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Research article

A novel effect of glycine on the growth and starch biosynthesis of storage root in sweetpotato (*Ipomoea batatas Lam.*)

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ABSTRACT

Sweetpotato (*Ipomoea batatas Lam.*) plays an indispensable role in feed, starch-based industries and ethanol biofuel production. Few studies have investigated on how external amino acids affect the growth and production of sweetpotato. In the study, we evaluated morphological, physiological and molecular effects of external glycine (Gly) on the root growth and starch metabolism of sweetpotato, Xushu16. At morphological level, the Xushu16 with Gly stimuli had larger plant biomass than that under control condition. At physiological level, the photosynthesis strength of the Xushu16 with Gly treatments showed significant differences relative to those under control condition. The relative content of plant hormone and starch in storage roots was higher under Gly conditions than that under control condition. At molecular level, a total of 4836 differentially expression genes were identified in the storage roots with different Gly treatments by RNA-Seq. Among them, as many as 1830 genes were involved in carbohydrate metabolism, which held maximum proportion among all the DEGs. Further, a few genes involved in starch biosynthesis were proved to be Gly-induced significantly by RT-qPCR. All the results indicated extrinsic Gly promotes the growth of storage roots by strengthening photosynthesis and increasing plant hormone, and enhances starch biosynthesis of storage roots by accelerating carbohydrate metabolism and regulating the expression of starch-related genes.

1. Introduction

Sweetpotato grows all over the world and has a great potential source of economic food and renewable energy due to its high yield, rich nutrient, high starch content, low input and strong adaptability (Bovell-Benjamin, 2007). The high productivity and economic value of sweetpotato are mainly originated from its edible storage roots. Sweetpotato is propagated through vegetative cuttings. Adventitious roots arise from cut ends or primordia on the nodes of the cuttings, which are further differentiated into fibrous roots, pencil roots, and storage roots (Lalusin et al., 2006; Villordon et al., 2009). Storage roots initiate from adventitious roots and the number of storage roots depends on the number of adventitious roots induced to storage roots (Lalusin et al., 2006; Villordon et al., 2009). The appearance of vascular cambium layer in adventitious roots indicates the initiation of storage roots, followed by anomalous primary and secondary cambial segments (Belehu et al., 2004; Lalusin et al., 2006). Then storage roots swell up through photosynthate accumulation and massive starch converting and filling (Ravi et al., 2009).

The formation and development of storage roots are the most important morphophysiological processes for high production of underground portion in sweetpotato (Firon et al., 2013). The processes are closely correlated with carbohydrate manipulation from photosynthetic tissues (leaves) to sink tissues (roots) in storage root-bearing plants (Zheng et al., 2012). The carbon can be fixed during the day and the carbohydrates are remobilized in the evening to support photosynthetic

Abbreviations: list: Gly, glycine; PC, primary cambium; ABA, abscisic acid; CK, cytokinin; IAA, indole-3-acetic acid; DWT, days with treatment; DEGs, differentially expressed genes; TF, transcription factor; URGs, up-regulated genes; DRGs, down-regulated genes; Glu, Glutamate; QC, quality control; FPKM, fragment per kilo bases per million mapped reads; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes

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carbohydrate metabolism in the photosynthetic tissues (Bahaji et al., 2014). The intensification of photosynthetic carbohydrate manipulation can enhance the yield of heterotrophic starch-storing organs (Katoh et al., 2015). Thereby, the physiological improvement of photosynthesis can increase the production of storage roots in sweetpotato.

The development process of storage roots coincides with an increase of cell numbers by cell division and proliferation and starch filling (Ravi et al., 2009; Lai et al., 2016). Starch is one of the primary carbohydrate sources and exists in the plastids of photosynthetic and non-photosynthetic tissues of higher plants. Starch accounts for 50–80% dry matter of storage roots in sweetpotato (Rukundo et al., 2013; Bahaji et al., 2014). Starch synthesis is derived from the cleaved products of imported photoassimilate sucrose (Li and Zhang, 2003). Sucrose is the major form in which carbohydrate is transported from aboveground portion to underground parts. Sucrose metabolism can affect starch synthesis and storage root development by starch-sugar interconversion in sweetpotato (Ravi et al., 2009; Schreiber et al., 2014). It was reported sucrose played a key role on the tuberous root induction of cassava in vitro (Wu et al., 2014). Tsubone et al. (2010) confirmed sucrose application increased tuberous root production and the weight ratio of tuberous root to total root in sweetpotato (Tsubone et al., 2010). Therefore, the enhancement of sucrose metabolism and starch biosynthesis contributes to high production of storage roots in sweetpotato.

Multiple external stimuli affect the formation and development of storage roots in the geophytes. For example, cultured cassava plants with fibrous roots easily generated tuberous roots in vitro under the combination of cytokinin N-benzyladenine (BA) at 0.5 mg l^{-1} and auxin α-naphthalene acetic acid (NAA) at 0.5 mg l^{-1} (Fan et al., 2011). Low humic acids can promote lily bulblet enlargement in vitro by enhancing root growth and carbohydrate metabolism (Wu et al., 2016). The optimal amount of potassium fertilizer (24 g K_2O m^2) can increase sucrose synthase by 16.47% and storage root yield by 36.42% in sweetpotato, respectively (Liu et al., 2013). Above indicated external stimuli play a dominant role in the formation and production of storage roots in storage root-bearing plants.

