Comparative proteomics analysis of proteins expressed in the I-1 and I-2 internodes of strawberry stolons

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Abstract
Background: Strawberries (Fragaria ananassa) reproduce asexually through stolons, which have strong tendencies to form adventitious roots at their second node. Understanding how the development of the proximal (I-1) and distal (I-2) internodes of stolons differ should facilitate nursery cultivation of strawberries.

Results: Herein, we compared the proteomic profiles of the strawberry stolon I-1 and I-2 internodes. Proteins extracted from the internodes were separated by two-dimensional gel electrophoresis, and 164 I-1 protein spots and 200 I-2 protein spots were examined further. Using mass spectrometry and database searches, 38 I-1 and 52 I-2 proteins were identified and categorized (8 and 10 groups, respectively) according to their cellular compartmentalization and functionality. Many of the identified proteins are enzymes necessary for carbohydrate metabolism and photosynthesis. Furthermore, identification of proteins that interact revealed that many of the I-2 proteins form a dynamic network during development. Finally, given our results, we present a mechanistic scheme for adventitious root formation of new clonal plants at the second node.

Conclusions: Comparative proteomic analysis of I-1 and I-2 proteins revealed that the ubiquitin-proteasome pathway and sugar-hormone pathways might be important during adventitious root formation at the second node of new clonal plants.

Background
Several stoloniferous species produce long, sympodial stolons with rooted rosettes (ramets) at their nodes [1,2]. For the garden strawberry (Fragaria ananassa), the mother plant forms plantlets on stolons during spring growth (Figure 1). The first stolon originates from an auxiliary leaf bud produced in the central crown and commonly contains only two nodes. The regions along the stolon and between the plant and the first node and the first and second nodes are the I-1 (proximal) and I-2 (distal) internodes, respectively. Although stolon growth requires internode elongation, the fates of the two nodes are dissimilar. First, I-1 elongates and terminates at the first node, which is non-productive, then I-2 elongates and terminates at the second node, which forms the main crown of the clonal plant.

Many studies have assessed the relationships between plant genotype and phenotype [3] using morphological differences caused by loss of function or altered expression of a single gene. To fully understand the function of a gene, however, the expressed protein must be characterized. Proteomics investigates the synthesis, turnover, and modification of proteins so that gene function and genotypes can be understood [4]. For example, a proteomic study of ripening strawberry fruit from plants of different genotypes identified constitutively and differentially expressed proteins that probably control the quality of the fruit [5]. Proteomics may, therefore, be used to address biochemical and physiological aspects of plant morphologies. Such approaches are increasingly used to elucidate the biochemistry and physiology of model species [6,7]. Yet proteomics is limited by the ability to identify proteins, which relies on the availability of sequence data. For plant species with unsequenced genomes, proteomics can still be applied but the number of identified proteins is usually smaller because their
identification relies on homology with proteins of other species. For example, although *F. ananassa* sequence data is very limited, two proteomic techniques, namely two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS), have been used to identify an *F. ananassa* protein homologous to the birch pollen allergen Bet v 1 [8].

Elongation of the strawberry stolon is considered to be the result of cell division and cell expansion [9], but little is known about how the I-1 and I-2 internodes develop. Such knowledge is required, however, if we are to improve the cultivation of nursery strawberries and understand in greater depth how clonal multiplication occurs.

For the work reported herein, we compared the proteomes of the *F. ananassa* I-1 and I-2 internodes to elucidate the differences in their growth and functional characteristics and establish reference maps by identifying the protein spots of their 2-DE maps in conjunction with MS peptide mass determination and database searches. We identified isoforms of several proteins and present a detailed analysis of the two proteomes, which allows us to begin to explore the different developmental mechanisms of the I-1 and I-2 internodes. The reference maps should be useful for investigation of strawberry physiology and for monitoring changes in protein expression in strawberry stolons in response to biotic and abiotic stresses.

**Materials and methods**

**Plant material**

The *F. ananassa* cultivar Hongjia was obtained from a nursery at the Hangzhou Academy of Agricultural Sciences, Zhejiang, China. The plants were grown in a tunnel greenhouse with a 10-h light/14-h dark cycle, a 30°C-day/26°C-night temperature cycle, 150 μmol m⁻² s⁻¹ light intensity, and a relative humidity of 60%. Plants were watered regularly and provided adequate nutrients.

In the morning, after transplantation, stolons that had formed from the leaf buds of three-month-old plants were collected and cut to isolate the I-1 and I-2 internodes (Figure 1). Daughter plants that had formed at the apices of stolons had not been allowed to root. For each experiment, 50 stolons were randomly chosen and removed from four or five plants. The I-1 and the I-2 internodes were pooled separately, rinsed with water to remove contaminants, quickly dried with paper towels, frozen in liquid nitrogen, and stored at -80°C prior to protein extraction.

**Protein extraction**

Proteins were extracted using acetone and trichloroacetic acid method. A portion (2 g) of each internode sample was pulverized with a pestle in a mortar that contained liquid nitrogen and then homogenized in 10 mL of 10% (w/v) trichloroacetic acid, 0.07% (v/v) 2-sulfanylethanol in acetone. Total protein was precipitated for 1 h or overnight at -20°C. The extracts were each centrifuged at 13000 × g for 20 min at 4°C. The pellets were washed three times with 0.07% (v/v) 2-sulfanylethanol in acetone, and vacuum dried for 30 min. The dried powders (30 mg) were each resuspended in 500 μL of 7 M urea, 2 M thiourea, 4% 3-[3-cholamidopropyl] dimethylammonio]-1-propanesulfonate (CHAPS), 0.75% dithiothreitol (DTT), 0.5% Biolyte (pH 3.0-10.0, Bio-Rad), 1 mM phenylmethanesulfonyl fluoride, and then shaken vigorously for 1 h at room temperature. Insoluble material was removed by centrifugation at 13000 × g for 15 min at 20°C. At least three replicates were prepared. Protein concentrations were determined using Bio-Rad Protein Assay kit reagents (standard Bradford method) with bovine serum albumin as the calibration standard [10].

