

Shoot–root carbon allocation, sugar signalling and their coupling with nitrogen uptake and assimilation

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Abstract. Roots and shoots are distantly located but functionally interdependent. The growth and development of these two organ systems compete for energy and nutrient resource, and yet, they keep a dynamic balance with each other for growth and development. The success of such a relationship depends on efficient root–shoot communication. Aside from the well-known signalling processes mediated by hormones such as auxin and cytokinin, sugars have recently been shown to act as a rapid signal to co-ordinate root and shoot development in response to endogenous and exogenous clues, in parallel to their function as carbon and energy resources for biomass production. New findings from studies on vascular fluids have provided molecular insights into the role of sugars in long-distance communications between shoot and root. In this review, we discussed phloem- and xylem- translocation of sugars and the impacts of sugar allocation and signalling on balancing root–shoot development. Also, we have taken the shoot–root carbon–nitrogen allocation as an example to illustrate the communication between the two organs through multi-layer root–shoot–root signalling circuits, comprising sugar, nitrogen, cytokinin, auxin and vascular small peptide signals.

Additional keywords: carbon partitioning, phloem and xylem, root development, shoot growth, sugar signalling.

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Introduction

Land plants possess two basic organ systems: the shoot (aboveground) and the root (underground). The former is responsible for capturing solar energy and atmospheric carbon dioxide, using photosynthesis to turn these into organic carbon (i.e. sugars), which is then used for development and biomass production of all plant organs. The latter, the roots, anchor the plant in and acquire water and inorganic nutrients from the soil. To optimise growth in an ever-changing environment, plants must balance the distribution between the two organ systems for energy, nutrient and water resources. The success of this co-ordination depends on effective root–shoot communications. For example, under soil water deficiency, quick stomatal closure is induced to minimise water loss by root-derived hormone, ABA, ahead of changes in leaf water status (Bates and Hall 1981; Stoll *et al.* 2000). Similarly, low leaf nitrogen status could generate a shoot-to-root signal to alter root physiology, leading to activation of nitrate uptake (Forde 2002). Numerous studies have established that a sophisticated hormonal signalling network regulates these root–shoot communications (e.g. Sauter *et al.* 2001; Dodd 2005; Schachtman and Goodger 2008; Puig *et al.* 2012). In contrast, much less attention has been paid to the impacts of carbon flow and sugar signalling on co-ordinating shoot and root development.

Sucrose (Suc) is the primary photoassimilate for long distance translocation through phloem in most higher plant species. Apart

from its crucial role as carbon source for energy and building blocks, Suc has also been shown to act as a long-distance signal to co-ordinate plant development (Kircher and Schopfer 2012; Mason *et al.* 2014). The rapid transport of Suc in the phloem (e.g. $\sim 150 \text{ cm h}^{-1}$ vs that of auxin at just $\sim 16 \text{ cm h}^{-1}$ in *Pisum sativum* cv. Torsdag, see Mason *et al.* 2014) highlights its ability for instantaneous response. It remains unknown in many cases whether it is the Suc itself or its derivatives, such as glucose (Glc), fructose (Fru) or trahalose-6-phosphate (T6P) that function as signalling molecules to regulate development (Ruan 2014).

There are many sugars also detected in the xylem sap (Schill *et al.* 1996; Iwai *et al.* 2003; Secchi and Zwieniecki 2012), which opens up the possibility of an acropetal sugar transport from roots to shoots and leaves. Together, it becomes possible for a circuit sugar signalling pathway to operate at the whole-plant level through the vascular system. Furthermore, recent studies have revealed significant interactions between sugars and other signalling components including hormones, microRNAs, reactive oxygen species (ROS) and many other signalling molecules (Considine and Foyer 2014; Matsoukas 2014; Tsai and Gazzarrini 2014; Ljung *et al.* 2015; Yu *et al.* 2015). Clearly, sugars are major players in the global signalling network regulating plant development, crop yield and response to biotic and abiotic stress (Ruan 2014).

