Transcriptomic comparison of Allium cepa var. agrogatum Don. cultivars with different facilitating potentials on tomato seedlings

Xuepeng Fu*, Shuqin Liu*, Mingqiang Li, Kai Pan and Fengzhi Wu

*Department of Life Science and Agroforestry, Qiqihar University, Qiqihar, People’s Republic of China; bSchool of Life Science, Bai Cheng Normal University, Baicheng Shi, People’s Republic of China; cHorticulture College, Northeast Agricultural University, Harbin, People’s Republic of China

ABSTRACT
Potato onion has facilitating effect on tomato seedlings; however, the mechanisms underlying the facilitating effect remain unknown. Here, we examined the effects of potato onion leaf volatiles and leachates on tomato seedling growth, and performed a transcriptomic comparison of two potato onion cultivars with different facilitating potentials (‘Suihua’: higher facilitating potential; ‘Qitaihe’: lower facilitating potential). Potato onion leaf volatiles and leachates decreased the stem length, but increased the shoot dry matter of tomato seedlings. Transcriptomic analysis showed that 640 genes were differentially expressed (DEGs) between ‘Suihua’ and ‘Qitaihe’. DEGs related to photosynthesis, energy metabolism, and hormone metabolism were higher expressed in ‘Suihua’ compared to that in ‘Qitaihe’, whereas genes related to phenylpropanoid metabolism and disease resistance were lower expressed. Pathway analysis showed the DEGs mainly participated in Metabolism, Biosynthesis of secondary metabolites and Plant hormone signal transduction.

Introduction
Interspecific plant–plant interactions, including competition and facilitation, are a very common phenomena in nature, and play key roles in regulating the compositions of communities and ecosystems (Brooker 2006). In recent years, the role of facilitating interactions between plant species has received increased attention (Moeller 2004; Zhang and Li 2003) and the facilitating interactions have also been used for crop growth regulation (Batish et al. 2001; Narwal and Zeng 2010). For example, potato onion, as a companion plant, can increase the growth of cucumber and tomato (Yang et al. 2011; Liu et al. 2013; Wu et al. 2016). The mechanisms whereby the effects of facilitating interactions are mediated are various and the release of chemicals is one of the reasons (Inderjit et al. 2011). Studies have demonstrated that these secondary metabolites include phenolics, flavonoids, alkaloids, and terpenes, and can be released by volatilization, leaching of aerial parts, and decomposition of plant debris in the environment (Rice 1984; Ni et al. 2007; Berendji et al. 2008). However, relatively little is currently known regarding the genes related to secondary metabolites.

Potato onion (Allium cepa var. agrogatum Don.), also commonly known as Chinese onion and tillering onion, is an annual herbaceous bulb vegetable in the family Alliaceae. It is a variant of onion known to have a rich genetic diversity, and is widely cultivated in North China as a nourishing vegetable (Liu et al. 2013; Jin et al. 2013). Our previous studies have shown that potato onions have facilitating potential, and that this potential is cultivar dependent. For example, the cultivars ‘Sui Hua’ and ‘Wu Chang Hongqishe’ have been shown to have higher facilitating potential with respect to promoting the germination and seedling growth of cucumber and tomato plants (Yang et al. 2011; Jin et al. 2013). Moreover, studies have demonstrated that a single potato onion variety can have different facilitating effects on cucumber and tomato (Yang et al. 2011). To date, however, the mechanisms underlying these facilitating effects have remained undetermined.

Furthermore, our previous study has demonstrated that the belowground interactions between potato onion and tomato can change the root architecture of tomato (Yu et al. 2017), whereas comparatively little consideration has been given to the aboveground interactions between the two plants. In the present study, we therefore aimed to explore the effects of the volatiles and leachates of potato onion leaves on tomato growth, and compared the gene expression profiles of two potato onion cultivars with different facilitating potentials in a potato onion/tomato intercropping system. The findings of this study will contribute to our understanding of the molecular mechanisms underlying the different facilitating effects of different potato onion cultivars on tomato growth.

