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Research Article

Energy Flow from Root to Shoot: A Comprehensive *In silico* Analysis

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Background: Root to shoot connection and transfer of information seems to be taken place mostly via the transmissions of signal molecules, secondary metabolites, amino acids, hormones and proteins, through xylem sap. Examination of earlier reports is indicative of relatively high levels of conservation in xylem sap protein compositions. Apparently these protein molecules are being synthesized in roots in response to environmental changes and get transported to aerial plant parts after secretion into xylem sap.
Objectives: In order to comprehend this so-called passive signaling, some questions need to be answered: 1) Do these proteins have the capability to act as signals? 2) How much energy does root spend for the biosynthesis of the secreted proteins? How similar is the amount of energy that root cells spent for the biosynthesis of intra- and extra-cellular proteins?
Materials and Methods: Reported xylem sap proteins curated from Arabidopsis, maize and soybean. Their sequences were put under scrutiny in terms of considering their mobility, and physical and chemical properties. Metabolic energy required for their biosynthesis along with the energy hidden in their peptide bonds were calculated and compared with random non-xylem sap proteins as control.
Results: Xylem sap proteins were significantly smaller than the root proteins, while they were bigger in size when compared to the leaf group. Xylem protein pIs were significantly higher than the control proteins in different plants. Similarly, the protein stability was higher for xylem sap proteins in comparison with roots and leaves in all analyzed plants, except for soybean that the stability was indifferent between xylem and root. The data were suggestive a significantly lower energy consumption for the synthesis of xylem sap proteins.
Conclusions: Lower energy consumption may suggest an economical route of communication between roots and shoots in plants that mainly rely on symplastic signaling.

Keywords: Energy Transfer; Plant Roots; Plant Shoots; Proteins; Xylem

1. Background

Vascular plants use xylem and phloem system to transport water and solutes. However, apart from opposite direction of material transfer in these two funneling systems, they are different in other means; concentrations of dynamic compounds, pH values, and ionic concentrations. Xylem pH (~5.8) is lower than phloem (7.9) with amino acid content of ~ 40× more than the phloem sap (1). The concentration of compounds and the presence/absence of molecules.
Within the vascular system are greatly influenced by the changes in surrounding environment. It has become apparent that apart from slow apoplastic transfer of the information to the aerial parts of plants, they can use a relatively faster strategy via xylem sap (2). Sugars, hormones (abscisic acid, gibberellin, cytokinins and ethylene), amino acids, peptides and proteins are the signaling molecules that convey the perceived changes in root to shoot (3). After solutes, the focus was on other major constituents of the xylem sap, i.e., proteins. Peroxidases, chitinases, proteases, protease inhibitors, lectins, proteins related to lipid metabolism, proteins involved in defense mechanism (pathogenesis-related proteins), and cell wall metabolism can be found in

xylem sap proteomes with a concentration of 0.05- 0.1 mg.mL^{-1} (4-7).

2. Objectives

The purpose of this study was to investigate the possible involvement of the previously identified proteins in the signal transduction processes from root to shoot, and to analyze the feasibility of energy investment by plants in such signaling action. The hypothesis was that the energy required for the synthesis of proteins in roots and exporting them via xylem sap to upper plant parts costs less.

3. Materials and Methods

3.1. Sequence Curation and Preliminary Analysis

According to earlier reports, a list of xylem sap proteins from Arabidopsis (31 proteins), soybean (14 proteins), and maize (54 proteins) were curated. The list contained proteins naturally occurring and the proteins that being produced under stress conditions. Similarly, the same numbers of proteins with no relevance to xylem sap were randomly picked from expression sites, i.e., roots and leaves, in 3 replicates. The sequences of the proteins were retrieved from TAIR (http://www.arabidopsis.org/index.jsp) (8) and NCBI (http://www.ncbi.nlm.nih.gov/) (9) and further analyzed via ProtParam (http://www.expasy.org/ProtParam) (10) to numerate the amino acid content and establish the stability index of each protein. SignalP (http://www.cbs.dtu.dk.service/SignalP) was used to define if the protein does in fact have a transit peptide. To investigate if the proteins are undergoing through secretory route towards apoplast, Target P (http://www.cbs.dtu.dk/services/TargetP/) (11) was implemented. TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) was used to eliminate non-membrane bound proteins.