The aim of this review was to evaluate the current understanding of sugar allocation and signalling in root–shoot

development, and to provide an insight into the global-level communication that integrates nutrition and growth signalling. First, we have discussed the possible routes for long-distance sugar transport and signalling. Then we have made an assessment of the roles of shoot-to-root sugar signalling in coupling root–shoot development. Finally, we have discussed root–shoot–root signalling circuits in co-ordinating the growth of these two distantly-located organs, using nitrogen-carbon balance as an example.

Long distance carbon flow and signalling circuit through phloem and xylem

Allocation of carbon resource from photosynthesising leaves (source) to non-photosynthetic organs (sink) relies on an efficient and highly controlled phloem transportation system. Among all the photosynthetically-fixed carbohydrates, only a few are able to be transported in the phloem over a long-distance (Lemoine *et al.* 2013). In this context, sucrose (Suc) is the primary form of assimilates translocated via phloem over a long-distance. The accumulation of Suc in the phloem of the source leaves attracts water osmotically, creating high turgor pressure, which drives mass flow of assimilates towards the sinks (Ruan *et al.* 1996). Recent reviews describing sugar transport pathways and transporters are also available elsewhere (Osorio *et al.* 2014; Chen *et al.* 2015; Fettke and Fernie 2015; Hedrich *et al.* 2015). Besides Suc, phloem hexose transport has also been reported for a limited number of species (van Bel and Hess 2008). However, these results were questioned by Liu *et al.* (2012). Oligosaccharides of raffinose family are indirectly involved in the building up of sugar concentrations in the phloem by polymer trapping (e.g. Rennie and Turgeon 2009). Polyols (mainly sorbitol and mannitol) are translocated through phloem, in some species such as apple (Noiraud *et al.* 2001). Overall, for most plant species, Suc is the main carbon resource moving from shoot to root or other sinks in the phloem, together with many other nutrients, transcripts, proteins and signalling molecules (Turgeon and Wolf 2009). For example, Suc was estimated to be 433 mM from phloem exudates of *Ricinus communis* L., accounting for 72% of total sap osmolality (Patrick 2013). Phloem sugar distribution is directly linked to the cellular pathways of assimilate transport and sugar metabolism in both ‘the giver’ and ‘the taker’ (Palmer *et al.* 2015), and can be affected by many environmental factors that alter shoot–root relationship (Liu and Vance 2010; Lemoine *et al.* 2013).

In contrast with the translocation of phloem sap from shoot to root, the root-to-shoot translocation is mediated by xylem sap flow driven by water loss through leaf transpiration. Some soluble sugars including Suc, Fru, and sugar polymers such as oligoarabinogalactan, oligoglycan and myo-inositol, have also been detected from the xylem sap of many species, such as maple (Schill *et al.* 1996), squash (Iwai *et al.* 2003), walnut (Améglia *et al.* 2004) and black poplar (Secchi and Zwieniecki 2012). However, it remains unclear how Suc or other sugars may have reached xylem vessels. One possibility is that Suc continuously leaks out from the phloem ((Minchin and McNaughton 1987; van Bel 2003) to the xylem/phloem parenchyma cells, which then diffuses to the transpiration stream. Indeed, apart from water and mineral elements, the xylem transpiration stream is full of

hormones, transcripts, proteins, carbohydrates and other small organic chemicals (Pérez-Alfocea *et al.* 2011).