Materials and methods
Evaluation of allelopathy
Cultivation of plants
The potato cultivar used in the present study was ‘Dongnong 708’ (provided by the Tomato Breeding Center of Northeast Agricultural University). The two potato onion cultivars we examined were ‘Suihua,’ which has a high facilitating potential, and ‘Qitaihe,’ which has a lower facilitating potential (Yang et al. 2011). Prior to planting, the potato onion bulbs were stored at 4°C in the Laboratory of Vegetable Physiological Ecology in the Northeast Agricultural University (Harbin, China). Tomato seeds were surface sterilized with 3.8% sodium hypochlorite for 10 min, rinsed three times with sterile distilled water, and
then germinated in a mixture of peat:perlite (1:1 v/v). Following the emergence of two true leaves, the seedlings were transplanted into plastic nutrition pots (10 cm × 10 cm), with one seedling planted in each pot. At the four-true leaf stage, the tomato seedlings were used for further study.

**Effect of potato onion leaf volatiles on tomato seedling growth**

A pot experiment was conducted outdoors in small plastic tunnels (1.5 m × 1.5 m × 1 m). In each tunnel, 40 tomato seedlings were placed, along with 0, 40, 80, or 120 potato onion seedlings. The tomato and potato onion seedlings were planted individually in plastic nutrition pots (10 cm × 10 cm). The tunnels were subsequently sealed but ventilated at 24-h intervals. The plastic tunnel treatments were assigned in a completely randomized block design, and each treatment was performed in triplicate, giving a total of 12 plastic tunnels. Within the tunnels, the tomato and potato onion seedlings were arranged in an alternating pattern, thereby replicating the arrangement used in potato onion/tomato intercropping. Thirty days after co-planting, the tomato seedlings were harvested for the measurement of stem length, stem diameter, and shoot dry matter weight.

**Effect of potato onion leaf leachates on tomato seedling growth**

Potato onion leaves were washed with sterile deionized water, and the collected washings were used as leaf leachate solutions to simulate the leaf leachates discharged by potato onion. The concentrations used (0, 0.1, 0.2, 0.3, and 0.4 g/mL) were defined in terms of fresh leaf weight per milliliter of deionized water. After the tomato seedlings had been transplanted, 5 mL of leaf leachate was watered into the tomato seedling rhizosphere once every 3 days. The 20 tomato seedlings used for each treatment were arranged in a completely randomized block design, and each treatment was performed in triplicate. Thirty days after transplanting, the tomato seedlings were harvested for the measurement of stem length, stem diameter, and shoot dry matter weight.

**Comparative transcriptomic analysis of potato onion leaves**

**Comparison of the botanical characters of 'Suihua' and 'Qitaie' and preparation for RNA-seq**

Potato onions were interplanted with tomatoes in a trough measuring 120 cm × 80 cm × 16 cm. The tomatoes were used as an inducing factor and cultured with different potato onion varieties for 1 month. At 30 days after planting, we assessed the botanical characters of potato onions, including leaf color, number of tillers, and the length and circumference of tubulose leaves. Furthermore, we separately randomly selected five seedlings of cultivars 'Suihua' and 'Qitaie,' the young leaves of which were harvested and mixed to give respective aggregate samples. The leaf samples were snap-frozen in liquid nitrogen and stored at −80°C until used for RNA extraction and RNA-seq library preparation. At maturity, we also measured the weight of single bulbs and yield per plant.

**RNA-seq library preparation and sequencing**

RNA-Seq library preparation and sequencing were conducted by BGI Tech (Shenzhen, China). Initially, the total RNAs of potato onion leaves were extracted using a Total RNA Purification Kit (NORGENT; Cat # 17200) and enriched eukaryotic mRNA was obtained. The mRNA samples obtained were then fragmented into short sequence lengths. Using these fragments as a template and a hexa-nucleotide as an arbitrary primer, first-strand cDNA was synthesized. Second strand synthesis was performed using DNA polymerase I, dNTPs, RNase H, and buffer solution. Subsequently, the cDNAs obtained were end-repaired, concatenated, and sequenced. The fragment sizes were determined using agarose gel electrophoresis and the fragments were PCR amplified. The sequencing database established was finally sequenced using an Illumina HiSeq 2000 sequencer. The raw sequencing data thus obtained was filtered using the following steps follows: (1) removal of 3′ adapter sequences; (2) removal of idling reads, namely, retaining only those sequences with 3′ adapters and those free of tags; (3) removal of poor quality tags, namely, the tags containing unknown N-nucleotides; (4) removal of over-long or over-short tags to retain tags of 21 bp (5); removal of duplicated tags (possibly caused by fault sequencing); and (6) obtaining clean tags.

