi-stress environments.

Some of the earliest transgenic plants expressed hormone biosynthesis genes from *A. tumefaciens* (*iaaM*, *iaaH*, *or ipt*) under constitutive promoters, but many of these had developmental or physiological abnormalities (reviewed in Smigocki and Owens 1999). This stimulated the production of transgenics where the same genes were placed under control of inducible- or tissue-specific promoters. Since

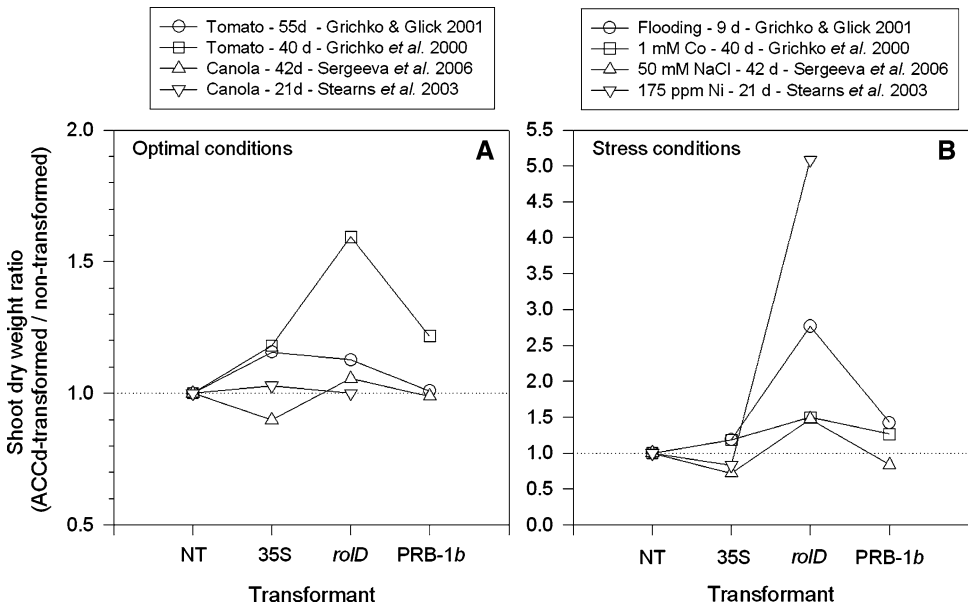

Fig. 3 Stimulation of shoot biomass by expression of a bacterial *ACC deaminase* gene under control of the *35S*, *rolD* and *PRB-1b* promoters compared with non-transformed plants under optimal (**a**) and stressful (**b**) conditions. Note the change of units on the *y*-axes. Data are means, error bars omitted for clarity (replotted from Grichko et al. 2001; Grichko and Glick 2001, Stearns et al. 2005; Sergeeva et al. 2006)

many "early-generation" constitutive *ipt* transformants were wilty, due to severely decreased root mass and/or CK-enhanced stomatal opening, one solution was to graft the transformant onto a WT-type rootstock to allow normal root development (Synková et al. 1999). Surprisingly, the physiological responses of reciprocal grafts (WT/*ipt*) were not evaluated. More recently, WT/*35S:ipt* tomato plants (scion/rootstock) were used to investigate the role of additional root CK production on plant responses to salinity (Ghanem et al. 2011, discussed in "Grafting, a horticultural tool to manipulate root-to-shoot hormonal signalling and abiotic stress responses" below). The traditional horticultural technique of grafting offers new possibilities to improve crop stress tolerance, and to unequivocally evaluate the role of root hormone synthesis in mediating plant responses.

Grafting, a horticultural tool to manipulate root-to-shoot hormonal signalling and abiotic stress responses

Grafting is the surgical alternative to the use of root-specific promoters that allow independent control of root and shoot genotypes. Introduced to control fusarium wilt in watermelon (Murata and Ohara 1936) and phylloxera on grapevine, its use has expanded to provide disease control for many other cucurbit and solanaceous crops (King et al. 2008). Grafting provides opportunities to exploit natural genetic variability for interesting root-specific traits by influencing a commercially desirable shoot phenotype. However, grafting also allows a transgenic rootstock to specifically influence the hormone status of a non-transformed shoot via manipulating root-to-shoot signalling. Although grafting can enhance scion performance under diverse environmental conditions, these effects have been often attributed to altered water and mineral uptake (Lee and Oda 2003). However, the rootstock can also affect scion performance by modifying root-to-shoot hormonal relationships (Pérez-Alfocea et al. 2010).