Interestingly, analysis in *Populus trichocarpa* Torr. & A. Gray has revealed that Suc infiltration into xylem resulted in a simultaneous physiological and molecular response, including reduction of the starch pool in xylem parenchyma cells and induction of expression of genes encoding aquaporins, amylases and sugar transporters, similar to the response induced by embolism, a formation of air bubble in xylem blocking or impeding long distance transport of xylem sap (Secchi and Zwieniecki 2011). Evidence provided from this study indicates Suc may function in sensing embolism and triggering the refilling in xylem vessels. Further studies are required to understand the dynamics of this xylem-mediated carbon flow and to determine whether it plays a role in root-to-shoot communication. In addition to simple sugars (Suc, Glc and Fru), some oligosaccharides may also function as elicitors in stress responses (Fry *et al.* 1993; John *et al.* 1997; Shibuya and Minami 2001), and probably be involved in long-distance signalling through xylem transportation (MacDougall *et al.* 1992; Iwai *et al.* 2003).

Notably, recent research centered on the secreted oligopeptides shed lights on a root-to-shoot-to-root signalling feedback circuit in plants (reviewed by Notaguchi and Okamoto 2015). In this scenario, signal molecules generated in root system in response to the soil environments could move towards shoot via the xylem, translocated to the phloem through shared vascular parenchyma cells and perceived by potential receptors in the phloem. The perception of root-derived signals could elicit molecular and biochemical responses in shoot, which could relay the same signal or generate new signals for delivery through the phloem sap to the rest of the plants including the root. Although the mechanism by which sugar signalling participates in root–shoot–root signalling circuit remains to be elucidated, such a possibility of bi-directional signalling does appear to be plausible based on the observations on the presence of sugars in both xylem and phloem and the intimate anatomic and functional coupling between the two vascular systems.

Shoot-to-root Suc transport in co-ordinating plant development

As the main assimilate transported from shoot to root, Suc is required as the original and primary carbon resource for root development. Recent findings from *Arabidopsis* seedlings highlight the importance of Suc basipetal translocation in coupling shoot–root development as discussed below.

In early seedling development, light is known as a key factor affecting shoot and root development (Salisbury *et al.* 2007; Page *et al.* 2011). A more recent study revealed that photosynthetic Suc transported from shoot (cotyledon) to the root function as a direct signal to activate and promote *Arabidopsis* root elongation in a light-dependent manner (Kircher and Schopfer 2012). The findings from this report and other studies support the following model on how young seedlings efficiently use their carbon resource to co-ordinate root–shoot growth with light availability and quality. Firstly, at the early germination stage, seedling may be in the dark or in the shade with no photosynthesis. Most of the carbon resource pre-stored in the cotyledons fuels the

hypocotyl elongation, whereas the root growth is stalled. At the meantime, a low ratio of red : far-red light under shade also induces hypocotyl elongation and promotes shoot over root growth (Page *et al.* 2011). As a result, cotyledons, together with shoot apex, are pushed towards the light. Thereafter, the de-etiolated seedling begins to photosynthesise to produce Suc acting as a shoot-to-root signal and nutrient and energy source to support root elongation to acquire belowground resources. The import of assimilate to roots is highly responsive to changes in Suc availability. For instance, studies in barley seedlings revealed a reduced carbon import to roots in response to either increased pool size of sugars within the roots (Farrar and Minchin 1991) or reduced supply from source leaves (Minchin *et al.* 1994).

Apart from Suc, other sugars such as Glc, were also reported to regulate root growth through: (i) driving target-of-rapamycin (TOR) signalling to instigate root meristem cell division (Xiong *et al.* 2013); (ii) interacting with auxin via heterotrimeric G protein complex to promote lateral root (LR) formation (Booker *et al.* 2010); and (iii) regulating brassinosteroid (BR) signalling and downstream auxin transportation to affect root directional responses and eventually root architecture (Singh *et al.* 2014). It may be that the phloem unloaded Suc may generate a Glc signal in the root, through the activities of Suc-cleavage enzymes, invertase and or sucrose synthase (Sus) to fulfil the reprogramming of gene expressions (Ruan 2014). Further research to determine the identity of sugar signalling pathway in the root is necessary to understand the nature of Suc- or Glc-mediated root growth.