**Screening of differentially expressed genes**

The expression quantity of the clean tags annotated against the reference genes was recorded and standardized by dividing by one million. To identify differential genes, it was necessary to calculate the probability of the equal expression of any specific gene in the two potato onion varieties using an accurate formula.

\[ P(y|x) = \left( \frac{N_2}{N_1} \right)^y \left( \frac{x + y}{x!y!} \left( \frac{N_2}{N_1} + \frac{y}{x+y+1} \right) \right) \]  \hspace{1cm} (a)

\[ P = 1 - \sum_{i=0}^{n-1} \left( \frac{M}{i} \left( \frac{N-M}{n-i} \right) \right) \frac{N}{n} \]  \hspace{1cm} (b)

Formula (a) was used to calculate the probability that gene A was equally expressed in two samples. The P-value represents the possible difference probability between transcripts. Its threshold was determined using the FDR obtained by repeated tests. N1 and N2 refer to the total numbers of clean tags in libraries M and S respectively, and x and y are the corresponding clean tags of gene A in M and S, respectively. GO and pathway analysis were based on the Gene Ontology database (http://www.geneontology.org/) and the KEGG database (http://www.genome.jp/kegg/), respectively. Formula (b) was used to calculate GO and KEGG pathways. Here, N refers to the number of genes with GO/KEGG annotation, n denotes the number of differential genes, M is the number of genes that are annotated to a certain GO/KEGG pathway, and M is the number of differential genes in M.

**Results**

**Effects of potato onion leaf volatiles and leachates on tomato seedling growth**

The number of potato onion seedlings placed within the small plastic tunnels had a significant effect on the growth of tomato seedlings, as shown in Table 1. Compared with tomato monocropping (no potato onion seedlings within the tunnel), in the treatment in which tomato seedlings
Effects of potato onion leaf volatiles on growth of tomato seedlings.

| Seeding number | Stem length (cm) | Stem diameter (cm) | Dry matter weight of shoot (g) |
|----------------|-----------------|-------------------|-------------------------------|
| 0              | 20.82 ± 0.45ab  | 0.56 ± 0.05a      | 0.58 ± 0.09b                  |
| 40             | 16.03 ± 0.87bc  | 0.57 ± 0.09a      | 0.50 ± 0.09b                  |
| 80             | 15.42 ± 0.82c   | 0.67 ± 0.15a      | 0.89 ± 0.04a                  |
| 120            | 21.67 ± 0.98a   | 0.45 ± 0.10a      | 0.45 ± 0.06a                  |

Note: Data are means ± standard deviation. Different letters following the data indicate significantly different (P < 0.05).

Effects of potato onion leaf leachates on growth of tomato seedlings.

| Concentration (g/mL) | Stem length (cm) | Stem diameter (cm) | Dry matter weight of shoot (g) |
|---------------------|-----------------|-------------------|-------------------------------|
| 0                   | 18.05 ± 0.108a  | 0.56 ± 0.06cd     | 0.63 ± 0.07b                  |
| 0.01                | 18.01 ± 0.79a   | 0.57 ± 0.09c      | 0.68 ± 0.09ab                 |
| 0.02                | 16.22 ± 0.63ab  | 0.62 ± 0.05b      | 0.88 ± 0.04a                  |
| 0.03                | 13.58 ± 1.27c   | 0.67 ± 0.03a      | 0.82 ± 0.05a                  |
| 0.04                | 15.84 ± 0.98bc  | 0.45 ± 0.06d      | 0.53 ± 0.06e                  |

Note: Data are means ± standard deviation. Different letters following the data indicate significantly different (P < 0.05). The Concentration was defined as fresh leaves weight per milliliter deionized water.

Botanical characteristics of the two potato onion cultivars

As shown in Table 3, there is a difference in the leaf color of the two cultivars, with that of the 'Suihua' cultivar being greener in color. The tiller number of potato onion seedlings was not significantly different between the two cultivars. However, both the average single bulb weight and yield per plant were higher in 'Suihua' than in 'Qitaihe' (P < 0.05). Although the average length of tubulose leaves did not differ significantly between the two cultivars, the average circumference of these leaves was higher in 'Suihua' than in 'Qitaihe'.

Transcriptomic analysis of potato onion leaves

RNA-seq library sequencing and sequencing quality validation

Two RNA-seq libraries ('Qitaihe' and 'Suihua') were constructed based on Illumina HiSeq 2000 sequences, and yielded 5,521,415 and 4,646,317 total raw tags, respectively. After discarding low-quality reads, a total of 5,079,819 and 4,112,398 clean reads remained for 'Qitaihe' and 'Suihua', respectively. The clean reads accounted for 92.00% and 88.51% of the 'Qitaihe' and 'Suihua' libraries, respectively. The number of clean reads that could be matched with the transcriptome database were 4,307,613 and 3,290,950, accounting for 84.80% and 80.03% of the total clean reads, respectively (Supplementary 1).