While rootstock capacity to induce salt tolerance in tomato has been related to the capacity of the plant to maintain shoot ionic (Na^+, K^+ and Cl^-) homeostasis (Estañ et al. 2005; Martínez-Rodriguez et al. 2008), no mechanistic explanations based on physiological or genetic markers (QTL's or candidate genes related to both vegetative traits and Na^+ and K^+ transport) have been proposed (Estañ et al. 2005; Asins et al. 2010). Grafting a commercial tomato cultivar (Boludo F1) onto recombinant inbred line (RIL) rootstocks derived from a *Solanum lycopersicum* × *Solanum cheesmaniae* cross, revealed that scion productivity under moderate salinity (75 mM NaCl) was related to leaf growth maintenance and delayed salt-induced senescence (Albacete et al. 2009). This occurred during the osmotic phase of salinity, before foliar Na^+ concentrations reached toxic levels. Indeed, rootstock-induced tolerance was related to the capacity to maintain xylem sap Na^+ concentrations over time, rather than absolute xylem sap or foliar Na^+ concentrations. Moreover, both leaf growth and delayed senescence were positively correlated with rootstock-sourced zeatin (Z) and K^+ concentrations in leaf xylem sap and also with hormonal ratios between CKs and ACC (Z/ACC and Z + ZR/ACC), while the ratio ACC/ABA was negatively correlated with leaf biomass. A mechanistic hypothesis proposed that the early hormonal signals coming from the roots positively (CKs, ABA) or negatively (ACC) influenced both leaf growth and senescence, thus providing more energy to maintain ionic homeostasis by acting on both root ion uptake (K^+) and efflux (Na^+) and by diluting toxic ions through growth (Pérez-Alfocea et al. 2010).

The difficulty of defining the contribution of specific hormones to this rootstock-mediated improvement in scion vigour has started to be addressed using rootstocks with altered hormone biosynthesis. Roots have been historically regarded as a major organ for cytokinin synthesis (Letham 1994). Salinity-induced decreases in shoot CK concentrations may be due to both diminished transport from the roots and/or increased foliar catabolism by CKX (Albacete et al. 2008; Ghanem et al. 2008). Although enhanced leaf CKX activity in response to drought or osmotic stress may contribute to decreased leaf CK status (Kudoyarova et al. 2007), no change in leaf CKX activity was detected at the time that foliar CK concentration declined following salinisation (Ghanem et al. 2008), suggesting that diminished CK transport from the roots could moderate shoot CK status. In support of this contention, a rootstock constitutively expressing *ipt* increased *trans*-zeatin concentration in developing fruits (1.5- to twofold) and fruit yield (30%) when grown under a moderate salinity (75 mM NaCl) for 3 months (Ghanem et al. 2011, Fig. 4). This improvement was essentially due to increased shoot development, as suggested by the 25% increase in fruit number, and to an additional significant 5% increase in fruit weight, thus supporting the positive effect of root-sourced CKs in regulating source–sink relations under salinity (Pérez-Alfocea et al. 2010; Ghanem et al. 2011).

Grafting has also been used to explore the specific role of other hormones (ABA and the ethylene-precursor ACC) on shoot physiology, which may lead to agronomic applications. The role of root synthesised ABA in regulating stomatal and growth responses has been evaluated by reciprocal grafting of wild-type (WT), ABA-deficient and more recently ABA overproducing genotypes, especially using the *notabilis* (*not*) and *flacca* (*flc*) ABA-deficient tomato mutants. In both WT self-grafts and WT/*flc* grafts, WT scions showed similar stomatal closure