Compared with the seedling stage, the regulation of Suc on root development at late growth stage is less studied. However, deficiency of Suc has been identified as a major cause of grain or seed abortion under drought, possibly owing to reduced Glc that activates programmed cell death (Ruan 2012; Liu *et al.* 2013). Whether this is the case in roots of mature plants remains to be tested.

In addition to Suc or Glc, shoot-derived auxin has also been identified to regulate primary root elongation in a light-dependent manner (Sassi *et al.* 2012). To this end, Suc moves much faster to axillary buds than local depletion of auxin (indole-3-acetic acid, IAA) to activate bud outgrowth in pea (Mason *et al.* 2014). This is likely due to the fact that Suc is translocated through mass flow in the phloem, whereas the cell-to-cell movement of auxin from shoot apex to axillary bud is probably mediated through the AUXs, PINs, auxin influxers and effluxers respectively (Wang and Ruan 2013). By deduction, it seems possible that the shoot-to-root Suc translocation may act as a light-induced signal, ahead of auxin, to promote root development. However, one cannot rule out the possibility that sugar and auxin may crosstalk with each other to modulate root development, as shown in other physiological and developmental contexts (Mishra *et al.* 2009; Wang and Ruan 2013; Ljung *et al.* 2015).

Clearly, the shoot-to-root Suc translocation is a critical factor in co-ordinating plant development, and must be tightly balanced. Brauner *et al.* (2014) provided an example that disruption of this balance limited the growths of both shoot and root. In the plastidial phosphoglucomutase (*PGM*) loss-of-function mutant (*pgm*), the inability of leaves to synthesise starch leads to the accumulation of Suc, Glc and Fru in the light, which are rapidly depleted during early night under a long-day light period, and

resulted in carbon starvation during the remaining night (Apelt *et al.* 2015). However, the *pgm* mutant exhibited dramatically increased shoot-to-root sugar supply during the day and the first 4 h of the night, leading to exaggerated root respiration in *pgm* (Brauner *et al.* 2014) and inhibited protein synthesis and increased protein turnover at night (Ishihara *et al.* 2015). Consequently, the *pgm* mutant displayed restricted biomass formation in both the root and the shoot. We note that the sugar uptake by root largely exceeded its metabolic demand (Brauner *et al.* 2014). It remains unknown whether the enhanced Suc export by shoot functions as a mechanism to maintain leaf photosynthesis which was not altered in the *pgm* mutant, or Suc is ‘drawn’ to the root by an error signal derived from root. Further investigation of the dynamics of sugar metabolism in *pgm* root, especially the activities of Suc-cleavage enzymes and sugar transporters, and the subcellular compartmentation of the sugars (e.g. vacuole vs cytoplasm), could provide valuable information in understanding how root achieves this over-demanding for sugars.

Root-shoot-root signalling: coupling between C and N uptake and assimilation

Sugars also crosstalk with many other signalling and metabolic processes to regulate local and systemic signalling pathways (Bailey-Serres and Voesenek 2010; Eveland and Jackson 2012; Ruan 2014; Tsai and Gazzarrini 2014; Ljung *et al.* 2015). As an example, we evaluated below the current understanding on Suc signalling in controlling plant nitrogen (N) balancing. Recent studies have revealed intriguing interactions among sugars, nitrate and many other long-distance mobile signals including cytokinin, auxin, CEP/CLV peptides (Hartig and Beck 2006; Ruffel *et al.* 2011; Singh *et al.* 2014; Notaguchi and Okamoto 2015).