GO and pathway enrichment analyses for DEGs

NOISEq analysis yielded a total of 640 DEGs between 'Qitaihe' and 'Suihua', among which 347 were upregulated and 293 were downregulated, as shown in Figure 1 and Supplementary 5. GO analysis was used to determine the functions of all DEGs. The DEGs were categorized into three groups as shown in Figure 2. In the cellular component group, cellular internal function was the most abundant GO term, accounting for 78.10% of the total. In the biological process categories, binding with catalytic activity and catalytic activity itself were dominant, with proportions of 63.0% and 61.1%, respectively. Biological process classification implied that metabolic validation.

RNA-seq library sequencing and sequencing quality validation

Two RNA-seq libraries ('Qitaihe' and 'Suihua') were constructed based on Illumina HiSeq 2000 sequences, and yielded 5,521,415 and 4,646,317 total raw tags, respectively. After discarding low-quality reads, a total of 5,079,819 and 4,112,398 clean reads remained for 'Qitaihe' and 'Suihua', respectively. The clean reads accounted for 92.00% and 88.51% of the 'Qitaihe' and 'Suihua' libraries, respectively. The number of clean reads that could be matched with the transcriptome database were 4,307,613 and 3,290,950, accounting for 84.80% and 80.03% of the total clean reads, respectively (Supplementary 1).

The expression distribution and alignment results for the clean tags indicated the tag quantity and type of the two varieties exhibited large differences within different tag abundance classifications (Supplementary 2 and Supplementary 3). For the genes with high expression in the differentially expressed gene (DEG) database, there were few types but these were present in higher quantities, whereas there were a larger number of types with low expression but these were present in lower quantities. For example, the tags (>100) of 'Qitaihe' with high copy number comprised a high proportion of 60.99%, whereas the tag types accounted for only 3.2% of the total tags. Conversely the tags with low copy number (2–5) comprised only 7.96% of the total clean tags, whereas the proportion of different types therein reached 63.97% (Supplementary 2). Comparison of these results revealed that the two cultivars 'Qitaihe' and 'Suihua' showed relatively more clean tags, with a perfect match of 78.68% and 73.6%, respectively. Sequencing saturation analysis revealed that the sequences obtained for all samples were of good quality and thus suitable for further analysis (Supplementary 4).

GO and pathway enrichment analyses for DEGs

NOISEq analysis yielded a total of 640 DEGs between 'Qitaihe' and 'Suihua', among which 347 were upregulated and 293 were downregulated, as shown in Figure 1 and Supplementary 5. GO analysis was used to determine the functions of all DEGs. The DEGs were categorized into three groups as shown in Figure 2. In the cellular component group, cellular internal function was the most abundant GO term, accounting for 78.10% of the total. In the biological process categories, binding with catalytic activity and catalytic activity itself were dominant, with proportions of 63.0% and 61.1%, respectively. Biological process classification implied that metabolic validation.
The pathway was a significantly enriched GO-term, accounting for 72.3%. Annotation of the 640 significant differential genes using the KEGG database yielded 205 annotated differential genes. These genes were found to participate in 78 metabolic pathways, among which pathways related to metabolism had the highest representation (21.8%), followed by the biosynthesis of secondary metabolites (9.87%), and signal transduction of plant hormones (5.28%) (Table 4).

**DEGs analysis**

Among the 205 annotated DEGs (Supplementary 6), 26 were annotated to photosynthesis and, interestingly, all 26 DEGs were higher expressed in 'Suihua' compared with those in 'Qitaihe' (Table 5), including photosystem I subunit H (Unigene21442), chloroplast chlorophyll a/b binding protein (Unigene21159), and photosystem II protein K (Unigene22918). Similar to the photosynthesis-related genes, genes that participate in energy metabolism were also higher expressed in 'Suihua' compared with those in 'Qitaihe', including H-quinone oxidoreductase subunit H, H-quinone oxidoreductase subunit K, NADH-plastoquinone oxidoreductase subunit 5, F0F1-type ATP synthase beta subunit, NADH dehydrogenase subunit F, NADH dehydrogenase [ubiquinone] iron-sulphur protein 4 (Table 5).