**Two-dimensional gel electrophoresis**

Each sample contained 300 μg protein in 350 μL of 8 M urea, 2 M thiourea, 2% CHAPS, 0.5% Biolyte (pH 3-10), 0.75% M DTT, 0.002% Bromophenol Blue. Each sample was each loaded onto a 17-cm immobilized pH (3-10) gradient strip (Bio-Rad). The strips were rehydrated for 12 h at 50 V. Isoelectric focusing used a linear ramp from 0 to 250 V in 15 min, a linear ramp from 250 to 10000 V in 1 h, and 10000 V for 5 h, all at 20°C. After isoelectric focusing, the strips were equilibrated in 50 mM Tris-HCl, pH 8.8, 6 M urea, 20% glycerol, 2% sodium dodecyl sulfate (SDS), 2% DTT, and then in a solution of the same composition that also contained 2.5% (w/v) iodoacetamide, (the time of each incubation was 15 min). The strips were then each placed onto a 1-mm-thick SDS (12.5% (w/v)) polyacrylamide gel and sealed with 1% (w/v) agarose. Electrophoresis was carried out in a Bio-Rad PROTEAN apparatus at...
24 mA/gel. The gels were stained using a modified silver-staining method that is compatible with MS [11]. Image analysis was subsequently performed. These procedures were replicated three times.

Image acquisition and analysis
The three replicates of the I-1 and I-2 2-DE gels were scanned using a calibrated densitometer (GS-800, Bio-Rad), and the spot patterns were characterized using PDQuest software (ver. 8.0.1, Bio-Rad). Image analysis steps included image filtration, spot detection and measurement, background subtraction, and spot matching. One I-1 gel served as the reference, and the spots of the other five gels were referenced to it. Initially, spots were automatically matched, and the positions of unmatched spots were then manually determined. The molecular mass (kDa) of each protein was estimated by comparison with those of a standard marker set, and the isoelectric points (pis) were determined by the spot positions along the immobilized pH gradient strips.

In-gel protein digestion and mass spectrometry
The silver-stained protein spots were manually excised from the gels, and each was placed into a well of a 96-well microplate. The gel pieces were destained in a solution prepared from a 1:1 (v/v) mixture of 30 mM potassium ferricyanide and 100 mM sodium thiosulfate at room temperature for 10 min, vortexed until destained, washed three times with 300 µL of Milli-Q water (each time for 5 min) and dehydrated in 150 µL of acetonitrile. Then the gel samples were swollen in 50 mM NH₄HCO₃ containing 12.5 ng/µL trypsin (Sigma, Cat. No. 089K6048) at 4°C for 30 min, and at 37°C for longer than 12 h. For each digest, the peptides were extracted from the gels twice with 5% trifluoroacetic acid in 0.1% trifluoroacetic acid/50% acetonitrile, and resuspended at room temperature for 10 min. Then the proteins were not included in the database or because a protein spot contained more than one protein. For protein identification, the peptide mass fingerprinting data were used in conjunction with a search of the complete Swissprot Viridiplantae taxonomic database as only 1017 F. ananassa protein sequences were available therein. Respectively, 164 and 200 spots from the I-1 and I-2 gels were selected for MS, and 38 and 52 proteins were identified, i.e., ~25% of the total in each case (Tables 1 and 2). No unambiguous matches were made for the other 274 proteins, probably because the proteins were not included in the database or because a protein spot contained more than one protein. For the 90 identified proteins, 5 (6.5%) were common to both proteomes, which indicated that the two internodes had some proteins (probably housekeeping proteins) in common. Certain proteins (35% of the gene products) were specific to I-1, whereas 58.5% were specific to I-2 (Figure 3). More than 50% of the proteins could be correlated with annotated proteins from at least one dicotyledon species (Figure 4). Of the F. ananassa proteins identified in our study, 13 I-1 and 25 I-2 proteins were matched to Arabidopsis thaliana proteins, and others were matched to Oryza sativa proteins (two from I-1, six from I-2) and Zea mays proteins (three from I-1, two from I-2). Only five I-1 proteins and three I-2 proteins could be matched to those found in the F. ananassa database, possibly because the number of annotated proteins

Gene ontology (GO) annotation and protein classification
The UniProt database (http://www.uniprot.org) was searched to determine the functions of the identified proteins. Three independent ontological sets in the Viridiplantae taxonomic database were used to annotate and group the proteins according to biological processes, molecular function, and cellular compartmentalization.

Protein-protein interactions (PPIs)
PPIs were predicted by Cytoprophet, which is a plug-in of Cytoscape. We sent all the data for the identified proteins in I-1 and I-2 to the Cytoprophet server along with the set cover approach maximum specificity set cover (MSSC). The algorithm was used to predict the interaction network(s).

Results and discussion
Proteome analysis and protein identification
We investigated the differences between the I-1 and I-2 protein profiles. More than 503 I-1 and 1127 I-2 protein spots were reproducibly detected (Figure 2). The pis of the protein spots ranged from 3.5 to 9.3, and the molecular masses ranged from 7.1 to 60.2 kDa. For protein identification, the peptide mass fingerprinting data were used in conjunction with a search of the complete Swissprot Viridiplantae taxonomic database as only 1017 F. ananassa protein sequences were available therein. Respectively, 164 and 200 spots from the I-1 and I-2 gels were selected for MS, and 38 and 52 proteins were identified, i.e., ~25% of the total in each case (Tables 1 and 2). No unambiguous matches were made for the other 274 proteins, probably because the proteins were not included in the database or because a protein spot contained more than one protein. For the 90 identified proteins, 5 (6.5%) were common to both proteomes, which indicated that the two internodes had some proteins (probably housekeeping proteins) in common. Certain proteins (35% of the gene products) were specific to I-1, whereas 58.5% were specific to I-2 (Figure 3). More than 50% of the proteins could be correlated with annotated proteins from at least one dicotyledon species (Figure 4). Of the F. ananassa proteins identified in our study, 13 I-1 and 25 I-2 proteins were matched to Arabidopsis thaliana proteins, and others were matched to Oryza sativa proteins (two from I-1, six from I-2) and Zea mays proteins (three from I-1, two from I-2). Only five I-1 proteins and three I-2 proteins could be matched to those found in the F. ananassa database, possibly because the number of annotated proteins
contained in the database is relatively small compared with that for the *A. thaliana* database.

**Protein isoforms and subunits of protein complexes**

Isoforms for malate dehydrogenase (spots 15, 90, and 91), glucan endo-1,3-β-glucosidase 6 (spots 29 and 31), triosephosphate isomerase (spots 69 and 72), oxygen-evolving enhancer protein 1 (spots 126 and 127), and a putative F-box/kelch-repeat protein (spots 62 and 151) were found in the I-1 proteome, and isoforms for malate dehydrogenase (spots 129 and 141), U-box domain-containing protein 34 (spots 153 and 175), and the probable protein ABIL5 (spots 12 and 48) were found for I-2 (Tables 1 and 2). Therefore, respectively, ~29% and ~12% of the identified I-1 and I-2 proteins exist as isoforms. Previous studies reported that ~70% of maize or Arabidopsis proteins exist as isoforms [12,13]. The experimental masses and/or pIs of certain proteins differed from their theoretical values, possibly owing to co-translational and/or post-translational modification (e.g., glycosylation, phosphorylation, and/or proteolysis), translation from alternatively spliced mRNAs [14-17], or post-translational modification by a nonprotein component(s) [18].