Nitrogen is the most abundant inorganic nutrient for plants. Most plant species use nitrate (NO_3^-) as a major N source, along with ammonium (NH_4^+) salts (Bloom 2015). Depending on species and soil NO_3^- abundance, root NO_3^- can be either assimilated in the root or translocated to the aerial parts of the plants through xylem sap for assimilation in the shoot (Andrews 1986). Typically, shoot dominates NO_3^- assimilation at high soil NO_3^- environment in most plants (Krapp 2015). Phloem loading of NO_3^- has been shown in both source leaves (Fan *et al.* 2009; Kiba *et al.* 2011; Hsu and Tsay 2013) and root companion cells (Wang and Tsay 2011), mediated by phloem-localised NO_3^- transporters. The former process contributes to NO_3^- remobilisation to younger leaves during N deficiency, whereas the latter facilitates NO_3^- loading into the root phloem for downward transport in the roots.

Carbon (C) skeletons are essential for the assimilation of inorganic N into amino acids, proteins and nucleic acids. Thus, N uptake is highly integrated with the availability of sugars (Ruffel *et al.* 2014). In contrast, N-deficiency negatively affects photosynthetic output, which can be recovered by N re-supply (Coruzzi and Bush 2001). On the other hand, the assimilation of NO_3^- is a highly energy-consuming process (Bloom 2015). Thus, increasing C supply promotes NO_3^- uptake and assimilation and *vice versa* (Cross *et al.* 2006; Weigelt *et al.* 2009; Schofield *et al.* 2009). The C : N ratio also

affects NO_3^- root-to-shoot transportation. For example, under low soil NO_3^- level, root C:N ratio is high, which creates a condition to assimilate most of the NO_3^- into amino acid for synthesising proteins in the root, thereby stimulating root growth and the initiation of lateral roots (LR), but with little NO_3^- delivered to the shoot (Zheng 2009). As soil NO_3^- increases or root C reduces, unassimilated NO_3^- could be transported to the shoot through xylem, stimulating shoot growth (Takei *et al.* 2001, 2002). Similarly, high leaf N promotes shoot development and its sink strength. Under this scenario, the photosynthetic C tends to be retained in the shoot with less C exported to roots. In contrast, low leaf N limits shoot growth and more C is likely to be translocated to the root (Bloom *et al.* 1993). The exact NO_3^- level and root C:N ratio below or above which plant growth may be affected vary from species to species since different species have different normal C:N ratio. For example, the root C:N ratio of canola, mustard and wheat is ~44, which is higher than chickpea and pea with their root C:N ratio being 33 and 18 respectively (Gan *et al.* 2011). The low C:N ratio of the latter two legume species may reflect their ability of N_2 -fixation. Interestingly, survey from three oilseed, three pulse crops and spring wheat revealed that root C:N ratio ranged from 17 to 75, higher than that in straw (shoot) of 14–55 and seed of 6–17 (Gan *et al.* 2011).

At the molecular level, the balance between C and N affects the expression of more than half of *Arabidopsis* genes (Palenchar *et al.* 2004; Gutiérrez *et al.* 2007), indicating a sophisticated C:N response network. Research in *Arabidopsis* roots has identified three candidates which probably sense and respond to C:N status. A putative glutamate receptor AtGLR1.1 was first reported as a sensor of intracellular Suc and NO_3^- status in *Arabidopsis*. This proposition was based on the following observations: (i) *AtGLR1.1*-deficient (*antiArGLR1.1*) seed failed to germinate in the presence of Suc, but not Glc, mannitol or sorbitol, while the germination could be restored upon co-incubation with NO_3^- , but not NH_4^+ ; (ii) the *antiArGLR1.1* seedlings exhibited a conditional phenotype that was sensitive to the C:N ratio (Kang and Turano 2003). Moreover, a putative methyltransferase AtOSU1 was identified as a critical modulator in balancing C and N nutrient response in *Arabidopsis* (Gao *et al.* 2008). The *Atosu1* mutant was more sensitive than the wild type to both low C/high N and high C/low N conditions, of which the *AtOSU1* mutation strongly upregulated the expression of *Asn synthetase isoform 1* (*AtASNI*) and *MYB75* transcription factor in response to low C/high N and high C/low N respectively (Gao *et al.* 2008).