We also identified some DEGs related to plant hormone metabolism and signal transduction. Among these, the DEGs involved in auxin metabolism and signal transduction were all higher expressed (Table 6). We found that the key gene for gibberellin biosynthesis (gibberellin 3-oxidase) was also higher expressed, whereas the gene for gibberellin degradation (gibberellin 2-beta-dioxygenase) was lower expressed (Table 6). The genes linoleate 9S-lipoxygenase 5 and allene oxide cyclase, which are involved in jasmonic acid biosynthesis, were both lower expressed (Table 6).

Genes involved in the phenylpropanoid metabolic pathway, such as phenylalanine lyase, caffeoyl CoA-methyltransferase, 4-coumarate:coenzyme A ligase, and cinnamoyl CoA reductase, were all lower expressed, whereas the gene encoding flavonol synthase/flavanone 3-hydroxylase-like was higher expressed (Table 7). Furthermore, with the exception of polygalacturonase-inhibiting protein, which was higher expressed, the genes related to disease resistance were all lower expressed (Table 7).

**Discussion**

In our previous studies, we found that potato onion is an effective partner for intercropping and relay intercropping. Intercropping with potato onion increased the growth of tomato

---

**Table 4. Top 20 enriched KEGG pathways.**

| NO. | Pathway                          | Unigenes with pathway annotation | Pathway ID |
|-----|---------------------------------|---------------------------------|------------|
| 1   | Metabolic pathways              | 4040 (21.85%)                  | ko01100    |
| 2   | Biosynthesis of secondary metabolites | 1825 (9.87%)          | ko01110    |
| 3   | Plant hormone signal transduction | 976 (5.28%)              | ko04075    |
| 4   | Plant-pathogen interaction      | 959 (5.19%)                  | ko04626    |
| 5   | Ribosome biogenesis in eukaryotes | 789 (4.27%)              | ko03008    |
| 6   | Spliceosome                     | 775 (4.19%)                  | ko03040    |
| 7   | RNA transport                   | 757 (4.09%)                  | ko03013    |
| 8   | Purine metabolism               | 740 (4.00%)                  | ko00230    |
| 9   | RNA degradation                 | 689 (3.73%)                  | ko03018    |
| 10  | Endocytosis                     | 660 (3.57%)                  | ko04144    |
| 11  | Glycerophospholipid metabolism  | 627 (3.39%)                  | ko00564    |
| 12  | Protein processing in endoplasmic reticulum | 551 (2.98%) | ko04141    |
| 13  | Ether lipid metabolism          | 492 (2.66%)                  | ko00565    |
| 14  | Ribosome                        | 466 (2.52%)                  | ko03010    |
| 15  | Starch and sucrose metabolism   | 433 (2.34%)                  | ko00500    |
| 16  | mRNA surveillance pathway       | 430 (2.33%)                  | ko03015    |
| 17  | Ubiquitin mediated proteolysis  | 381 (2.06%)                  | ko04120    |
| 18  | Pyrimidine metabolism           | 354 (1.91%)                  | ko00240    |
| 19  | Phenylpropanoid biosynthesis    | 275 (1.49%)                  | ko00940    |
| 20  | Oxidative phosphorylation       | 255 (1.38%)                  | ko00190    |

*The percentage of total annotated unigenes.
Table 5. DEGs related to photosynthesis and energy metabolism.