The root growth and development of plants can be affected by external amino acid, which has been proved in the series studies of Walch-Liu et al. on the regulatory interactions between amino acids and root growth (Walch-Liu et al., 2006a, 2006b; Walch-Liu and Forde, 2008). For example, the presence of exogenous L-glutamate (Glu) slowed primary root growth and stimulated root branching of *Arabidopsis thaliana* (Walch-Liu et al., 2006a). Among potential growth factors for root growth of plants, Gly, Glu, serine, alanine, and aspartic acid are the most abundant organic nitrogen sources in the terrestrial ecosystems (Lipson and Näsholm, 2001; López-Bucio et al., 2003). One of them, Gly plays an important role in carbohydrate metabolism and is frequently used in plant uptake studies with the characteristics of low molecular, simple structure, and favouring acquisition (Mouillon et al., 1999; Thornton et al., 2007; Wang et al., 2013; Han et al., 2018). There have been evidences that exogenous Gly can affect root growth of plants. For instance, exogenous Gly inhibited root growth, stimulated root hair, and induced a significant accumulation of starch grains in the root apex of habanero pepper (Domínguez-May et al., 2013). Gly application inhibited primary root elongation of pak choi by enhancing ethylene production in roots (Han et al., 2018). However, few studies have been conducted to investigate how external Gly can affect root growth of storage root-bearing plants.

In the present study, we focused on the effect of Gly on the growth and starch biosynthesis of storage roots in sweetpotato. Cultivar Xushu16 was selected as plant material for growth evaluation including morphological measurement, physiological characterization, starch content and hormone content determination. RNA-Seq was conducted to profile gene expression of the Xushu16 with treatments of different levels of exogenous Gly. The differentially expressed genes (DEGs) involved in starch biosynthesis during storage root swelling were identified by both RNA-Seq and RT-qPCR. The study provided a fundamental theory on how Gly affected root growth and starch metabolism of Xushu16, which may advance the future prospects of Gly application in improving the yield of sweetpotato and highlight the molecular mechanism of external amino acids on root growth and development of storage root-bearing plants.

2. Materials and methods

2.1. Plant materials

Plant cuttings from a single clone of Xushu16 were pot-cultured in the greenhouse with 60–70% relative humidity, 16/8-h light/dark cycles, and an average temperature of 24 °C in Jiangsu province of China. Per pot (50 cm diameter × 50 cm height) holding 10 kg acid-washed quartz sand contained three cuttings for seedling culture, which was modified following the description by Hewitt (1966). The Xushu16 seedlings at 15-days-old were applied with Hoagland and Arnon solution lacking nitrogen (Supplemental Table 1, Hoagland and Arnon, 1950) directly and daily for 15 days. Then the Xushu16 seedlings at one-month-old were challenged with Gly by adding above Hoagland and Arnon solution containing respective 0, 1.0, and 3.0 mM Gly solution directly and daily. Each treatment contains 9 biological replicates with one seedling as one replicate.

2.2. Morphological measurement

To investigate the growth state of sweetpotato with different Gly treatments, a few morphological indices of the Xushu16 at 30 days with treatment (DWT30) were measured with nine biological repeats, including plantlet height, fresh plantlet weight, dry plantlet weight, number of lateral roots in adventitious root, adventitious root length, fresh storage root weight, dry storage root weight, fresh storage root diameter, and fresh storage root volume. The plant height and root length were measured with tape measure. The plant weight and storage root weight were determined by an electronic balance (Mettler Toledo, PL202-L, Switzerland). The storage root diameter was estimated by electronic digital vernier calliper (Links, China). The storage root volume was calculated by water soaking method. In detail, the storage roots were forced to be soaked in the measuring cylinder with constant water, and the excess volume was determined as storage root volume.

To observe developing primary cambium (PC) of sweetpotato with different Gly treatments, the adventitious roots of the Xushu16 at DWT30 were sampled and cut into 1 cm-thick slices transversely. The 76 μm-thick slices were prepared from the 1 cm-thick slices with freezing microtome (microm HM525, Germany) and primordially observed by stereomicroscope (Nikon SMZ-10, Japan).

2.3. Physiological and hormonal characterization

At 60 days with treatment (DWT60), chlorophyll content, photosynthetic intensity, transpiration rate, stomatal conductance, stomatal density and intercellular CO2 concentration of the Xushu16 were measured with three biological repeats following the procedures described by Guo et al. (2005). In addition, sucrose content in the leaves and stems, and starch content in the storage roots were quantified with three biological repeats by the methods of McCready et al. (1950) and Zheng et al. (2012). The relative content of ABA, CK and IAA in the storage roots were quantified according to the methods described by Tamaki and Mercier (2007) and Chen et al. (2008).

2.4. RNA-seq analysis

At DWT60, the storage roots of the Xushu16 with three biological replicates were thoroughly washed with ddH_2O for three times, then frozen in liquid nitrogen and stored at −80 °C for RNA-Seq with

Illumina Hi-seq2500.

Data analysis was conducted as follows: 1) examination of raw reads by FastQC (QC, quality control); 2) purification of raw reads by Cutadapt and Prinseq; 3) De novo assembling of clean reads by Trinity using paired-end method; 4) SSR detection of unigenes and transcript using MISA; 5) functional annotation of unigenes by KAAS; 6) alignment of QC sequences to assembled transcript by Bowtie 2; 7) RNA-Seq evaluation by RSeQC; 8) FPKM quantification by RSEM; 9) DEGs identification with DEseq algorithm (p-value < 0.001, fold change > 2).

The venn diagram of DEGs was drawn by VENNY2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html). The hierarchical clustering of DEGs was performed by Gene Cluster 3.0 and viewed by Treeview. The gene ontology (GO) database (http://geneontology.org/) was used for gene set enrichment analysis. The kyoto encyclopedia of genes and genomes (KEGG) database (http://www.kegg.jp/) was applied for significant pathway enrichment analysis. The corrected p-value of GO terms and KEGG pathways was < 0.05.