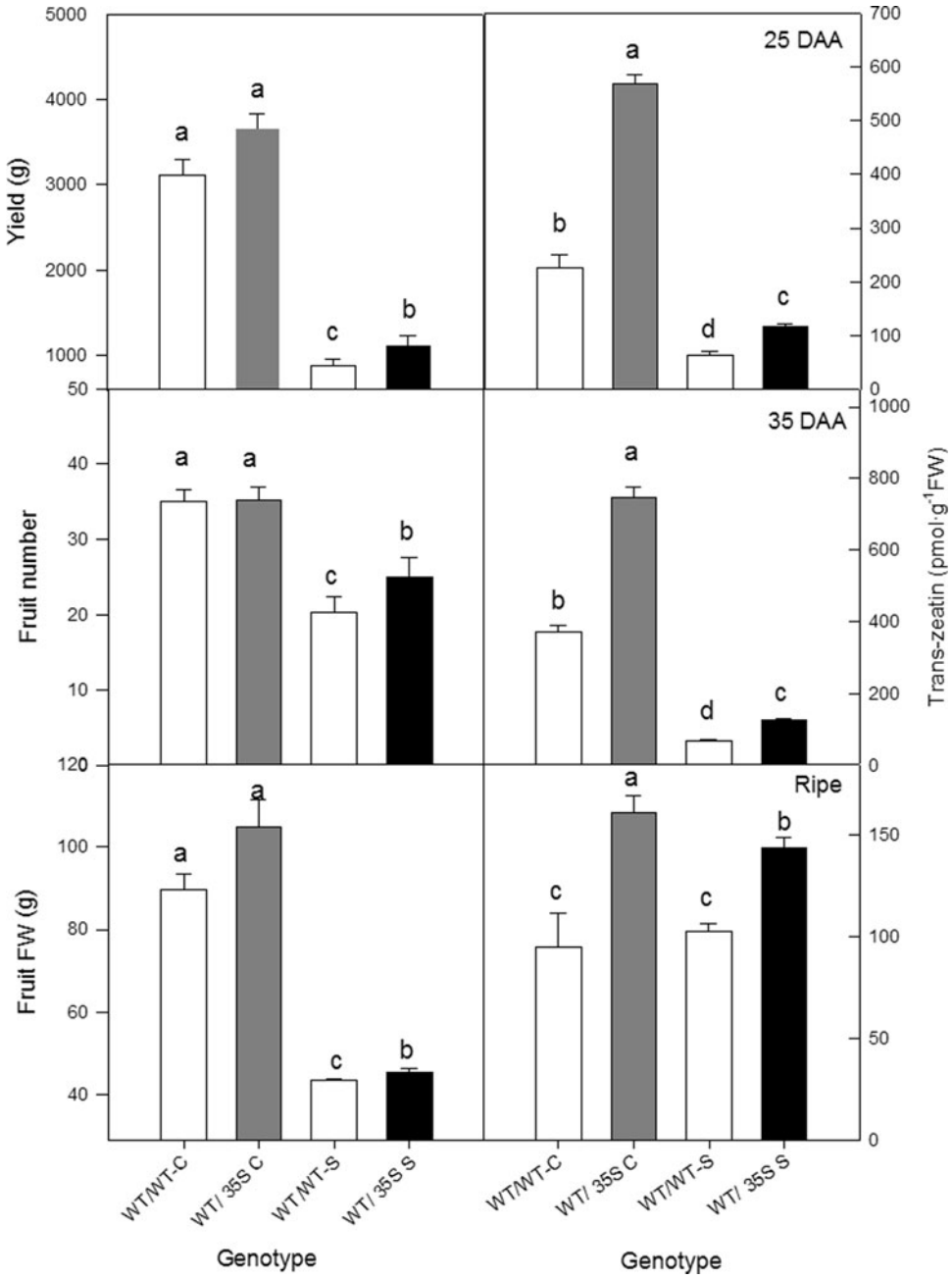

Fig. 4 Fruit yield (**a**), number (**b**), mean fresh weight of individual fruits (FW) (**c**) and *trans*-zeatin concentrations at 25 (**d**) or 35 (**e**) days after anthesis—DAA) or at ripeness (**f**) of grafted plants cultivated in the absence (control-C) or the presence (S) of 75 mM NaCl for 3 months. Data are means ± SE of five replicates. Genotypes were the cultivar P-73 either grafted onto rootstocks of the commercial cultivar UC-82B (WT/WT) or the same genotype overexpressing the *IPT* gene under control of the constitutive CaMV *35S* promoter (WT/*35S::IPT*). Values marked with *different letters* within each panel are significantly different according to a two-tailed *t* test for small sample size based on the t-distribution (replotted from Ghanem et al. 2011)