| NO. | Gene ID       | log2 ratio (Suihua/Qitaihe) | P-value | FDR     | blast nr                                                                 |
|-----|---------------|-----------------------------|---------|---------|--------------------------------------------------------------------------|
| 1   | Unigene22732  | 1.698894                    | 0       | 0       | gi|104763664|gb|ABF74605.1|/1e-46/chloroplast photosystem II 10 kDa protein [Agave tequilana] |
| 2   | Unigene18811  | 1.56921                     | 0       | 0       | gi|109156608|ref|YP_65227.1|/1e-17/cytochrome b6/f complex subunit V [Oryza sativa Indica Group] |
| 3   | Unigene53866  | 1.08499                     | 4.12E-10| 1.70E-08| gi|11273174|gb|AB120691.1|/2e-88/chloroplast FLU-like protein [Gymnadenia conopsea] |
| 4   | Unigene22195  | 1.13128                     | 2.12E-05| 0       | gi|11651921|gb|ABJ99590.1|/e-147/type III chlorophyll a/b-binding protein [Lycoris aurea] |
| 5   | Unigene21360  | 1.46756                     | 6.00E-15| 4.82E-13| gi|156598133|gb|ABU65331.1|/e-173/cytochrome f [Elaeis oleifera] |
| 6   | Unigene29146  | 1.28029                     | 1.26E-12| 6.02E-11| gi|183239004|ref|ACC60965.1|/0.0/phytochrome A [Vitis vinifera] |
| 7   | Unigene22450  | 1.310124                    | 6.75E-13| 3.91E-11| gi|195632817|gi|ACG36753.1|/8e-63/secondary metabolites (Table 4) |
| 8   | Unigene22836  | 1.426011                    | 2.02E-13| 1.30E-11| gi|255562087|ref|XP_002521161.1|/3e-38/photosystem I reaction center subunit NFA, chloroplastic precursor [Ricinus communis] |
| 9   | Unigene21442  | 8.30738                     | 2.57E-05| 0       | gi|268044989|gb|ACF29095.1|/5e-63/photosystem I subunit H [Lycoris radiata] |
| 10  | Unigene21159  | 2.01158                     | 1.28E-05| 0       | gi|285804077|gb|ADCC55797.1|/e-139/chloroplast chlorophyll a/b binding protein [Lycoris radiata] |
| 11  | Unigene21158  | 1.63864                     | 0       | 0       | gi|285804077|gb|ADCC55797.1|/e-140/chloroplast chlorophyll a/b binding protein [Lycoris radiata] |
| 12  | Unigene9322   | 2.096561                    | 0       | 0       | gi|28065147|ref|YP_003434033.1|/0.0/hypothetical chloroplast RF19 [Typha latifolia] |
| 13  | Unigene52301  | 1.083146                    | 5.17E-11| 2.34E-09| gi|29070712|gb|ADDC62970.1|/0.0/photosystem I P700 apoprotein A1 [Oryza australiensis] |
| 14  | Unigene22991  | 0.9200815                   | 3.55E-05| 0       | gi|29070766|gb|ADDC63023.1|/6e-33/photosystem II protein K [Potamogetum parviflorum] |
| 15  | Unigene23294  | 1.260515                    | 0       | 0       | gi|229559495|ref|XP_003540915.1|/5e-17/photosystem II protein I [Phoenix dactylifera] |
| 16  | Unigene19112  | 1.399941                    | 7.21E-08| 2.27E-06| gi|229559355|ref|XP_003540926.1|/4e-54/cytochrome c biogenesis protein [Phoenix dactylifera] |
| 17  | Unigene21647  | 1.59186                     | 0       | 0       | gi|229559363|ref|XP_003540999.1|/0.0/hypothetical chloroplast RF1 [Phoenix dactylifera] |
| 18  | Unigene10661  | 1.99081                     | 0       | 0       | gi|229559363|ref|XP_003540999.1|/0.0/hypothetical chloroplast RF1 [Phoenix dactylifera] |
| 19  | Unigene10590  | 2.205594                    | 1.77E-13| 1.16E-11| gi|357121842|ref|XP_003526266.1|/7e-7/PREDICTED: 29 kDa ribonucleoprotein, chloroplastic-like [Brachypodium distachyon] |
| 20  | Unigene19153  | 2.009815                    | 3.55E-05| 0       | gi|357123645|ref|XP_003563233.1|/3e-49/PREDICTED: sucrose synthase 2-like [Brachypodium distachyon] |
| 21  | Unigene52273  | 1.124513                    | 5.45E-06| 0       | gi|357470609|ref|XP_00365589.1|/4e-71/Cytochrome b559 subunit alpha [Medicago truncatula] |
| 22  | Unigene21299  | 1.305602                    | 5.33E-05| 0       | gi|30560327|gb|CAT10888.1|/3e-95/chloroplast chaperonin [Vitis vinifera] |
| 23  | Unigene10259  | 1.957487                    | 3.04E-10| 1.28E-08| gi|30560327|gb|CAT10888.1|/4e-99/chloroplast chaperonin [Vitis vinifera] |
| 24  | Unigene21185  | 1.583152                    | 9.12E-13| 5.22E-11| gi|392175919|gb|AAC20486.1|/0/photosystem II 47 kDa protein [Yucca schidigera] |
| 25  | Unigene21148  | 1.064335                    | 1.39E-07| 4.71E-06| gi|81176251|ref|YP_398330.1|/4e-41/photosystem I assembly protein Ycf2 [Lactuca sativa] |
| 26  | Unigene18291  | 2.384143                    | 6.71E-08| 2.13E-06| gi|28065143|ref|XP_003434019.1|/1e-43/hypothetical chloroplast RF21 [Typha latifolia] |