2.5. RT-qPCR analysis

RT-qPCR was conducted to determine the relative expression level of 11 putative genes involved in starch and sucrose metabolisms. Total RNA was extracted by Column Plant RNAout Kit (Tiandz, Beijing, China) and proceeded to reverse transcript first-strand cDNA synthesis by PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Dalian, China). RT-qPCR was performed using SYBR Premix Ex Taq™ (Takara, Dalian, China) with a BioRad CFX96™ Real-time System C1000 Thermal Cycler. The relative expression level of the genes was calculated by $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The primer information of reference gene (*Osactin*) and 11 DEGs were listed in Supplemental Table 2.

2.6. Statistic analyses

All the data in the study were represent as the mean and standard error of independent biological replicates. Statistical analysis of morphological and physiological attributes was by one-way ANOVA followed by Duncan's test (P < 0.05). Student's t-test (P < 0.05) was used to identify significant differences of RT-qPCR results.

3. Results

3.1. Morphological measurement of the Xushu16 with gly treatments

Nine morphological traits of the Xushu16 at DWT30 were measured with 9 biological repeats. The results showed that the Xushu16 had larger plant biomass with Gly treatments than that under control condition (Table 1). Under 1 mM Gly condition, the plantlet height, fresh plantlet weight, dry plantlet weight, number of lateral roots, fresh weight of storage root, dry weight of storage root, storage root diameter, and storage root volume were 1.15, 1.39, 1.41, 1.34, 2.02, 2.00, 3.00, 2.14 times higher than those under the control condition, respectively. Under 3 mM Gly, the indexes were 1.08, 1.56, 1.56, 1.28, 1.50, 1.50, 1.80, 1.84 times higher than those under the control condition, respectively. However, it exhibited a significant decrease in adventitious root length with 1 mM Gly treatment and a weak decrease by 3 mM Gly stimulus, compared to the control condition. The results showed 1 mM Gly stimulus affected plant biomass of the Xushu16 at DWT30.

Ultrathin sections of adventitious roots of the Xushu16 at DWT30 were carried out to observe the developing PC with Gly treatments. The PC diameter of adventitious roots was larger with Gly treatments than that under the control condition (Supplemental Fig. 1). And it was largest with 1 mM Gly treatment, which indicated 1 mM Gly stimulus induced the growth of adventitious roots of the Xushu16 at DWT30.

Table 1
The nine morphological traits of the Xushu16 at DWT30 with nine biological repeats.

Morphological traits	0 mM	1 mM	3 mM
Plantlet height (cm)	62 ± 6.7a	71 ± 5.9a	67 ± 8.4a
Fresh plantlet weight(g)	15.09 ± 1.6b	21.04 ± 5.6a	23.48 ± 4.8a
Dry plantlet weight(g)	2.68 ± 0.72b	3.78 ± 1.35a	4.16 ± 2.30a
Number of lateral roots in adventitious root	29 ± 2.7b	39 ± 3.7a	37 ± 3.2a
Adventitious root length (cm)	19.5 ± 4.8a	18.7 ± 1.9a	19.2 ± 2.6a
Fresh storage root weight(g)	10.78 ± 3.3b	21.83 ± 4.3a	16.27 ± 4.7a
Dry storage root weight(g)	2.63 ± 0.42b	5.26 ± 0.18a	3.94 ± 0.49a
Fresh storage root diameter (cm)	1.5 ± 0.5b	4.5 ± 1.2a	2.7 ± 0.6a
Fresh storage root volume (cm^3)	113 ± 22b	242 ± 45a	208 ± 24a

Note: The Xushu16 seedlings at one-month-old were challenged with Gly by adding 1 L Hoagland and Arnon solution lacking nitrogen and containing respective 0, 1.0, and 3.0 mM Gly solution directly and daily. Nine morphological traits of the Xushu16 were measured at DWT30 with nine biological repeats. The plant height and root length were measured with tape measure. The plant weight and storage root weight were determined by an electronic balance (Mettler Toledo, PL202-L, Switzerland). The storage root diameter was estimated by electronic digital vernier calliper (Links, China). The storage root volume was calculated by water soaking method. Statistical analysis of the morphological traits was by one-way ANOVA followed by Duncan's test (P < 0.05).

3.2. Physiological characterization of the Xushu16 with gly treatments

Six physiological parameters associated with photosynthesis of the Xushu16 at DWT60 were characterized. As shown in Fig. 1, the chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance and stomatal density of the Xushu16 with Gly treatments were significantly higher than those under the control condition. However, there was no significant difference in intercellular CO2 concentration (Fig. 1). In addition, the leaf color of the Xushu16 with Gly treatments was obviously darker than that under the control condition (Supplemental Fig. 2). The physiological state with 1 mM Gly treatment was significantly better than that under 3 mM Gly and control conditions. The results indicated 1 mM Gly stimulus strengthened the photosynthesis of the Xushu16 at DWT60.

3.3. Sucrose and starch content determination of the Xushu16 with gly treatments

The relative content of sucrose and starch were determined in the Xushu16 at DWT60. As shown in Fig. 2, the sucrose content in the leaves and stems were highest and starch content in the storage roots was lowest under the control condition, implicating there may be less translocation from photosynthetic source tissues (leaves) to sink tissues (storage roots) without Gly stimulus. The starch content in the storage roots peaked while the sucrose content in the leaves and stems were lowest under 1 mM Gly, indicating sucrose unloading from aboveground to underground and starch filling in storage roots were more efficient with 1 mM Gly stimulus than those under 3 mM Gly (Fig. 2). Moreover, the storage roots with Gly treatments were obviously larger than that under the control condition (Supplemental Fig. 2). The phenomenon indicated 1 mM Gly application had a promotive effect on sucrose utilization and starch biosynthesis of the Xushu16 at DWT60.