Of the 90 identified proteins, 16 (more than 17%) form complexes [19]. For example, two I-1 spots corresponded to the α and β subunits of chloroplastic ATP synthase. The β-subunit of the DNA-directed RNA polymerase subunit was detected in both the I-1 and I-2 proteomes. For some complexes, all component subunits were detected; for instance, both the small and large subunits of ribulose-bisphosphate carboxylase/oxygenase (RuBisCo) were identified in the I-1 proteome. The fact that several complexes were identified will facilitate future studies of how complex formation is regulated. The components of a protein complex would be expected to be coordinately expressed and regulated to keep a system in functional balance. Thus, our 2-DE reference maps can be used to compare the levels of functionally related polypeptides (isoforms and subunits of complexes) and may provide insights into protein function and participation in molecular networks.

**Functional classification and subcellular localization of the identified proteins**

Identification of proteins that are differentially expressed in I-1 and I-2 is important for our understanding of internodal development and differentiation. The identified proteins were grouped according to their biological processes, i.e., photosynthesis, protein synthesis, protein folding, transcription, transport, stress, and development, and cellular locations using the GO annotation in the Viridiplantae taxonomic databases (Figures 5 and 6). Proteins involved in metabolism and photosynthesis accounted for 32% and 18%, respectively, of the I-1 proteins. The numbers of I-1 proteins involved in transcription (13%) and energy production (8%) were also substantial. Interestingly, for I-2, although still the largest two groups, metabolic proteins accounted for only 15% and those involved in photosynthesis for 16%. I-2 proteins involved in protein synthesis and DNA transcription each accounted for 12% of the total. The function
| Spot No. | Protein | Accession Number | Molecular Function | Reference Organism | Theoretical kDa/pI | Experimental kDa/pI | Score SC (%) | Matched/Unmatched queries |
|----------|---------|------------------|--------------------|--------------------|-------------------|--------------------|--------------|-----------------------------|
| 15       | Malate dehydrogenase | P83373           | L-malate dehydrogenase activity | Fragaria ananassa | 35.8/8.7         | 13.5/5.3         | 99           | 41/943                      |
| 29       | Glucan endo-1,3-beta-glucosidase 6 | Q93Z08        | glucan endo-1,3-beta-D-glucosidase activity | Arabidopsis thaliana | 52.6/5.6 | 20.3/5.8 | 58 | 27/635                      |
| 31       | Glucan endo-1,3-beta-glucosidase 6 | Q93Z08        | glucan endo-1,3-beta-D-glucosidase activity | Arabidopsis thaliana | 52.6/5.6 | 20.4/5.0 | 63 | 21/542                      |
| 40       | Unicase | Q04420           | urate oxidase activity | Arabidopsis thaliana | 35.0/8.6 | 23.4/6.1 | 58 | 38/1047                     |
| 42       | GDSL esterase/lipase At4g16220 | O23469 | hydrolase activity, acting on ester bonds | Arabidopsis thaliana | 26.7/8.9 | 23.2/7.8 | 57 | 37/636                      |
| 90       | Malate dehydrogenase, mitochondrial | P83373       | L-malate dehydrogenase activity | Fragaria ananassa | 35.8/8.7 | 40.3/8.2 | 122 | 51/1050                     |
| 91       | Malate dehydrogenase, mitochondrial | P83373 | L-malate dehydrogenase activity | Fragaria ananassa | 35.8/8.7 | 43.3/8.3 | 100 | 50/945                      |
| 111      | S-adenosylmethionine synthase | Q8W3Y4 | metal ion binding | Phaseolus lunatus | 43.5/5.6 | 47.4/5.8 | 74 | 24/79                       |
| 118      | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase | P30792 | manganese ion binding | Zea mays | 60.7/5.2 | 41.3/4.9 | 59 | 24/827                       |
| 133      | Fructokinase-1 | A2WXV8 | ATP binding | Oryza sativa | 34878/5.1 | 36.1/3.8 | 76 | 23/615                       |
| 142      | S-adenosylmethionine synthase 4 | A9PHC5 | metal ion binding | Populus trichocarpa | 42999/5.7 | 46.5/6.3 | 74 | 18/52                        |
| 162      | Phosphoglucomutase | P93804 | magnesium ion binding | Zea mays | 63286/5.4 | 60.2/6.1 | 63 | 17/930                       |
| 152      | ATP synthase subunit beta, chloroplastic | Q9MR99 | ATP binding | Brasenia schreberi | 53.8/5.2 | 45.3/4.8 | 76 | 27/819                       |
| 153      | ATP synthase subunit beta | Q01859 | ATP binding | Oryza sativa | 59.0/5.9 | 48.1/5.4 | 134 | 44/1945                     |
| 155      | ATP synthase subunit beta | Q6QBP2 | ATP binding | Castanea sativa | 53.8/5.3 | 45.9/4.4 | 108 | 37/1526                     |
| 156      | ATP synthase subunit alpha | A4QJA0 | ATP binding | Aethionema cordifolium | 55.3/5.2 | 58.5/3.5 | 114 | 22/1013                     |