To date, however, the effects of potato onion leaf volatiles and leachates have remained virtually unexplored. In present study, we found that tomato seedlings were co-planted with 80 potato onion seedlings in a plastic tunnel, the stem length of these tomatoes was decreased, whereas there was an increase in seedling shoot dry matter weight. In contrast, no comparable significant changes were observed when the tomato seedlings were interplanted with 40 and 120 potato onion seedlings (Table 1), indicating that although potato onion leaf volatiles have an effect on tomato seedling growth, the effect is concentration dependent. Similarly, the leachates of potato onion leaves can decrease the stem length of tomato seedlings but increase the stem diameter and shoot dry weight at a concentration of 0.03 g/mL (Table 2). These results indicate that the leaf volatiles and leachates of potato onion seedlings have facilitating effects on tomato seedlings in terms biomass under certain conditions. In contrast, several studies have shown that the aqueous extracts of leaves from a number of different plant species had inhibitory effects on other plants (Zhang et al. 2010; Georgieva and Nikolova 2016).

Our previous studies revealed that potato onions possess facilitating potential, and that this potential is cultivar dependent. For example, the cultivars 'Sui Hua' and 'Wu Chang Hongqishe' possess higher facilitating potential with respect to promoting the germination and seedling growth of cucumber and tomato plants (Yang et al. 2011; Jin et al. 2013). And in the present study, we demonstrated that the leaf volatiles and leachates of potato onion had facilitating effects on tomato growth. In an effort to identify the factors that underlie the different effects of potato onion leaf volatiles and leachates on tomato seedlings, we conducted transcriptomic analyses of two varieties of potato onion ('Suihua' and 'Qitaihe') with different facilitating potentials. Pathway function analysis revealed that these genes participate in 78 metabolic pathways, among which pathways related to metabolism had the highest representation (29.7%), followed by biosynthesis of secondary metabolites (Table 4).
The majority of the genes that were differentially expressed between 'Suihua' and 'Qitaihe', and for which we obtained functional annotations, are related to photosynthesis and energy metabolism, and it is of interest to note that these DEGs were all higher expressed in 'Suihua' compared to those in Qitaihe (Table 5). A similar relationship has been observed in rice, in which the allelopathic variety has stronger photosynthetic ability than the non-allelopathic variety, which was consistent with the patterns of those in Qitaihe (Table 6). It can thus be speculated that, of the two varieties, 'Suihua' is the more vigorous in terms of photosynthetic rate, stomatal conductance, transpiration rate, and energy metabolism, and it is of interest to note that these terms of growth, which is consistent with the patterns of gene expression relating to photosynthesis and energy metabolism, and also the botanical characters of these two varieties (Tables 3 and 5).

Several studies have demonstrated that phenolic acids are mainly responsible for the allelopathic inhibiting potential of potato onion plants (Wu et al. 2001; Zhang et al. 2010; Techer et al. 2016; Georgieva and Nikolova 2016). In present study, we found that in the 'Suihua' variety of potato onion, the genes involved in phenylpropanoid metabolic pathways, which play an important role in phenylpropanoid biosynthesis, were all lower expressed (Table 7). This may be speculated that phenylpropanoid metabolism is weak in 'Suihua'. He et al. (2012) and Xiong et al. (2012) detected higher expression of the genes involved in the phenylpropanoid metabolism and correspondingly higher contents of phenolic compounds in the allelopathic rice cultivar PI312777, which were consistent with the higher rates of *Echinocloa crus-galli* L. inhibition attributed to this variety.

In conclusion, potato onion leaf volatiles and leachates have facilitating effect on tomato seedlings growth. Compared with the 'Qitaihe' cultivar of potato onion, the 'Suihua' cultivar (with higher facilitating potential) has higher expression of genes related to photosynthesis, energy metabolism, and gibberellin and auxin metabolism, and conversely, the 'Suihua' has lower expression of genes related to phenylpropanoid metabolism.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This research was funded by the National Natural Science Foundation of China (No. 31672200), the Natural Science Foundation of Heilongjiang Province (No. C2017068), Foundation of Heilongjiang Educational and Scientific Research Projects (No. 2017H5017).
Committee (YSTSXK201888, 135109254), and the Science and Technology Foundation in Qiqihar (No. NYGG-201616).