3.4. Hormone content detection of the Xushu16 with gly treatments

The relative content of plant hormone in storage roots was measured in the Xushu16 at DWT60. The results showed that the relative content of CK, ABA and IAA in the storage roots with 1 mM Gly treatment were 1.24, 1.41 and 1.19 times significantly higher than that

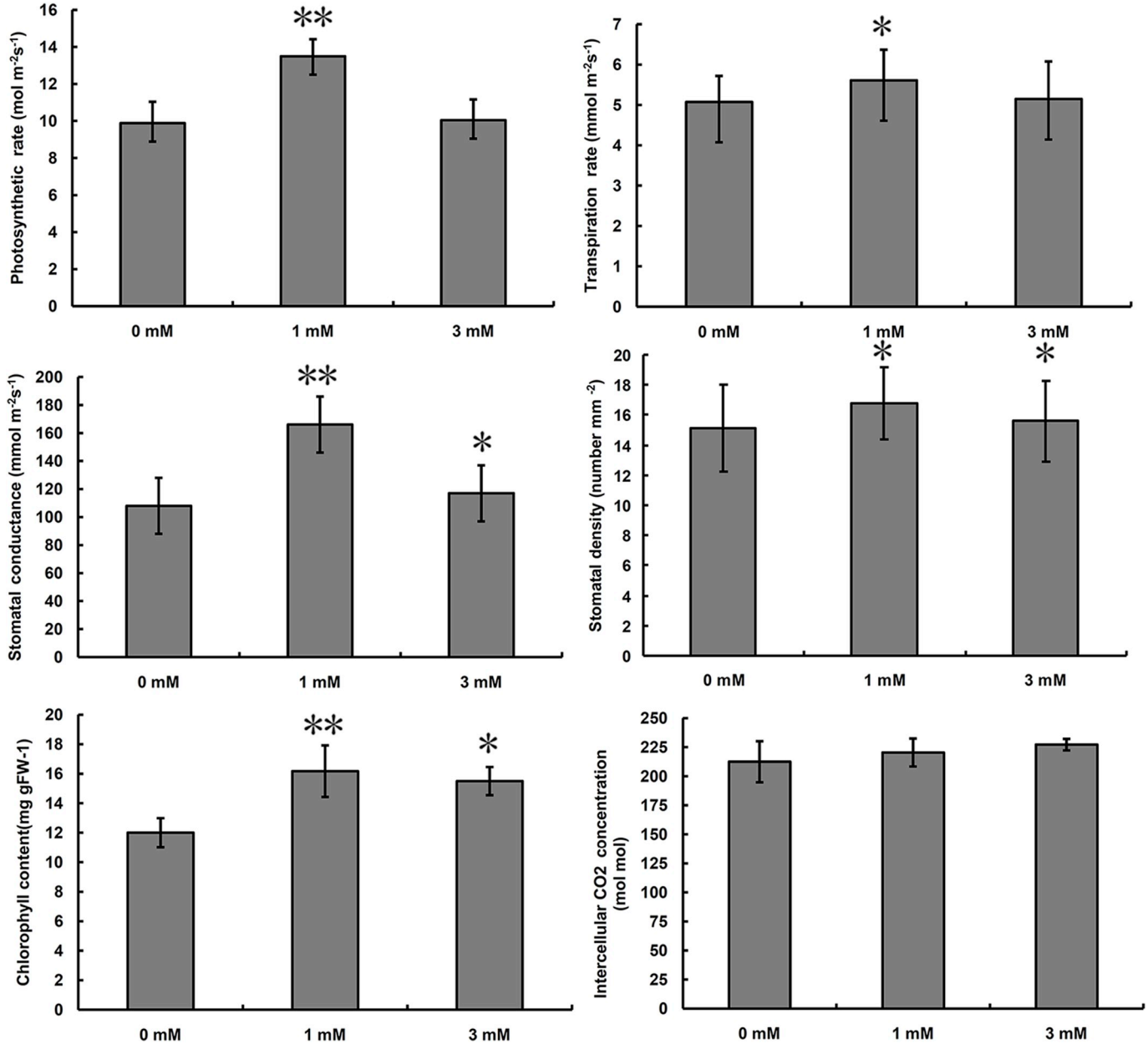

Fig. 1. Physiological characterization of the Xushu16 at DWT60. The Xushu16 seedlings at one-month-old were challenged with Gly by adding 1 L Hoagland and Arnon solution lacking nitrogen and containing respective 0, 1.0, and 3.0 mM Gly solution directly and daily. Six physiological traits of the Xushu16 were measured at DWT60 with three biological repeats. * indicates significant, ** indicates high significant.

under the control condition. And the values were 1.09, 1.02 and 1.07 with 3 mM Gly treatment, compared to control condition (Fig. 3). The results suggested that 1 mM Gly has a positive effect on plant hormone content of the Xushu16 at DWT60.

3.5. DEGs identification by RNA-Seq

The transcriptome data profiled a total of 34,406,268 raw reads with average length in 150 bp, resulted 34,314,847 clean reads with average length in 143 bp, which contained 182,148 transcript, 132,038 unigenes, and 28,171 annotated contigs. More up-regulated genes (URGs) were detected than down-regulated genes (DRGs) with Gly treatments. There were 9175 DEGs including 6497 URGs and 2678 DRGs identified with 1 mM Gly treatment, compared to control condition. A total of 6989 DEGs including 4413 URGs and 2576 DRGs were identified with 3 mM Gly treatment, compared to control condition. And 1282 URGs and 2035 DRGs were identified with 3 mM Gly treatment, compared to 1 mM Gly stimulus (Fig. 4, Supplemental Excel 1).