**Energy**

| Spot No. | Protein | Accession Number | Molecular Function | Reference Organism | Theoretical kDa/pI | Experimental kDa/pI | Score SC (%) | Matched/Unmatched queries |
|----------|---------|------------------|--------------------|--------------------|-------------------|--------------------|--------------|-----------------------------|
| 2        | Ribulose bisphosphate carboxylase small chain 1A | P10795 | monooxygenase activity | Arabidopsis thaliana | 20.5/7.6 | 10.7/7.8 | 62 | 43/7101                     |
| 37       | Ribulose bisphosphate carboxylase large chain (Fragment) | O98681 | ribulose-bisphosphate carboxylase activity | Zamioculcas zamifolia | 49.9/6.3 | 21.4/4.9 | 79 | 25/1078                     |
| 46       | Ribulose bisphosphate carboxylase large chain | P28439 | magnesium ion binding | Pelargonium hortorum | 53.3/6.3 | 23.7/7.5 | 62 | 32/13104                    |
| 69       | Triosephosphate isomerase | Q9M458 | triose-phosphate isomerase activity | Fragaria ananassa | 33.7/7.6 | 30.0/5.1 | 152 | 55/19112                    |
| 72       | Triosephosphate isomerase | Q9M458 | triose-phosphate isomerase activity | Fragaria ananassa | 33.7/7.6 | 31.3/5.6 | 126 | 59/1677                     |
| 126      | Oxygen-evolving enhancer protein 1 | P26320 | calcium ion binding | Solanum tuberosum | 35.6/5.8 | 35.6/4.4 | 111 | 33/88                        |
| 127      | Oxygen-evolving enhancer protein 1 | P26320 | calcium ion binding | Solanum tuberosum | 35.6/5.8 | 34.9/4.8 | 113 | 28/921                        |
could not be determined for 3% of the identified I-1 proteins and ~13% of the I-2 proteins. The percentage of unidentified I-2 proteins is similar to those found for maize [20], rice [21] or oilseed rape [22]. The substantial differences in the numbers of I-1 and I-2 proteins involved in metabolism, energy production, and protein synthesis suggest that the functions of these proteins during development deserve further attention.

Subcellular localization provides important information about a protein’s physiological function [23,24]. Recently, GO annotation has been widely used to predict the locations of proteins [25-29], because the two are strongly correlated. We found most of the identified proteins to be located in chloroplasts and mitochondria, which is congruent with total genomic data available for plants [30]. Interestingly, most of these proteins are involved in metabolism or energy production, which is consistent with the large number of proteins that we classified as metabolic or photosynthetic. We could not identify the cellular location of ~11% and ~25% I-1 and I-2 proteins, respectively.

### Table 1 Identified proteins from the strawberry stolon I-1 internode (Continued)

|     | Transcription | Protein binding | Arabidopsis thaliana |     |     |     |     |     |     |
|-----|---------------|-----------------|----------------------|-----|-----|-----|-----|-----|-----|
| 6   | Histone acetyltransferase GCN5 | Q9AR19          | 63.5/6.0 | 14.2/4.1 | 59 | 29 | 11/66 |
| 18  | Pentatricopeptide repeat-containing protein At3g09650 | Q9SF38          | 84.4/6.5 | 11.2/6.2 | 80 | 23 | 14/49 |
| 27  | Transcription factor bHLH145 | Q9FGB0          | 35.2/5.1 | 18.3/5.1 | 62 | 28 | 10/63 |
| 92  | DNA-directed RNA polymerase subunit beta | B1VKHS         | 139.6/9.4 | 44.6/8.1 | 59 | 12 | 11/65 |
| 136 | DNA polymerase | P10582          | 108.2/8.6 | 55.3/8.5 | 58 | 15 | 8/23  |
|     | Protein synthesis |             |                     |     |     |     |     |     |     |
| 154 | 5S ribosomal protein L33 | B2LML4         | 7.8/9.7 | 56.4/5.1 | 62 | 42 | 5/11  |
|     | Protein folding, degradation and assembly |             |                     |     |     |     |     |     |     |
| 45  | Heat shock protein B1-3 | P51818          | 80.2/5.0 | 26.7/8.2 | 59 | 19 | 9/85  |
| 73  | Proteasome subunit alpha type-6 | O48551        | 27.5/5.8 | 28.3/6.1 | 58 | 31 | 6/62  |
|     | Transport |             |                     |     |     |     |     |     |     |
| 23  | NAD(P)H-quinone oxidoreductase subunit H, chloroplastic OS | A6MMH1          | 45.8/5.4 | 18.5/7.3 | 75 | 26 | 12/66 |
| 52  | Ras-related protein ARA-4 | P28187         | 24.1/5.0 | 28.6/8.4 | 61 | 28 | 10/101|
|     | Stress Related |             |                     |     |     |     |     |     |     |
| 62  | Putative F-box/kelch-repeat protein At1g19330 | O65704         | 62.9/7.1 | 27.3/5.7 | 59 | 25 | 10/82 |
| 151 | Putative F-box/kelch-repeat protein At1g19330 | O65704         | 62.8/7.1 | 44.3/4.8 | 65 | 21 | 8/75  |
|     | Development |             |                     |     |     |     |     |     |     |
| 53  | 3-ketoacyl-CoA synthase 5 fatty acid elongase activity | Q9C6L5          | 56.3/9.0 | 27.7/8.1 | 61 | 40 | 12/19 |
| 81  | Cytoplasmic dynein 2 heavy chain 1 | Q9SMH5         | 483.5/6.1 | 40.0/8.6 | 58 | 7  | 22/48 |
|     | Unknown |             |                     |     |     |     |     |     |     |
| 8   | Embryonic abundant protein VF30.1 OS = Vicia faba PE = 2 SV = 1 | P21745 | 30.1/6.4 | 18.1/4.2 | 67 | 33 | 7/67  |