in response to drying soil (Holbrook et al. 2002), stomatal conductance and shoot growth in well-watered soil (Dodd et al. 2009) and shoot growth under control and saline (75 mM NaCl) conditions when grown hydroponically (Chen et al. 2003). In contrast, leaf area and xylem sap ABA concentration of *flc*/WT (scion/rootstock) plants increased 1.6-fold and threefold compared with *flc* self-grafts, respectively, while xylem sap ACC concentration and leaf ethylene evolution was decreased to WT levels (Dodd et al. 2009). These data suggest little impact of the root system on shoot physiology except in the case of ABA-deficient scions.

Tomato plants overexpressing a gene encoding *nced3* (an enzyme that catalyses a key rate-limiting step in ABA biosynthesis) had significantly increased ABA concentrations, thus decreasing stomatal conductance and increasing root hydraulic conductivity under well-watered glasshouse conditions (Thompson et al. 2007). However, reciprocal grafting of WT and ABA-overexpressing genotypes showed no influence of the ABA overexpressing rootstock on WT shoot growth under well watered conditions (only an increase in root hydraulic conductivity—Thompson et al., personal communication), suggesting that root-sourced ABA was not enough to change shoot ABA

homeostasis or physiology. However, rootstock changes in root hydraulic conductivity may alter shoot behaviour under certain conditions (see "Physiological root-targeted approaches to increase crop resource capture"). Similarly, WT self-grafts and WT/*flc* grafts showed no difference in stomatal response or leaf water potential when grown under low evaporative demand in a controlled environment (Dodd et al. 2009), but WT/*flc* had a lower water potential under higher evaporative demand (Fig. 5) presumably due to lower root hydraulic conductivity (Chen et al. 2003).

Hence, rootstock-mediated impacts on hormone status could influence shoot performance and yield depending on environmental conditions. Positive effects seem more prevalent under suboptimal (resource limiting) conditions (Albacete et al. 2009; Ghanem et al. 2011), with no penalties under optimal (non-resource limiting) conditions. While root-sourced CKs, ABA and ACC seem key in controlling shoot performance under abiotic stress, gaining further insights about the physiological and genetic determinants and their regulation will certainly facilitate rootstock genetic improvement (Asins et al. 2010). Rootstock hormone status may also be important in determining tolerance to biotic stress, another highly desirable agronomic trait.

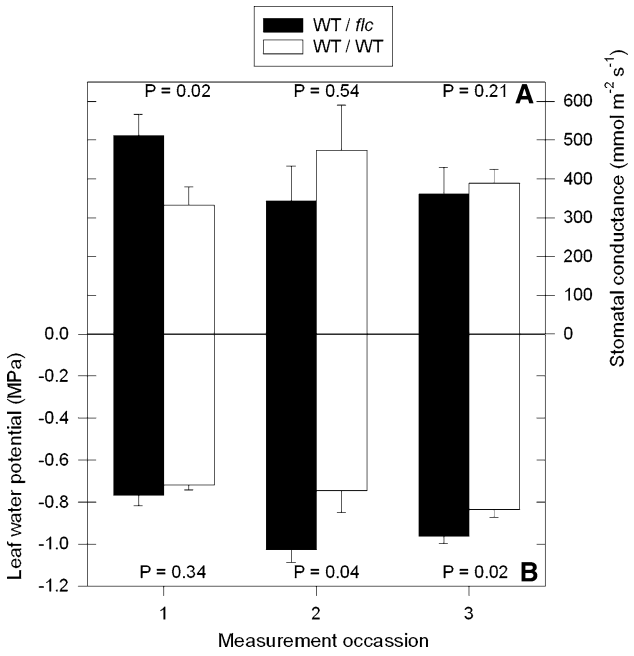

Fig. 5 Stomatal conductance (**a**) and leaf water potential (**b**) of WT self-grafts (*hollow bars*) and WT/*flc* grafts (*filled bars*) maintained under well-watered conditions ($\Psi_{soil} > -0.01$ MPa) in a greenhouse at the Lancaster Environment Centre on three consecutive days when atmospheric evaporative demand during the time of measurement was 1.9, 3.7 and 3.2 kPa, respectively. *P* values between graft combinations indicated. For further details of methodology, see Dodd et al. (2009). Data are means ± SE of eight replicates (I.C Dodd, unpublished results)