The numbers of DEGs that were specific to control VS 1 mM, control VS 3 mM, and 1 mM VS 3 mM were 4273 (3140/1133), 1530 (842/688) and 2694 (1055/1639), respectively. As many as 4836 (3344 URGs/1492 DRGs) DEGs were shared in the control VS 1 mM and control VS 3 mM comparisons, and 557 (214/343) DEGs were shared in the control VS 3 mM and 1 mM VS 3 mM comparisons. A total of 66 DEGs (1.2%) including 13 URGs and 53 DRGs were shared in the three comparisons (Fig. 4, Supplemental Excel 1).

3.6. DEGs clustering and annotation

The hierarchical clustering indicated that the expression of DEGs in the Gly treatments was significantly different from those under the control condition. The genes in the 1 mM and 3 mM Gly treatments

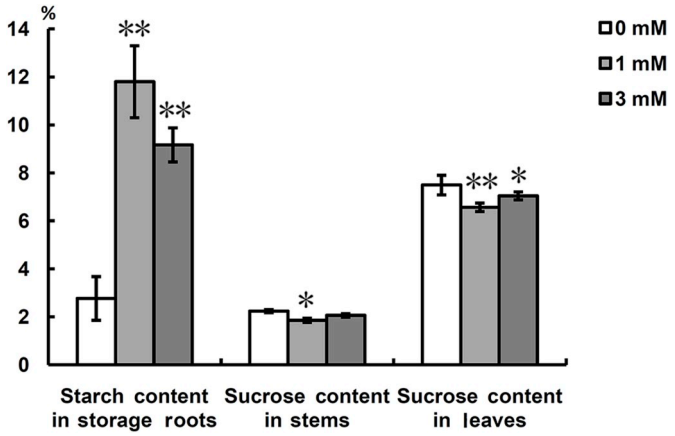

Fig. 2. Sucrose and starch content determination in the Xushu16 at DWT60. The Xushu16 seedlings at one-month-old were challenged with Gly by adding 1 L Hoagland and Arnon solution lacking nitrogen and containing respective 0, 1.0, and 3.0 mM Gly solution directly and daily. The sucrose content in the leaves and stems and starch content in the storage roots of the Xushu16 were measured at DWT60 with three biological repeats. * indicates significant, ** indicates high significant.

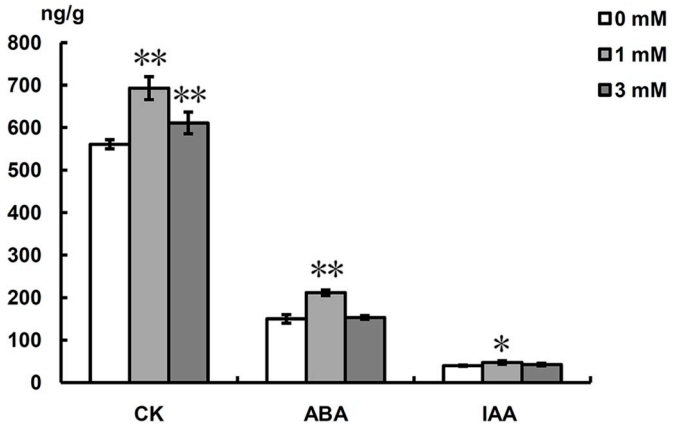

Fig. 3. Hormone content determinations in the storage roots of Xushu16 at DWT60. The Xushu16 seedlings at one-month-old were challenged with Gly by adding 1 L Hoagland and Arnon solution lacking nitrogen and containing respective 0, 1.0, and 3.0 mM Gly solution directly and daily. The sucrose content in the leaves and stems and starch content in the storage roots of the Xushu16 were measured at DWT60 with three biological repeats. * indicates significant, ** indicates high significant.

were classified into same subgroup horizontally, compared to those under the control condition, which were also classified into 6 clusters longitudinally. In the comparison of control VS 1 mM Gly, the cluster 1, 4 and 5 genes were up-regulated, the cluster 2 and 6 were down-regulated, and the cluster 3 was a mixture of up- and down-regulated. However, in the comparison of control VS 3 mM Gly, the up-regulated were in cluster 3 and 5, the down-regulated were in cluster 1 and 4, and the mix were in cluster 2 and 6 (Fig. 5).

According to enriched GO classification, all the DEGs were mainly associated with multiple biological processes such as biological regulation, metabolic process, cellular process, response to stimulus, cellular component organization or biogenesis. Followed by cellular components including cell, membrane, organelle and so on. The last was molecular function, for example, catalytic activity, TF (transcription factor) activity, receptor activity, binding, transporter activity, enzyme regulator activity and molecular transducer activity (Fig. 6A).