*As indicated in Figure 2. *Swissprot database accession number. *Molecular functions were inferred from those reported in the UniProt database. *Theoretical isoelectric point and molecular mass. *Experimental isoelectric point and molecular mass. *Mascot score. *Sequence coverage. *Number of matched and unmatched peptides.
| Spot No. | Protein | Accession 
Number | Molecular Function | Reference Organism | Theoretical 
kDa/pI | Experimental 
kDa/pI | Score | SC (%) | Matched/Unmatched 
queries |
|---------|---------|-----------------|-------------------|----------------|-------------|--------------|--------|--------|------------------|
| **Metabolism** | | | | | | | | | |
| 91 Fructokinase-1 | A2WXV8 | ATP binding | Oryza sativa | 34.9/5.1 | 35.8/3.4 | 76 | 20 | 6/59 |
| 95 Caffeic acid 3-O- methyltransferase | Q8GULZ5 | caffeate O- methyltransferase activity | Rosa chinensis | 40.1/5.6 | 43.7/4.4 | 110 | 35 | 12/30 |
| 129 Malate dehydrogenase, mitochondrial | P83373 | Oxidoreductase | Fragaria ananassa | 35.8/8.7 | 39.1/7.8 | 74 | 31 | 8/28 |
| 139 Glyceraldehyde-3-phosphate dehydrogenase, cytosolic | P25858 | Oxidoreductase | Arabidopsis thaliana | 37.0/6.6 | 40.1/9.3 | 74 | 29 | 9/27 |
| 141 Malate dehydrogenase, mitochondrial | P83373 | Oxidoreductase | Fragaria ananassa | 35.8/8.7 | 41.0/9.1 | 65 | 27 | 6/23 |
| 172 S-adenosylmethionine synthase 2 | Q9FUZ1 | ATP binding | Brassica juncea | 43.3/5.3 | 43.9/5.9 | 110 | 35 | 12/30 |
| 173 S-adenosylmethionine synthase | Q9FUZ1 | ATP binding | Arabidopsis thaliana | 43.7/5.6 | 38.5/6.8 | 178 | 52 | 15/17 |
| 174 S-adenosylmethionine synthase | Q9FUZ1 | ATP binding | Phaseolus lunatus | 43.5/5.6 | 41.2/6.7 | 132 | 48 | 15/40 |
| **Energy** | | | | | | | | | |
| 176 ATP synthase subunit beta, plastid | Q8MBG5 | ATP binding | Cuscuta pentagona | 53.2/5.5 | 53.4/6.7 | 61 | 32 | 10/53 |
| **Photosynthesis** | | | | | | | | | |
| 17 Probable alpha,alpha-trehalose-phosphate synthase (UDP-forming) 4OS | Q9T079 | alpha,alpha-trehalose-phosphate synthase (UDP-forming) activity | Arabidopsis thaliana | 90.3/6.1 | 14.2/6.3 | 58 | 25 | 12/66 |
| 33 Ribulose bisphosphate carboxylase large chain (Fragment) 4OS | P2B261 | magnesium ion binding | Nypa fruticans | 51.5/6.2 | 22.3/5.1 | 123 | 23 | 13/61 |
| 35 Probable granule-bound starch synthase 1, chloroplastic/amyloplastic | Q9MAQ0 | starch synthase activity | Arabidopsis thaliana | 67.5/8.7 | 23.0/4.5 | 60 | 24 | 9/37 |
| 52 Ribulose bisphosphate carboxylase large chain (Fragment) 4OS | P2B261 | magnesium ion binding | Ceratopetalum gummiferum | 51.3/6.2 | 24.0/6.9 | 104 | 22 | 11/63 |
| 64 Ribulose-phosphate 3-epimerase, chloroplastic | Q43157 | ribulose-phosphate 3-epimerase activity | Spinacia oleracea | 30.6/8.2 | 27.2/7.9 | 58 | 31 | 6/51 |
| 82 Triosephosphate isomerase, chloroplastic | Q9M4S0 | triose-phosphate isomerase activity | Arabidopsis thaliana | 33.7/7.6 | 31.5/5.2 | 154 | 51 | 16/71 |
| 90 Oxygen-evolving enhancer protein 1, chloroplastic | P26320 | calcium ion binding | Solanum tuberosum | 35.6/5.8 | 34.5/4.7 | 98 | 31 | 10/48 |
| 103 Coproporphyrinogen-III oxidase, chloroplastic | P35055 | coproporphyrinogen oxidase activity | Glycine max | 43.6/6.7 | 42.2/5.2 | 84 | 31 | 10/32 |
| 104 Glutamyl-tRNA reductase 1, chloroplastic | Q42843 | NADP or NADPH binding | Hordeum vulgare | 58.1/8.7 | 42.1/4.9 | 70 | 24 | 11/48 |
| **Transcription** | | | | | | | | | |
| 18 Homeobox-leucine zipper protein GLABRA 2 OS | P46607 | sequence-specific DNA binding | Arabidopsis thaliana | 83.7/6.1 | 15.0/6.4 | 63 | 31 | 20/90 |
| 47 Protein HIRA | Q32SG6 | transcription regulator activity | Zea mays | 106.5/7.8 | 19.7/7.1 | 60 | 22 | 16/90 |
| 121 Two-component response regulator ARR9 | O80366 | two-component response regulator activity | Arabidopsis thaliana | 26.2/5.2 | 34.6/8.1 | 58 | 35 | 5/43 |
| 128 DNA-directed RNA polymerase subunit beta | P12465 | DNA binding | Chlorella vulgaris | 179.4/9.9 | 36.9/8.0 | 60 | 9 | 11/34 |
| 149 B3 domain-containing protein Os07g0679700 | Q6Z3U0 | DNA binding | Oryza sativa | 105.4/6.5 | 53.5/7.9 | 63 | 11 | 9/27 |
| 168 Protein HIRA | Q32SG6 | transcription regulator activity | Zea mays | 106.5/7.8 | 41.1/6.8 | 60 | 22 | 16/90 |
Table 2 Identified proteins from the strawberry stolon I-2 internode (Continued)

| **Protein synthesis** | **Protein** | **Function** | **Organism** | **Expression** |
|-----------------------|-------------|--------------|--------------|---------------|
| 28                    | Eukaryotic translation initiation factor 5A-2 OS | translation initiation factor activity | *Medicago sativa* | 17.5/5.4 17.3/4.7 74 26 5/39 |
| 37                    | Eukaryotic translation initiation factor 5A | translation initiation factor activity | *Dianthus canyphyllus* | 17.6/5.6 20.3/5.9 88 45 7/28 |
| 45                    | Glutathione gamma-glutamylcysteine transferase 2 | acyltransferase activity | *Arabidopsis thaliana* | 52.3/6.6 16.4/6.6 57 15 8/32 |
| 66                    | Phospho-2-dehydro-3-deoxyheptonate aldolase 1, chloroplastic | 3-deoxy-7-phosphoheptulonate synthase activity | *Solanum tuberosum* | 60.0/8.9 27.9/7.3 62 31 11/71 |
| 87                    | Molybdenum cofactor sulfurase | lyase activity | *Oryza sativa* | 92.9/7.1 27.2/5.2 60 12 6/22 |
| 136                   | Probable beta-1,3-galactosyltransferase 18 | galactosyltransferase activity | *Arabidopsis thaliana* | 77.9/8.7 35.1/9.1 66 17 7/27 |

**Protein folding, degradation and assembly**

| **Protein** | **Function** | **Organism** | **Expression** |
|-------------|--------------|--------------|---------------|
| 148         | Anaphase-promoting complex subunit 2 | ubiquitin protein ligase binding | *Arabidopsis thaliana* | 98.4/4.8 43.5/7.9 64 12 7/20 |
| 153         | U-box domain-containing protein 34 | ubiquitin-protein ligase activity | *Arabidopsis thaliana* | 91.7/9.1 39.6/6.9 63 18 12/51 |
| 171         | Ubiquitin carboxyl-terminal hydrolase 6 | ubiquitin-specific protease activity | *Arabidopsis thaliana* | 54.0/5.8 41.6/5.8 58 19 8/35 |
| 175         | U-box domain-containing protein 34 | ubiquitin-protein ligase activity | *Arabidopsis thaliana* | 91.7/9.1 44.7/6.2 58 19 12/67 |