3.2. Energy Calculation for Amino Acid Biosynthesis

The energy required for the biosynthesis of each and every amino acids (12) in plants was calculated regarding to the biochemical pathways and biological cycles. First, sucrose was considered as the initiation point for all amino acids. Equivalent to each FADH2 molecule, two ATP molecules and for each NADH and NADPH molecule, 3 ATP molecules were considered. Total number of ATPs required for biosynthesis of amino acids was calculated and the data transformed and presented based on kJ.mol^{-1} (1 ATP = 30.6 kJ) (13). Later, energy involved in the biosynthesis of each and every protein was calculated as follows:

1. Energy formation for disulfide bond: Sulfide group of cysteines can form disulfide bridges internally and with other protein subunits. The formation of each bond consumes an average of 70 kJ.mol^{-1} energy (14).

$$\text{Disulfide bond energy formation} = 70 \times \frac{number\ of\ disulfide\ bonds\ formed\ in\ each\ linkage}{total\ number\ of\ amino\ acids}$$

2. Protein metabolic energy = $\sum_{i=1}^{n}(ea_i \times n_i)$

where n_i = the number of amino acids in desired proteins and ea_i = metabolic biosynthetic energy of desired amino acids

3. Peptide bond energy: a bond that is created between two amino acids called peptide bond. In which 2.4 kCal.mol^{-1} of energy is required for its formation (15).

Peptide bond energy = 2.4 × (number of amino acids in protein - 1)

4. Total energy = (*metabolic energy*) + (*peptide bond energy*) + (*disulfide bond energy*)

4. Results

4.1. Protein Features

According to ProtParam, the average-numbers of amino acids of xylem sap proteins were lower than the random proteins (**Fig. 1a**). Numbers of proteins with an average pI value of greater than 6.1 higher (5 ≤ pH ≤ 6.1) were higher for the xylem sap proteins compared to the control set (**Fig. 1b**). The xylem proteins appeared to be more stable (stability index < 40) as opposed to randomly selected proteins of root and leaf (**Fig. 1c**). Most of the xylem sap proteins, as opposed to randomly-picked proteins, had a secretory signal peptide (**Fig. 1d**) that leads them to the apoplastic milieu (**Fig. 1e**).

4.2. The Amount of Energy Laid in the Xylem Sap Proteins

Arabidopsis: In all three replication, total biosynthetic energy of xylem sap proteins for 26 proteins was different to that of random proteins in leaves and roots. Total energies required for the biosynthesis of random. Proteins of roots and leaves in average were 36579 kJ. mol^{-1} and 27694 kJ. mol^{-1} respectively (**Table 1**). The average biosynthetic energy for xylem sap proteins was 21655 kJ. mol^{-1} and it was significantly smaller than that of the random proteins from leaves and roots.

Maize: In all three replicates, total biosynthetic energy of xylem sap proteins for 48 proteins was different to that of random proteins of leaves and root. Total energy required for the biosynthesis of random proteins of roots and leaves in average were 64122 kJ. mol^{-1} and 72387 kJ. mol^{-1}, respectively (**Table 2**). The average biosynthetic energy for xylem sap proteins was 59383 kJ. mol^{-1} and significantly smaller than the random proteins from leaves and roots.

Soybean: In all three replicates, total biosynthetic energy of xylem sap proteins for 48 proteins was different to that of random proteins of leaves and root. Total energy required for the biosynthesis of random proteins of roots and leaves in average were 13335 kJ. mol^{-1} and 13941 kJ. mol^{-1}, respectively (**Table 3**). The average biosynthetic energy for xylem sap proteins was

5698 kJ.mol^{-1} and significantly smaller than the random proteins from leaves and roots.