Further, the significantly enriched pathways of all the DEGs were investigated. There were five types of pathways based on KEGG classification: cellular processes, genetic information processes,

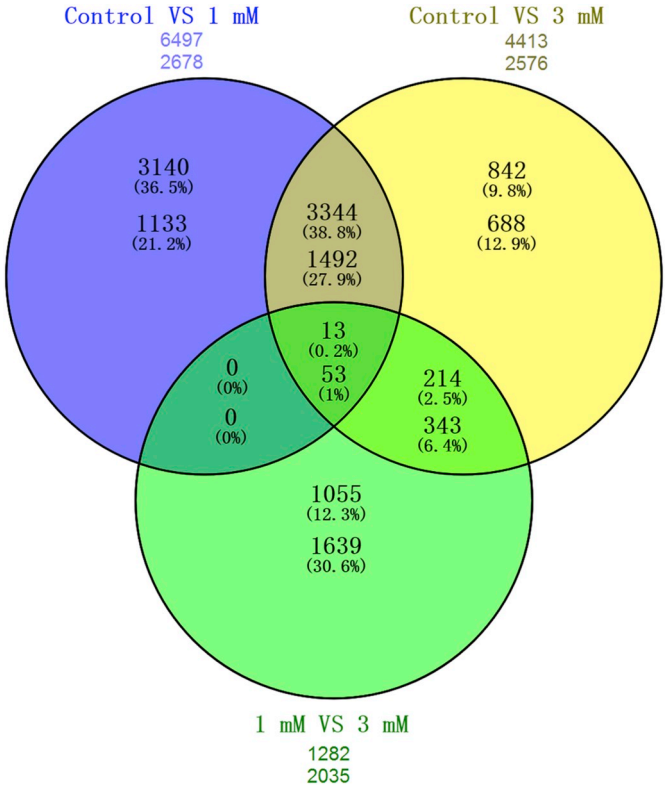

Fig. 4. Venn diagram of DEGs in the Xushu16 at DWT60. Venn diagram of DEGs was drawn by VENNY2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html). The numbers on top denote URGs and those on below denote DRGs, respectively.

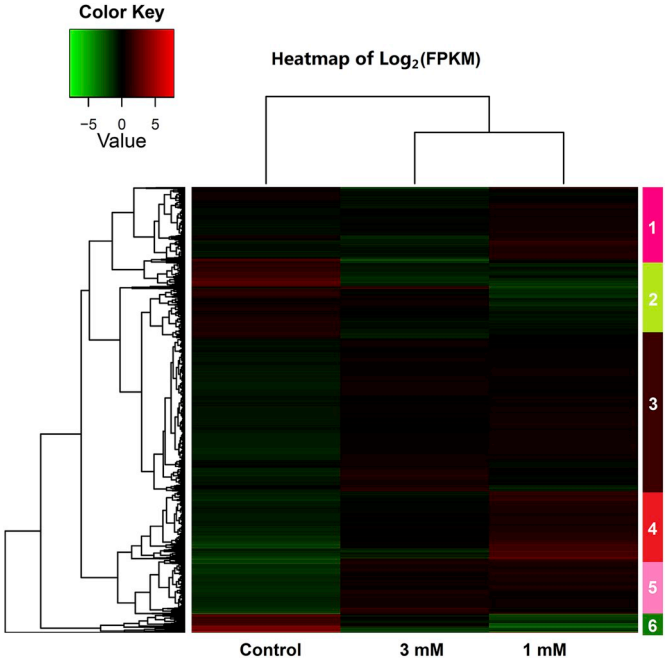

Fig. 5. Heatmap of DEGs in the Xushu16 at DWT60. The hierarchical clustering of DEGs was performed by Gene Cluster 3.0 and viewed by Treeview. Red and green colors indicate URGs and DRGs, respectively. The colorful vertical bars denote different gene clusters. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