**Transport**

| **Protein** | **Function** | **Organism** | **Expression** |
|-------------|--------------|--------------|---------------|
| 24          | Magnesium transporter MRS2-8 OS | metal ion transmembrane transporter activity | *Arabidopsis thaliana* | 43.1/5.3 13.6/4.4 58 22 6/34 |
| 54          | Putative copper-transporting ATPase 3 | ATP binding | *Arabidopsis thaliana* | 109.0/6.0 29.0/8.0 65 18 14/48 |

**Stress Related**

| **Protein** | **Function** | **Organism** | **Expression** |
|-------------|--------------|--------------|---------------|
| 4           | Ninja-family protein AFP4 OS | protein binding | *Arabidopsis thaliana* | 35.6/8.5 7.1/5.0 57 40 9/70 |
| 9           | Glutathione S-transferase 6 | glutathione binding | *Arabidopsis thaliana* | 29.2/8.5 11.9/6.2 58 25 5/46 |
| 20          | Annexin D6 OS | calcium ion binding | *Arabidopsis thaliana* | 36.6/7.7 14.0/5.1 62 20 7/39 |
| 41          | Monodehydroascorbate reductase, chloroplastic | monodehydroascorbate reductase (NADH) activity | *Arabidopsis thaliana* | 53.5/8.1 16.2/5.7 63 26 8/76 |
| 154         | Monodehydroascorbate reductase | monodehydroascorbate reductase (NADH) activity | *Pisum sativum* | 47.3/5.8 43.5/6.8 58 22 7/31 |

**Development**

| **Protein** | **Function** | **Organism** | **Expression** |
|-------------|--------------|--------------|---------------|
| 2           | Pentatricopeptide repeat-containing protein At3g06430, chloroplastic OS | N/A | *Arabidopsis thaliana* | 56.2/7.8 9.5/6.7 58 25 11/59 |
| 11          | Protein BRUSHERY 1 | protein binding | *Arabidopsis thaliana* | 148.8/5.5 12.0/5.9 63 24 21/71 |
| 38          | Protein PAIR1 | protein binding | *Oryza sativa* | 53.8/9.8 17.2/5.5 66 19 10/34 |
| 68          | 1-aminoacyclopropane-1-carboxylate synthase 7 | 1-aminoacyclopropane-1-carboxylate synthase activity | *Arabidopsis thaliana* | 51.0/5.9 26.2/6.3 57 19 10/72 |

**Unknown**

| **Protein** | **Function** | **Organism** | **Expression** |
|-------------|--------------|--------------|---------------|
| 12          | Probable protein ABIL5 OS | N/A | *Oryza sativa* | 28.3/8.3 15.2/7.6 59 40 7/42 |
| 16          | Pentatricopeptide repeat-containing protein At4g26800 OS | N/A | *Arabidopsis thaliana* | 58.4/9.1 16.1/6.8 64 32 12/49 |
| 31          | Putative F-box protein At1g20795 OS | N/A | *Arabidopsis thaliana* | 48.5/8.6 20.3/5.1 60 21 8/55 |

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Proteins involved in metabolism

For I-1, identified proteins were found for lipid metabolism (spot 42), the citric acid cycle (spots 15, 90, and 91), starch and sucrose metabolism (spots 29, 31, 118, 133, and 162), and one-carbon metabolism (spots 111 and 142). For I-2, we found additional proteins involved in one-carbon metabolism (spots 172, 173, and 174) but fewer and different proteins involved in starch and sucrose metabolism (spots 91 and 139). Many more proteins involved in metabolism were found for I-1 than for I-2 (Tables 1 and 2, Figure 5). The activities of extracellular lipase (spot 42) and \( \beta \)-1,3-glucanase (spots 29 and 31) have been implicated in pollen germination, fertilization, response to wounding, and cell division [31,32], all or any of which may be related to I-1 elongation via cell division. Sugars are involved in energy metabolism and act as signaling molecules. For I-1, we identified 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (spot 118) and phosphoglucomutase (spot 162), and for I-2, glyceraldehyde-3-phosphate dehydrogenase (spot 159), all of which are glycolytic enzymes. Interestingly, malate dehydrogenase (spots 90 and 129), fructokinase-1 (spots 155 and 91), and S-adenosyl-l-methionine (AdoMet) synthase (spots 111 and 175) were identified in both I-1 and I-2 proteomes. Malate dehydrogenase is a citric-acid-cycle enzyme that converts malate into oxaloacetate (using NAD). Fructokinase is involved in sucrose and fructose metabolism and may regulate starch synthesis in conjunction with sucrose synthase, which first metabolizes plant sink tissue in, for example, potatoes [33]. AdoMet synthase catalyzes the formation of AdoMet from methionine and ATP [34], which is the main methyl group donor and is involved in transmethyllations of many secondary metabolites [36,37] and can be decarboxylated to generate a propylamine donor for polyamine biosynthesis [38]. Polyamines are required for cell proliferation and may play a role in the rapid growth of bloom-forming dinoflagellates [39]. In plants, AdoMet participates in ethylene biosynthesis [40] and is the methyl group donor in transmethylation of alkaloids [41]. Cell and life cycle variation in AdoMet synthase expression has been observed in yeast and apicomplexa [42,43].