The nitrate transporter AtNRT2.1 was also proposed to act either as a high C/low N sensor or signal transducer to coordinate the development of the root system with nutritional cues (Little *et al.* 2005). Several molecular components have been identified to regulate *AtNRT2.1* expression. A dual affinity NO_3^- transporter AtNTR1.1 was shown to act as a NO_3^- transceptor (transporter/sensor, Gojon *et al.* 2011), required for both local induction (short term) and repression (long term) of a high-affinity nitrate transporter gene *AtNRT2.1* (Muños *et al.* 2004; Krouk *et al.* 2006; Ho *et al.* 2009). The phosphorylation of AtNRT1.1 by CBL-interacting protein kinase AtCIPK23 switches AtNRT1.1 to the high-affinity function leading to a weak induction of *AtNRT2.1* (Ho *et al.* 2009; Parker and Newstead 2014; Sun *et al.* 2014). Another CBL-interacting protein kinase AtCIPK8 (Hu *et al.* 2009) and a putative transcription factor NIN-Like

Protein 7 (AtNLP7, Castaings *et al.* 2009; Marchive *et al.* 2013) were also found to be involved in the induction of *AtNRT2.1* expression by NO_3^- . In addition, *Arabidopsis* high nitrogen-insensitive 9 (AtHNI9)/INTERACT WITH SPT6 (AtIWS1), an evolutionarily-conserved component of the RNA polymerase II complex, acts in roots to repress *AtNRT2.1* transcription in response to high N supply (Girin *et al.* 2010; Widiez *et al.* 2011). Furthermore, the transcription factors AtLBD37/38/39 seem to mimic the effect of organic N compounds at high N status, and act as a long-distance N signalling to downregulate nitrate transport *AtNTR2.1* (Rubin *et al.* 2009). These findings indicate a mechanism of managing whole-plant level NO_3^- (for recent reviews see Krapp *et al.* 2014; Ruffel *et al.* 2014; Bloom 2015; Krapp 2015).

de Jong and co-workers (2014) demonstrated in *Arabidopsis* that Glc is coupled to nitrate uptake and assimilation through hexose kinase1 (HXK1)-mediated oxidative pentose phosphate pathway (OPPP). The interaction regulates the expression and activity of NO_3^- transporter NRT2.1 from transcriptional to post-translational levels. Reda (2015) reported Suc signalling from shoot enhances the nitrate reductase (NR) activity in *Arabidopsis* root by inducing *NIA* genes (encoding NR) expression and NR activation state, and probably through an HXK1-independent pathway.

Regulatory proteins and hormonal pathways involved in balancing shoot–root C:N status

Along with the root-shoot-root N–C signal circuit as outlined above, other signalling components are also involved in modulating C and N metabolism and allocation. To this end, SnRK1 (SNF1-related protein kinase-1) is a plant protein kinase activated by low levels of Glc, but inhibited by glucose 6-phosphate, trehalose-6-phosphate and Suc (Toroser *et al.* 2000; Halford *et al.* 2003; Zhang *et al.* 2009; Ruan 2014). In addition to participating carbon allocation by modulation gene expression of Sus, ADP glucose pyrophosphorylase (AGPase) and α -amylase (Laurie *et al.* 2003; McKibbin *et al.* 2006), SnRK1-mediated signalling was also revealed to regulate nitrogen assimilation and amino acid biosynthesis through phosphorylation and inactivation of NR, and regulation of asparagine synthase gene expression (Baena-González *et al.* 2007). These findings indicate the potential role of SnRK1 in C–N balancing.