Impacts of root hormone status and volatile emission on rhizosphere biotic interactions

The role of hormones in rhizosphere biotic interactions is well documented (Okubara and Paulitz 2005; Dodd et al. 2010). Cytokinins specifically have been linked to improved defense responses in plants. Endogenously overproduced or exogenously supplied cytokinins are known to trigger the induction of systemic acquired resistance (SAR) and salicylic acid (SA) activated expression of pathogenesis-related (PR) genes (Ward et al. 1991; Martineau et al. 1994; van Loon and van Strien 1999). Nevertheless, some authors suggest that elevated levels of cytokinins in mycorrhizal roots could suppress the induction of some PR-protein genes, specifically chitinase and glucanase genes (Spanu et al. 1989; Shaul et al. 2000). In addition, cytokinins have been correlated with the *in planta* accumulation of secondary metabolites, many of which plays a significant role in plant defense responses (Chilton 1997; Smigocki et al. 1997, 2000). Cotton yields were higher and insect populations reduced in field foliar applications of commercial formulations of cytokinins, most likely due to the relatively high levels of four secondary metabolites, all known to be toxic to tobacco budworm (*Heliothis virescens* F.), a major pest of cotton (Hedin and McCarty 1994).

In transgenic plants that overexpress the *A. tumefaciens ipt* gene under a potato tuber wound-inducible *Pin-II* gene promoter (Smigocki et al. 1993; Smigocki 1995) typical cytokinin effects (dark green leaves, reduced height, shorter internodes, delayed senescence) were observed concomitant with a 70-fold increase in cytokinin levels and enhanced tolerance to leaf feeding pests *Manduca sexta* and *Myzus persicae*. The insecticidal activity was localised primarily to leaf surfaces, was lethal to tobacco hornworm larvae and reduced egg hatch (Smigocki et al. 1997, 2000, 2003). Activity of the extracts was genotype dependent and likely influenced by the overall endogenous cytokinin content since *ipt N. plumbaginifolia* leaves had more than 20 times the activity of extracts from *ipt N. tabacum*.

Partial purification of the insecticidal extracts identified secondary metabolites as the active compounds. Interestingly, a gene for a cytochrome P450 (*CYP72A2*) was among the genes found to be up-regulated in the insect resistant *ipt Nicotiana* plants (Mujer and Smigocki 2001). Secondary metabolic pathways are catalysed by various cytochrome P450 enzymes and *CYP72A2* expression was shown to be inducible by cytokinin, insect feeding and mechanical wounding (Mujer and Smigocki 2001). Heterologous expression in *N. tabacum* of the sense (co-suppression) or antisense *CYP72A2* gene produced shorter plants with branched stems, smaller leaves and deformed flowers (Smigocki and Wilson 2004). Several reports have

correlated heterologous cytochrome P450 gene expression with enhanced insect and pathogen resistance (Smigocki and Wilson 2004; Takemoto et al. 1999; Wang et al. 2001). In addition, Barna et al. (2008) showed that production of cytokinins in *ipt* transgenic tobacco suppressed HR symptoms induced by incompatible bacteria and concomitantly increased antioxidative enzyme levels in the infected tissues. Thus, enhancing plant cytokinin status using root-targeted technologies (see "Root-specific promoters to localise transgenic gene expression" and "Grafting, a horticultural tool to manipulate root-to-shoot hormonal signalling and abiotic stress responses" above) offers promise to decrease insect and pathogen attack and root damage.

Unlike the studies on phytohormones in rhizospheres (Okubara and Paulitz 2005; Dodd et al. 2010), other chemicals (both non-volatile and volatile) exuded or emitted by roots have received less attention. Non-volatile compounds have long been studied in the context of allelopathy mainly in crop/weed interactions (Belz 2007). Above-ground volatiles mediate plant interactions with the surrounding environment including plant reproduction (attraction of pollinators, seeds dissemination), defence mechanisms (plant-plant signalling, repellence of parasites or herbivores, attraction of parasitoids, antifungal or antimicrobial effects) and abiotic stress protection (heat or ozone protection) (Dudareva et al. 2006). Despite their potential to exchange information between organisms, volatile compounds emitted by roots are frequently neglected, and their effects on plant-environment interactions require further study.