Proteins involved in energy production

More identified I-1 proteins (11%) were found to be involved in energy production than were I-2 proteins (2%, Figure 5). Both the \( \alpha \) and \( \beta \) subunits of chloroplastic proteins were found for lipid metabolism (spot 42), the citric acid cycle (spots 15, 90, and 91), starch and sucrose metabolism (spots 29, 31, 118, 133, and 162), and one-carbon metabolism (spots 111 and 142). For I-2, we found additional proteins involved in one-carbon metabolism (spots 172, 173, and 174) but fewer and different proteins involved in starch and sucrose metabolism (spots 91 and 139). Many more proteins involved in metabolism were found for I-1 than for I-2 (Tables 1 and 2, Figure 5). The activities of extracellular lipase (spot 42) and \( \beta \)-1,3-glucanase (spots 29 and 31) have been implicated in pollen germination, fertilization, response to wounding, and cell division [31,32], all or any of which may be related to I-1 elongation via cell division. Sugars are involved in energy metabolism and act as signaling molecules. For I-1, we identified 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (spot 118) and phosphoglucomutase (spot 162), and for I-2, glyceraldehyde-3-phosphate dehydrogenase (spot 159), all of which are glycolytic enzymes. Interestingly, malate dehydrogenase (spots 90 and 129), fructokinase-1 (spots 155 and 91), and S-adenosyl-l-methionine (AdoMet) synthase (spots 111 and 175) were identified in both I-1 and I-2 proteomes. Malate dehydrogenase is a citric-acid-cycle enzyme that converts malate into oxaloacetate (using NAD). Fructokinase is involved in sucrose and fructose metabolism and may regulate starch synthesis in conjunction with sucrose synthase, which first metabolizes plant sink tissue in, for example, potatoes [33]. AdoMet synthase catalyzes the formation of AdoMet from methionine and ATP [34], which is the main methyl group donor and is involved in transmethyllations of many secondary metabolites [36,37] and can be decarboxylated to generate a propylamine donor for polyamine biosynthesis [38]. Polyamines are required for cell proliferation and may play a role in the rapid growth of bloom-forming dinoflagellates [39]. In plants, AdoMet participates in ethylene biosynthesis [40] and is the methyl group donor in transmethylation of alkaloids [41]. Cell and life cycle variation in AdoMet synthase expression has been observed in yeast and apicomplexa [42,43].

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Proteins involved in energy production

More identified I-1 proteins (11%) were found to be involved in energy production than were I-2 proteins (2%, Figure 5). Both the \( \alpha \) and \( \beta \) subunits of chloroplastic
ATP synthase (spots 156 and 152) were identified for I-1, whereas only the ATP synthase β-subunit was detected in the I-2 proteome. ATP synthase is a very large complex (>500 kDa) embedded in the inner membranes of chloroplasts and mitochondria. It utilizes the products of fat and carbohydrate breakdown to generate proton gradients across membranes, which then drive ATP synthesis.

Proteins involved in photosynthesis
Photosynthesis uses light energy and chlorophyll to synthesize simple sugars from carbon dioxide and water and to capture the energy as phosphate bonds in ATP. ATP is then available as an energy source, and the sugars are used as building blocks to produce other cell structural and storage components. Photosynthetic enzymes including RuBisCo (spots 2, 37, 46, and 33), oxygen-evolving enhancer protein 1 (spots 126 and 90), triosephosphate isomerase (spots 69, 72, and 82) and triosephosphate isomerase (spots 69, 72, and 82) were found in the I-1 and I-2 proteomes.

Interestingly, many other proteins involved in sugar synthesis were found only in the I-2 proteome, i.e., alpha, alpha-trehalose-phosphate synthase (UDP-forming, spot 17), granule-bound starch synthase 1 (spot 35), and ribulose-phosphate 3-epimerase (spot 64). Alpha, alpha-trehalose-phosphate synthase (UDP-forming) synthesizes alpha, alpha-trehalose 6-phosphate from D-glucose 6-phosphate, and is then dephosphorylated to trehalose by trehalose 6-phosphate phosphatase. Trehalose metabolism, a side-branch of carbon flux in bacteria, yeast, and plants, has recently drawn attention because it may partially regulate plant growth, development, and stress resistance [44]. Granule-bound starch synthase 1 is required for the synthesis of amylase, and ribulose-phosphate 3-epimerase (pentose-5-phosphate 3-epimerase) converts d-ribulose 5-phosphate into d-xylulose 5-phosphate as part of the reductive pentose phosphate (Calvin) cycle. These aforementioned enzymes are very important for sugar synthesis. Sugars can act as signaling molecules in microorganisms, animals, and plants. During plant growth and development, sugars modulate seed germination, seedling development, root and leaf differentiation, floral transition, fruit ripening, embryogenesis, senescence, and responses to light, stress, and pathogens [45-53]. For strawberries, the clonal plant is usually found at the second node, so the identification of specific sugar-related enzymes found only in the I-2 proteome should increase our understanding of the different internodal developmental mechanisms. Our results indicate that positive interactions between sugar synthesis and hormonal signaling in I-2 may be necessary for asexual strawberry reproduction.
Proteins involved in protein synthesis

We identified only one I-1 protein associated with protein synthesis, namely the 50S ribosomal protein L33 (spot 154). Cell growth and division require the synthesis of new proteins and ribosomes. L33 is involved in the biogenesis of both the small and large ribosomal subunits. For I-2, we found the eukaryotic translation initiation factor 5A (spots 28 and 37), which is a highly conserved eukaryotic protein. Eukaryotic translation initiation factor 5A appears to be involved in RNA metabolism and trafficking in mammals and yeast, thus regulating cell proliferation, cell growth, and programmed cell death [54]. In plants, however, its physiological function is not known.

Proteins involved in protein folding and protein degradation

For I-1, two proteins involved in protein folding and processing were identified, and for I-2, four proteins involved in protein modification and degradation were found. The heat shock protein 81-3 (spot 45) found in the I-1 proteome is a molecular chaperone and likely involved in signal transduction and development associated with certain hormone receptors and kinases [55]. Interestingly, three of the I-2 proteins, anaphase-promoting complex subunit 2 (APC2; spot 148), U-box domain-containing protein 34 (spots 153 and 175), and ubiquitin carboxyl-terminal hydrolase 6 (spot 171), are all ubiquitin-dependent proteins involved in catabolism. The ubiquitin-proteasome pathway is responsible in large part for protein degradation and consequently regulates many aspects of development. The identification of the aforementioned proteins suggests that research on the ubiquitin conjugation pathway might illuminate the mechanism of I-2 clonal multiplication.

Proteins involved in transcription

We identified five I-1 proteins, histone acetyltransferase GCN5 (spot 6), transcription factor bHLH145 (spot 27), DNA polymerase (spot 136), pentatricopeptide repeat-containing protein (spot 18), and DNA-directed RNA polymerase (spot 92), that are involved in transcription. GCN5 is a coactivator of transcriptional regulation [56,57]. The I-2 homeodomain-leucine zipper protein (spot 18) is a putative transcription factor required for correct morphological development and maturation of trichomes as well as for normal development of seed coat mucilage [58]. The function of histone regulator protein (spots 47 and 168) has yet to be determined; however, it may be involved in maintaining knox genes silencing throughout leaf development [59]. The two-component response regulator (spot 121) is involved in the His-to-Asp phosphorelay signal transduction system [60].