Plant 14-3-3 proteins could also act as regulators of carbon and nitrogen metabolism through direct interaction with many essential enzymes such as the plasma membrane H^+ -ATPase, sucrose phosphate synthase, ADP-glucose pyrophosphorylase, NR, and glutamine synthase (Chung *et al.* 1999) and their overall control over the TCA cycle (Diaz *et al.* 2011). Overexpression of 14-3-3 protein in *Arabidopsis* results in hypersensitivity to C–N stress conditions, which could further be aggravated by the absence of its upstream regulator ubiquitin ligases ATL31 (Sato *et al.* 2011). The observations suggest that ATL31 regulates C–N response by degrading the 14-3-3 proteins. Finally, Dof1 (DNA binding with one finger), a gene expression activator for organic acid metabolism, has been shown to improve N assimilation under low-nitrogen condition in *Arabidopsis*. Overexpression of *Dof1* was accompanied with

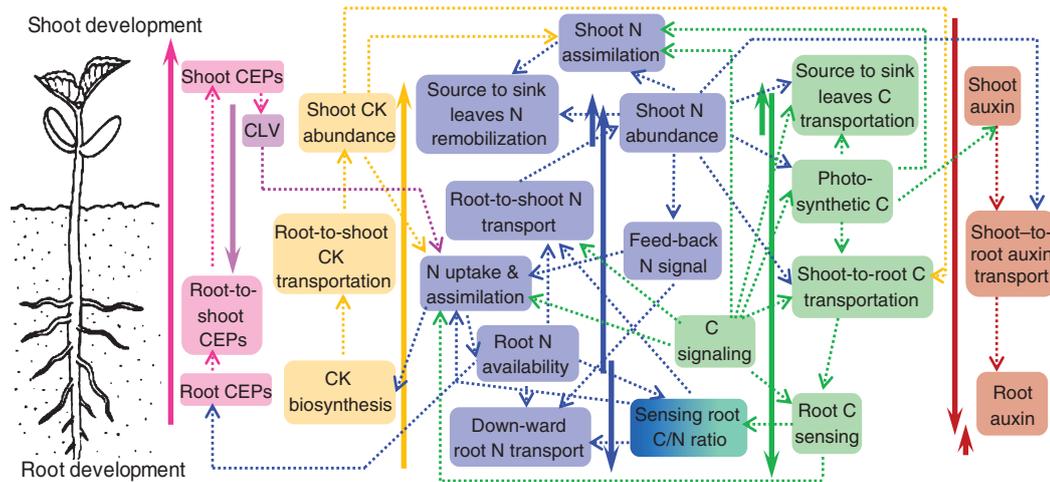


Fig. 1. Schematic representation of multi-layer root–shoot–root signalling circuits in balancing C–N distribution between shoot and root. The model comprises sugar (green), nitrogen (blue), cytokinin (yellow), auxin (dark red) and CEPs (pink)/CLV (purple) signalling components. Arrows with solid line and dot line represent the mass flow and signalling interactions respectively.

reduced Glc level and increased gene expressions responsible for carbon-skeleton production, required for amino acid biosynthesis (Yanagisawa *et al.* 2004). Similarly, introduction of a maize orthologue, *ZmDof1*, into rice also enhanced N and C assimilations under N-deficiency (Kurai *et al.* 2011). Together, a root–shoot–root signal circuit in sensing C–N status is equipped with a cohort of regulatory proteins to achieve C–N balancing at the whole-plant level (Fig. 1).