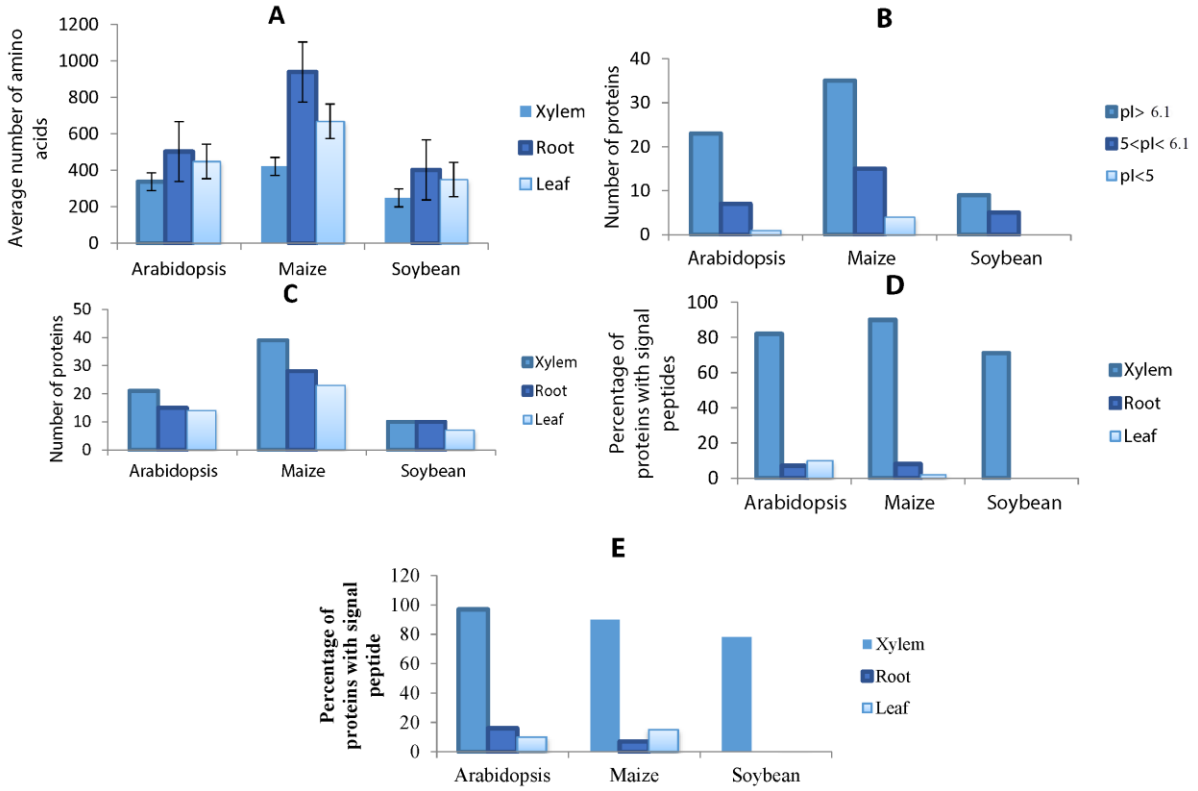

Figure 1. Statistics of xylem sap vs. random proteins chosen from leaves and roots in Arabidopsis, maize and soybean. A) Average number of amino acids B) Isoelectric point C) Number of proteins with instability index of smaller than 40. D) Percentage of proteins with signal peptides E). The relative abundance of proteins that enter the secretory pathways. In cases where bar charts are missing the number is zero.

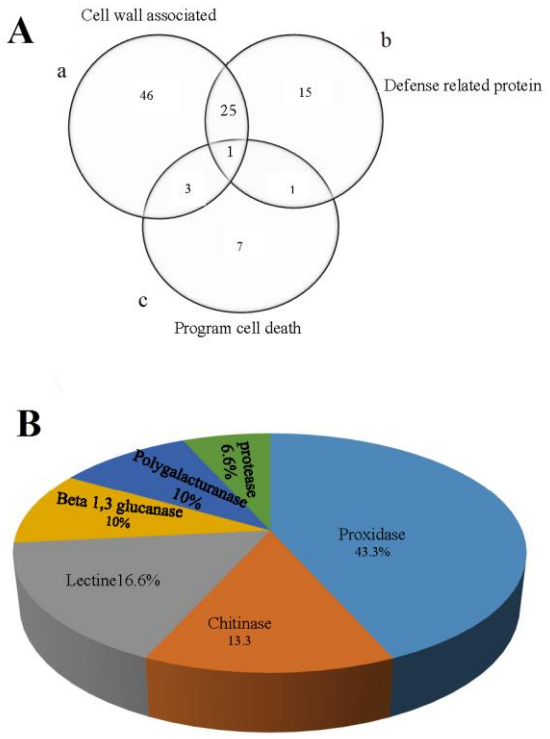

Figure 2. A. Proteins involved in cell wall (a), responsive proteins to pathogens (b) proteins involved in program cell death (c). B. Distribution of xylem sap proteins into major classes.