Belowground volatile organic compounds are involved in plant defence against various organisms found in the rhizosphere (insects, nematodes, pathogenic bacteria or fungi). Direct effects can be observed by release of antimicrobial compounds (e.g. 1,8-cineole) or anti-herbivore substances while indirect effects involve tritrophic interactions such as attraction of enemies of root-feeding herbivores (Dudareva et al. 2006). In maize, feeding by the western corn root worm induces the emission of (*E*)-β-caryophyllene which attracts nematodes that in turn infect the insects. A maize line that was unable to emit this compound was transformed, using the maize ubiquitin promoter, to re-establish the emission of (*E*)-β-caryophyllene thus decreasing insect root damage (Degenhardt et al. 2009). Thus, it is possible to enhance biological control and hence reduce the use of pesticides by the release of a volatile belowground compound. Ideally, agronomic research should focus more on inducible signal(s) against a biotic agent but with a broad spectrum of action (e.g. weed and pest control).

Belowground and aboveground mechanisms are frequently considered as two completely distinct systems. This is surprising since it is well known that plant roots synthesise many compounds that are toxic for leaf attackers (e.g. nicotine) and are translocated to the shoot, and that intense signalling takes place between the two plant parts (Erb et al. 2009). External application of jasmonic acid can trigger herbivore-induced responses in plants. The site of jasmonic acid induction (root or shoot) affects the composition of aboveground volatile compounds emitted, resulting in different efficiency of insect parasite attraction (van Dam et al. 2010). Volatile compounds emitted by plants below ground not only induce root defense mechanism(s) but also impact on plant interactions with numerous beneficial soil-dwelling microorganisms (Wenke et al. 2010).

Traditional breeding and biotechnology offer opportunities to exploit these phenomenon but several areas require further investigation. First, the induction, biosynthesis and the emission of organic volatile compounds by belowground plant organs require detailed study both within the laboratory and in realistic field conditions during the complete plant life development cycle. To achieve this goal, analytical methods allowing the sampling, separation, identification and quantification of belowground volatile compounds must be developed. Volatiles released by roots are typically a complex mixture of up to one hundred different compounds. Solid-phase micro extraction or dynamic headspace sampling both combined with gas chromatography mass spectrometry are powerful analytical methods. However, they are not sensitive enough to detect subtle and/or rapid modifications in volatile composition (Tholl et al. 2006). Accurate and reproducible volatile compound sampling in field conditions is challenging, especially for belowground compounds. Since the rhizosphere is a complex medium, it is also important to study the degradation of volatile compounds once released by roots. Chemical degradation like oxidation or biotransformation by soil microorganisms can occur and modify (increase and decrease) biological effects (Macias et al. 2007). Finally, since soil composition, structure and moisture status can strongly influence the diffusion of belowground volatile compounds it is crucial to individually model their transport and compartmentation in the soil.

Conclusions

Increasingly, it is being perceived that root system engineering provides new opportunities to maintain sustainable crop production under changing environmental conditions. Root-specific traits such as root system architecture, sensing of edaphic stress and root-to-shoot communication can be exploited to improve resource capture and plant development under adverse conditions. These responses are often mediated by hormones, which can be manipulated via

conventional (e.g. grafting) or biotechnological genetic approaches (e.g. root-specific promoters) to directly improve those traits and therefore, plant performance. Recent evidence suggests that root-synthesised cytokinins can ameliorate shoot growth inhibition caused by environmental stress (Ghanem et al. 2011), and also mediate disease and pest resistance (Smigocki et al. 1997, 2000). Alternative approaches to modify the existing cytokinin metabolic pathways are needed to gain a better understanding of cytokinin participation in plant defense responses. Manipulating biotic interactions within the rhizosphere at the communication level (hormones and other bioactive compounds) may improve such interactions to benefit crop performance. Research should focus on gaining insights into the physiological and genetic determinants of such performance and about the mechanisms for optimal *in radix* regulation.

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