In parallel to regulatory proteins involved, plants also recruit hormone signals for root–shoot communications with respect of C–N status. Root cytokinins (CKs), for example, are transported acropetally in the xylem sap to increase the expression of N-uptake/assimilation genes in both shoot and root, and to promote lateral root (LR) development (Takei *et al.* 2001; Kiba *et al.* 2011). Reciprocally, the supply of NO_3^- induces CK biosynthesis (Kiba *et al.* 2011). The synergistic NO_3^- –CK relay provides a positive feedback system contributing to N uptake and assimilation (Ruffel *et al.* 2011) and allows the root to actively ‘forage’ soil NO_3^- by increasing lateral root formation in N-limited conditions (Ruffel *et al.* 2011). We note that CK deficiency in the *Arabidopsis* cytokinin oxidase (*CKX*) overexpressing plants leads to high root to shoot ratio, largely owing to stunted shoot growth with enhanced root system, a phenomenon probably reflecting a shift of carbon allocation from shoot to root (Werner *et al.* 2003, 2008). The findings suggest a linkage between N–CK and C–N loop under N-limitation condition: CK-deficiency signal may trigger more C allocated from shoot to root whereby promoting root growth. The N–CK positive feedback loop then kicks in to strengthen root N uptake and assimilation, and to induce CK biosynthesis. The increased CK, once translocated to the shoot, could shift root-over-shoot growth back to a balanced shoot–root development (Fig. 1).

Similar to CK, auxin also exerts sophisticated interactions with both N and sugar signalling pathways (Ljung *et al.* 2015). Auxin was found to be a positive regulator for NO_3^- accumulation in leaves. Treatment with exogenous auxin or introduction of the auxin overproducing mutation in *Arabidopsis*

resulted in a strong increase in the transcription of *AtNRT1.1*, a dual-affinity NO_3^- transporter/sensor, in root tips (Guo *et al.* 2002). N-deficiency trends to trigger auxin translocated from shoot to root (Caba *et al.* 2000; Krouk *et al.* 2010), which could promote root growth and LR formation (Giehl and von Wirén 2014). Further, *AtNRT1.1* not only transports NO_3^- but also facilitates auxin translocation from shoot to root when *Arabidopsis* seedlings were grown in medium containing less than 0.2 mM NO_3^- (Krouk *et al.* 2010). Together with strong upregulation of IAA biosynthesis by soluble sugars (LeClere *et al.* 2010; Lilley *et al.* 2012; Sairanen *et al.* 2012), the basipetal allocation of auxin probably function as a shoot-to-root feedback signal to stimulate root development under low N condition in accompany with C flow from shoots (Fig. 1). Further addition to the shoot–root bio-directional C–N signalling loop comes from the recently identified xylem signalling peptides C-TERMINALLY ENCODED PEPTIDE (CEP) and their receptor protein CLAVATAV (CLV) in phloem. Tabata *et al.* (2014) identified two CEP1 receptors in *Arabidopsis*, XIP1/CEPR1 and CEPR2. Application of CEP1 peptides into roots resulted in upregulation of *NRT2.1*, in a shoot CEPs-dependent manner. The findings suggest a root–shoot–root CEP/CLV signalling pathway in promoting N uptake. Together, a multi-layer global signalling circuit appears to have been employed by plants to balance C–N status and co-ordinate root–shoot development (Fig. 1).

Concluding Remarks

Significant progress has been made over the last several decades on roles of C allocation and sugar signalling in shoot–root communication and development. Plants have clearly evolved sophisticated mechanisms to manage the allocation of C, N and other nutrients between shoot and root through sugar signalling and other interacting pathways. The advances in genomic and molecular tools including large scale data analyses have provided great opportunities to study the systemic sugar signalling network

along with the roles of sugars as building blocks and energy resource. Among various challenges, tracing the spatial-temporal changes of sugars at the cellular and subcellular levels remains essential for better understating shoot-root communication. New technologies such as biosensors and magnetic resonance imaging (MRI) have opened up new windows to tackle this challenge. The former are genetically encoded fluorescent protein-based sensors for ion and metabolites including Suc and Glc, allowing *in vivo* visualisation of the spatial and temporal dynamics of sugars and other small molecules at the cellular and subcellular levels (e.g. Jones *et al.* 2013). The MRI permits non-destructive monitoring of sap flow through phloem and xylem at the whole-plant level (e.g. Windt *et al.* 2009). Application of these techniques together with other molecular and analytical tools will undoubtedly deepen and broaden our understanding on roles of sugars in co-ordinating shoot-root nutrient allocation and development.

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