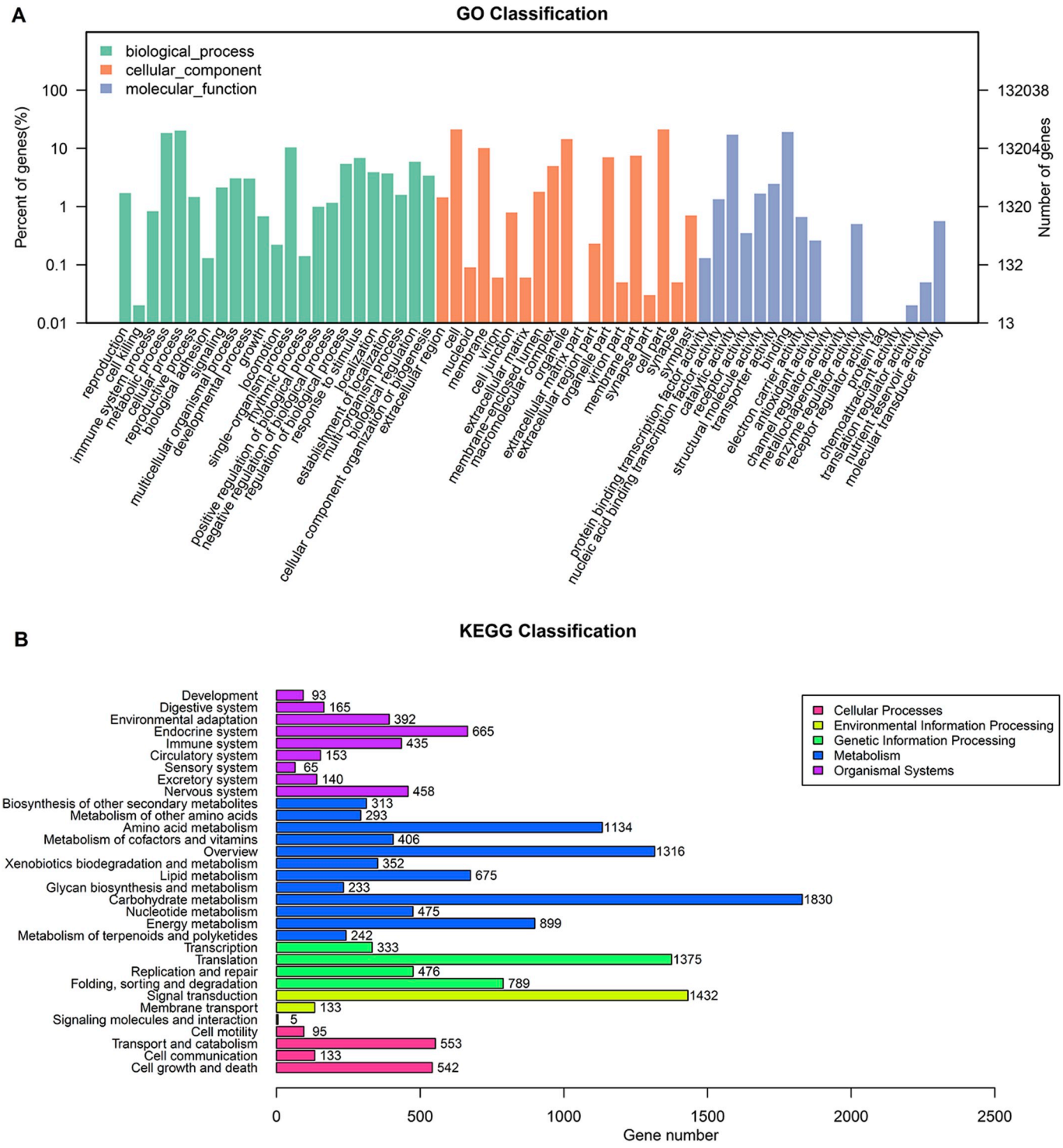

Fig. 6. GO and KEGG classification of DEGs in the Xushu16 at DWT60. A, GO classification; B, KEGG classification. The GO database (http://geneontology.org/) was used for gene set enrichment analysis. The KEGG database (http://www.kegg.jp/) was applied for significant pathway enrichment analysis. The corrected p-value of GO terms and KEGG pathways was < 0.05.

metabolism, organismal systems and environmental information processing. The largest fraction of pathways was involved in metabolism, mainly including carbohydrate metabolism, amino acid metabolism, energy metabolism etc.. The pathway involved in carbohydrate metabolism (1830 genes) accounted for the largest part in all pathways, followed by the pathway associated with signal transduction with 1432 genes (Fig. 6B).

3.7. DEGs validation by RT-qPCR

To verify RNA-Seq results, we conducted RT-qPCR to quantify the relative expression level of 11 DEGs associated with starch and sucrose metabolisms, including 7 URGs and 4 DRGs (Supplemental Table 2). The results indicated that the expression trend of the 11 DEGs detected by RT-qPCR was consistent with that profiled by RNA-Seq (Fig. 7). The relative expression level of 7 URGs with 1 mM Gly treatment was 7.51,

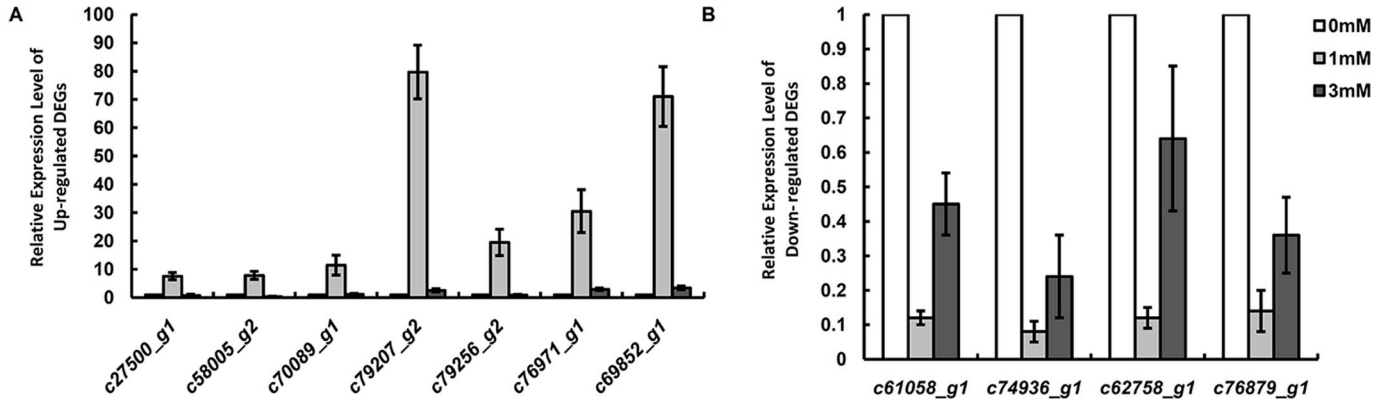

Fig. 7. RT-qPCR validation of DEGs in the Xushu16 at DWT60. A, the relative expression level of URGs; B, the relative expression level of DRGs. Student's t-test ($P < 0.05$) was used to identify significant difference of RT-qPCR results with three biological repeats.

7.81, 11.41, 79.65, 19.43, 30.49, and 70.98 folds higher than that under the control condition, respectively. However, the expression change of the URGs was relatively small with 3 mM Gly treatment, compared to control condition (Fig. 7A). The transcript abundance of 4 DRGs in the 1 mM Gly treatment were 8.3, 12.5, 8.3, 7.14 folds lower than that under the control condition, respectively (Fig. 7B). The decreased folds of the DRGs were 2.2, 4.2, 1.6, 2.8 with 3 mM Gly treatment, compared to control condition, respectively. The results indicated that Gly stimulus regulated the expression of DEGs and 1 mM Gly induced the expression of starch-related genes significantly.