Table 1. The energy required for the biosynthesis of xylem sap and random proteins obtained from leaves and roots in Arabidopsis (kJ/mol)

	Replication						
		1st		2nd		3rd	
	Xylem	Root	Leaf	Root	Leaf	Root	Leaf
Formation of disulfide bond	0.3	0.2	0.11	0.2	0.1	0.2	0.08
Metabolic	27.34	28.62	28.35	28	28	28.4	28.41
Peptide bond	21628	32616	26872	40732	28005.6	36304.2	28122
Total	21655.64	32644.82	26900.46	40760.2	28033.7	36332.8	28150.49

Table 2. The energy required for the biosynthesis of xylem sap and random proteins obtained from leaves and roots in Maize (kJ/mol)

	Replication						
		1st		2nd		3rd	
	Xylem	Root	Leaf	Root	Leaf	Root	Leaf
Formation of disulfide bond	0.2	0.2	0.2	0.2	0.2	0.26	0.11
Metabolic	27.52	28.8	28.69	28.64	28.35	28.77	28.57
Peptide bond	59356	61543.4	72532.8	70948.8	74668.8	59788	69876
Total	59383.72	61572.4	72560.89	70977.64	74697.35	59817.03	69904.68

Table 3. The energy required for the biosynthesis of total xylem sap and random proteins obtained from leaves and roots in Soybean (kJ.mol)

	Replication						
		1st		2nd		3rd	
	Xylem	Root	Leaf	Root	Leaf	Root	Leaf
Formation of disulfide bond	0.1	0.2	0.2	0.04	0.1	0.2	0.01
Metabolic	27.79	28.8	28.97	28.69	28.27	28.62	27.91
Peptide bond	5671	13516.8	13089.6	14476.8	13687.2	11926	14961.6
Total	5698.89	13545.8	13118.77	14505.53	13715.57	11954.82	14988.92

4.3. The Functional Classification of Xylem Sap Proteins

The xylem sap proteins in all investigated plant species in this study can be classified in three groups as cell wall-related, defense-related, and programmed cell death-related. Peroxidase, chitinase, protease, polyglacturonase, β-(1,3)-glucanase and lectin were similar over the plant species (**Figs. 2a** and **b**).

5. Discussion

It seems that one way of disseminating the information through plants would be via chemical signals in order to respond to internal and external changes (6, 16, 17). In this study, the putative role of proteins as signaling molecules through xylem sap from roots to shoots of Arabidopsis, soybean and maize were investigated. In this regard, the economy of energy flow and the level of conservation in terms of biodiversity of protein molecules between species were also put under scrutiny. It seems such signals are being generated in plant roots and transferred to shoots via xylem sap. Among which, a population of secretory proteins mostly involved in defense mechanisms and some being functionally conserved seems to play such signaling role. It has been reported that some proteins in xylem sap are conserved among the various plant species (18), mostly bearing signal peptides (19, 20). Thus, some of the root proteome profiles were checked for their predicted transit peptides, secreting into the xylem. To confirm the predictions, the candidate proteins were checked against earlier xylem proteomics data (4-7). The generated protein list was checked for their function through homology based searches and classified into pathogen-responsive proteins, stress response proteins, cell wall metabolism and programmed cell death. Analyses of protein lengths between root-secreted and other places were indicated that the earlier was significantly shorter; proposing less expenditure of energy for so-called signaling molecules. Furthermore, they appeared to be relatively stable in extra-cytoplasmic milieu (instability index smaller than 40) (12, 21, 22). Generally, the protein pIs were more than 6.1 that make the overall protein surface charge distribution positive. Since the apoplastic net charge due to the action of membrane H^+-ATPases are generally positive then the protein transfer within the xylem (pH 5.8) would be smooth with little to no interaction with surrounding cell wall. On average the spent energy for synthesis of reported xylem proteins (4, 5, 7) were much smaller than randomly chosen proteins from other tissues. Also this suggests that the protein molecules have the potential to be used as signal carriers from roots to the aerial parts. In addition of being signals, xylem resident proteins play pivotal functions in suppressing invading pathogens and invigorating surrounding cell wall. Studies performed on the function of protein showed that xylem sap protein have a variety of roles in plants, such as defense, cell wall metabolism, program cell death, and tolerance to salt stress (23-26). The majority of enzymes were cell wall-related proteins: peroxidase (43.3%), lectins (16.6%), chitinases (13.3%), β-(1→3)-glucanase, polygalacturonase (10%) and proteases (6.6%). In summary, it seems that the production of these proteins at the root and its transmission to the shoot in all three plants are economical.

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