References

Batish DR, Singh HP, Kohli RK, Kaur S. 2001. Crop allelopathy and its role in ecological agriculture. J Crop Prod. 4:121–161.

Berendji S, Asghari JB, Matin AA. 2008. Allelopathic potential of rice (Oryza sativa) varieties on seedling growth of barnyardgrass (Echinochloa crus-galli). J Plant Interact. 3(3):175–180.

Brooker RW. 2006. Plant-plant interactions and environmental change. New Phytol. 171(2):271–284.

Georgieva N, Nikolova I. 2016. Allelopathic facilitating tolerance of pea cultivars to Sorghum halepense L. (Pers.) extracts. Pestic Phytomed (Belgrade). 31:59–67.

He H, Wang H, Fang C, Wu H, Guo X, Liu C, Lin Z, Lin W. 2012. Barnyard grass stress up regulates the biosynthesis of phenolic compounds in allelopathic facilitating rice. J Plant Physiol. 169(17):1747–1753.

Inderjit, Wardle DA, Karban R, Callaway RM. 2011. The ecosystem and evolutionary contexts of allelopathy. Trends Ecol Evol. 26:655–662.

Jin X, Zhou X, Liu SW, Liu SQ, Wu F. 2013. 48 ISSR Markers of 48 Tillering Onion(Allium cepa var. aggregatum)Germplasms Genetic Diversity and Analysis of Their Agronomic Traits. China Vegetables. 1:27–34. Chinese.

Liu S, Wu F, Wen X. 2013. Allelopathic facilitating effects of root exudates of Chinese onion on tomato growth and the pathogen Fusarium oxysporum (Schl) f.sp. lycopersici. Allelopathy J. 31:387–404.

Moeller DA. 2004. Facilitative interactions among plants via shared pollinators. Ecology. 85(12):3289–3301.

Narwali SS, Zeng RS. 2010. Allelopathy in ecological sustainable organic agriculture. Allelopathy J. 25:537–564.

Ni GY, Li FL, Chen BM, Song LY, Peng SL. 2007. Allelopathic facilitating plants. 21. Mikania micrantha H.B.K. Allelopathy J. 19:287–296.

Rice EL. 1984. Allelopathy. 2nd ed. New York: Academic Press.

Techer D, Fontaine P, Personne A, Viot S, Thomas M. 2016. Allelopathic facilitating potential and ecotoxicity evaluation of gallic and nonanoic acids to prevent cyanobacterial growth in lentic systems: A preliminary mesocosm study. Sci Total Environ. 547:157–165.

Wang H, He H, Ye C, Qiu L, Fang C, Lin W. 2008. Photosynthetic physiology of different allelopathic facilitating rice accessions at seedling stage under potassium stress. Chin J Eco-Agric. 16(6):1474–1477. Chinese.

Wu H, Haig T, Pratley J, Lemerle D, An M. 2001. Allelochemicals in wheat (Triticum aestivum L.): variation of phenolic acids in root tissues. J Chem Ecol. 27(1):125–35.

Wu X, Wu F, Zhou X, Fu X, Yue T, Xu W, Pan K, Liu S. 2016. Effects of intercropping with potato onion on the growth of tomato and rhizosphere alkaline phosphatase genes diversity. Front Plant Sci. 7:846.

Xiong J, Lin H, Li Z, Fang C, Han Q, Lin W. 2012. Analysis of rhizosphere microbial community structure of weak and strong allelopathic facilitating rice varieties under dry paddy field. Acta Ecol Sinica. 32(19):6100–6109. Chinese.

Yang Y, Wu F, Liu S. 2011. Allelopathic facilitating effects of root exudates of Chinese onion accessions on cucumber yield and Fusarium oxysporum f.sp. cucumerinum. Allelopathy J. 27:75–86.

Yu H, Chen S, Zhou X, Wu F. 2017. Root interactions and tomato growth in tomato/potato onion companion-cropping system under different phosphorus level. J Plant Interact. 12(1):438–446.

Zhang F, Li L. 2003. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. Plant Soil. 248(1–2):305–312.

Zhang RM, Wang YZH, Hou P, Wen GSH, Gao Y. 2010. Physiological responses to allelopathy of aquatic stem and leaf extract of Artemisia frigida in seedling of several pasture plants. Acta Ecol Sinica. 30:2197–2204. Chinese.