Proteins involved in stress response and development

Twelve of the identified I-1 and I-2 proteins are associated with stress and development. One I-1 protein (spots 62 and 151) and five I-2 proteins (e.g., spots 4, 9, 20, 41, and 154) are involved in stress responses, and two I-1 proteins (spots 53 and 81) and four I-2 proteins (spots 2, 11, 38, and 68) are associated with development. Spot 53 is 3-ketoacyl-CoA synthase, which mediates the synthesis of very long chain fatty acids (26 to 30 carbons). Spot 81 is the cytoplasmic dynein 2 heavy chain1, which is an intracellular motor for retrograde vesicle and organelle motility along microtubules.

For I-2, monodehydroascorbate reductase (spots 41 and 154) was identified and is an oxidoreductase that oxidizes NADH or NADPH using a quinone as the oxidant during the glutathione-ascorbate cycle, a major plant antioxidant system that protects against reactive oxygen species. Monodehydroascorbate reductase activity has been found in chloroplasts, the cytosol, mitochondria, glyoxysomes, and leaf peroxisomes [61]. 1-aminocyclopropane-1-carboxylate synthase 7 (spot 68) catalyzes the conversion of AdoMet into 1-aminocyclopropane-1-carboxylate, a precursor of ethylene.

Comparison of metabolic pathways in I-1 and I-2

Interestingly, many of the proteins involved in central metabolic pathways (e.g., glycolysis, the citric acid cycle, pyruvate metabolism) were identified in both the I-1 and I-2 proteomes. Using the KEGG PATHWAY database (http://www.genome.jp/kegg/pathway.html), we classified more of the I-1 proteins than the I-2 proteins as involved in carbon fixation, glyoxylate and dicarboxylate metabolism, glycolysis/gluconeogenesis, and oxidative phosphorylation (Figure 7). For the two internodes, seven enzymes (15 spots) were classified as carbon fixing, and 13.2% of the I-1 proteins belonged to this category, whereas only 7.7% of those from I-2 did. The difference is related to the number of spots found for the RuBisCo complex, i.e., more were found for I-1 than for I-2. Most of the enzymes of the citrate cycle, pyruvate metabolism, fructose and mannose metabolism, and starch and sucrose metabolism were identified in the two proteomes, and the numbers of proteins found for each pathway were similar, suggesting that the housekeeping pathways are needed for stolon viability. Many enzymes involved in the ubiquitin-proteasome pathway were also identified but were greater in relative number in the I-2 proteome (Figure 7). In plants, regulated protein degradation by the ubiquitin-proteasome system contributes substantially to development by affecting many processes, e.g., embryogenesis, hormone signaling, and senescence, which suggests that the ubiquitin-proteasome system may play a central role in I-2 morphogenesis. The I-1 and I-2 proteomes, which we have
described herein, will allow us to analyze and compare proteins in metabolic pathways and may provide new insights into the regulation and expression of various molecular networks.

**Possible PPI networks and adventitious root-formation mechanisms in I-2**

Proteins are the main catalysts, structural elements, signaling messengers, and molecular machines of biological tissues [62]. PPIs are extremely important in orchestrating cellular events. Protein interaction networks provide road maps of cellular pathways. Therefore, many methods are used to characterize PPIs, which include physical interactions and functional linkages [63].

Although we could not use Cytoprophet to delineate a PPI network for I-1 because of limited information, a possible PPI network was found for I-2 (Figure 8). U-box domain-containing protein 34 (PUB34) (Q8S8S7_ARATH) is the central core protein of the signaling network, as it interacts with many other proteins, e.g., alpha, alpha-trenthalose-phosphate synthase (TPS4) (Q9T079_ARATH), APC2 (Q8H1U5_ARATH), 1-aminocyclopropane-1-carboxylate synthase 7 (ACS7) (1A17_ARATH), and EIF5A. APC2 is an E3 ubiquitin ligase that is a component of the SCF family ubiquitin ligases, which catalyze the attachment of ubiquitin to the lysine side chains of securin and mitotic cyclins [64-66]. EIF5A interacts with BRUSHY1 (BRU1_ARATH), which is required for the proper arrangement of cells in the root and shoot apical meristems. Ubiquitin-mediated protein degradation probably affects meristem structural formation by modulating the concentration of cell-cycle regulators and transcription factors [67]. Therefore, the ubiquitin system may be vital during the morphogenesis of clonal plants.

Adventitious roots develop from the second node at the end of I-2 before clonal plant formation. We developed a model for adventitious root formation in I-2 based on published data [64-69] and our findings; the model includes four regulated pathways (Figure 9). Regulated protein degradation has repeatedly been identified as a key component of cell-cycle regulation. Securin inhibits a protease called separase, which cleaves cohesins allowing anaphase onset. Activated APCcdc20 targets securin for degradation, which initiates the metaphase-to-anaphase transition [68]. In addition, biochemical and molecular studies have shown that EIF-5A is crucial for plant growth and development as it regulates cell division and cell growth [69]. Continuous cell division, elongation, and differentiation can cause the formation of root primordia, so the APC complex-related and EIF-5A-related biological processes may be two important pathways that regulate the formation of adventitious roots. Moreover, ACS7 catalyzes the conversion of AdoMet into 1-aminocyclopropane-1-carboxylate, a direct precursor of ethylene, whereas ACS7 is ubiquitinated. Ubiquitination probably leads to its subsequent degradation, thus controlling ethylene production. Ethylene can regulate root initiation and emergence. Conversely, as an important catalytic enzyme, TPS4 plays a central role in the complex signaling network that links sugars and hormones with its interacting partner PUB34. Together, the four pathways work synergistically to induce formation of adventitious roots.
For this research, we compared the proteomes of the I-1 and I-2 internodes of the strawberry stolon to begin to elucidate the how the differences in the proteomes affect growth and functional characteristics of the two internodes as only the second node, at the end of I-2, tends to form adventitious roots and a clonal plant. In I-1, the majority of the proteins were involved in metabolism, photosynthesis, energy, and transcription. In I-2, relatively more proteins were involved in photosynthesis, carbohydrate metabolism, stress responses, and protein folding and degradation, indicating that these many different processes work synergistically to induce cell differentiation necessary for root and plant formation. Given our findings and those of others, we present a scheme for protein interactions that could be responsible for adventitious root and clonal plant formation in I-2.

### Conclusions

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### Authors’ contributions

XF designed and carried out experiments, analyzed data, and wrote the manuscript. HM designed the study, interpreted data, and assisted with the writing of the manuscript. DL did most of the MS analysis and assisted with the evaluation of MS data. HY conceived the study and participated in its design. WL designed and analyzed experiments. SR conceived of, designed, and coordinated the study, and assisted with the writing of the manuscript. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

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