4. Discussion

Sweetpotato is a promising complementary material for feed and energy in the whole world (Nedunchezhiyan et al., 2012). The high yield of sweetpotato depends on the sink potential of storage roots. The development process of storage roots can be affected by many intrinsic and extrinsic factors, such as photosynthesis, carbohydrate manipulation, sucrose metabolism, plant growth regulators and exogenous amino acids (López-Bucio et al., 2003; Walch-Liu and Forde, 2008; Tsubone et al., 2010; Fan et al., 2011; Zheng et al., 2012; Liu et al., 2013; Schreiber et al., 2014). There were both positive and negative effects of amino acids on the root growth of plants (Skinner and Street, 1954; Walch-Liu and Forde, 2008). For instance, the development of shoot bud to microshoots was enhanced by two fold in pigeonpea with supplemental amino acids like proline, Glu, asparagine and L-cysteine (Sudarsana et al., 2001); The root growth process was inhibited when applying external Glu to root tip in *Arabidopsis* (Sivaguru et al., 2003). In the study, we focused on how external Gly affected the root growth and starch biosynthesis of sweetpotato at morphological, physiological and molecular levels.

External amino acids are beneficial for plant culture by increasing regeneration rate and explants biomass (Sudarsana et al., 2001). In our study, low Gly promoted lateral root growth and inhibited root elongation, which kept in accordance with the previous studies in habanero pepper and pak choi with Gly treatments (Domínguez-May et al., 2013; Han et al., 2018). In addition, Gly stimulus strengthened photosynthesis and accordingly improved plant biomass of storage roots, including weight, diameter, and volume. It showed low Gly is beneficial for storage root growth at morphological and physiological levels.

ABA, CK, and IAA are common and naturally-occurring hormones, which play a prominent role in root growth and development (Gaspar et al., 1996; Kende and Zeevaart, 1997). ABA is an important phytohormone regulating various developmental and physiological processes in plants, including root growth (Fujii et al., 2007). CK plays a dominant role in many development processes of roots, including specifying root stem cells, regulating root apical meristem and lateral root formation (Werner and Schmülling, 2009). IAA regulates plant development processes by different physiological alterations, including root growth (Wang et al., 2001; Ali et al., 2009). In the other hand, a few studies highlighted that plant hormones were affected by nitrogen signaling in several plant species (Krouk et al., 2011). For example, N applied sidedress caused significant changes in ABA, IAA, and CK concentration in *Festulolium* plants (Pavlíková et al., 2012). In our study, the relative content of ABA, IAA, and CK in storage roots of the Xushu16 were significantly higher with 1 mM Gly treatment, compared to the control condition. It indicated low Gly may act as a kind of nitrogen signaling to coordinate the relative content of plant hormones, thus to affect storage root growth of sweetpotato.

Carbohydrates, in particular starch and sucrose, can provide both energy and carbon skeletons for nitrogen assimilation, and play important roles in growth and development of storage roots (Zheng et al., 2012; Bahaji et al., 2014; Schreiber et al., 2014). Starch synthesis can be affected by sucrose metabolism through starch-sugar interconversion (Schreiber et al., 2014). It was reported Gly and serine are two interconvertible amino acids that play an important role in C1 metabolism (Mouillon et al., 1999). In our study, the sucrose content was lowest in the leaves and stems and the starch content in the storage roots was largest with 1 mM Gly treatment, compared to control and 3 mM Gly conditions. And the starch content of storage roots was significantly improved with low Gly treatment, which was similar to significant accumulation of starch grains in habanero pepper by Gly stimulus (Domínguez-May et al., 2013). The results indicated low Gly contributes to starch converting and filling of sweetpotato by accelerating carbohydrate metabolism.

There were many genes involved in carbohydrate metabolism and starch biosynthesis up-regulated at an early stage of storage root formation (Firon et al., 2013). Transcriptome profile of storage roots by Zhang et al. (2017) showed that most DEGs involved in starch and sucrose metabolism were highly expressed, and correspondingly high rates of sucrose cleavage and interconversion of sucrose to starch occurred during storage root development in sweetpotato. In our study, a total of 4836 differentially expression genes were identified in the storage roots with different Gly treatments by RNA-Seq. Among them, as many as 1830 genes were involved in carbohydrate metabolism, which held maximum proportion among all the DEGs. It indicated low Gly markedly affected starch metabolic process by regulating the expression of starch-related genes.

AGP is a key regulatory enzyme in starch biosynthesis of higher plants, which consists of two small subunits and two large subunits. The wheat AGP small subunit, *TaAGPS1b*, played a significant role in increasing starch content and grain weight (Yang et al., 2017). Among the significantly induced URGs evaluated by RNA-Seq and RT-qPCR in the study, the URGs with ID as c27500_g1, c58005_g2 and c79256_g2 were

identified as ADP-glucose pyrophosphorylase (AGP) small subunit based on NCBI blastP. The gene c70089_g1 had 98% identity with isoamylase, which was required for normal synthesis of amylopectin (main component of starch) in plants (Hussain et al., 2003). The gene c79207_g2 had 99% identity with granule-bound starch synthase I, which was proved to be capable of synthesizing a significant number of crystalline structures within starch (Wattebled et al., 2002). And the gene c76971_g1 matched with starch branching enzyme I, which was of crucial importance for the quantity and quality of starch in plants (Kim et al., 1998). The URGs closely related with starch biosynthesis can be significantly induced by 1 mM Gly, which indicated low Gly played a promotive role in starch content by significantly up-regulating the genes involved in starch biosynthesis.

5. Conclusions

In the study, we interpreted how external Gly affected the root growth and starch biosynthesis of Xushu16. Morphological measurement showed the Xushu16 had growth advantages in the 1 mM Gly treatment, compared to control and 3 mM Gly conditions. Physiological characterization indicated 1 mM Gly application enhanced plant biomass by strengthening photosynthesis, and improved starch content of storage roots by accelerating source-sink conversion. Moreover, 1 mM Gly stimulus enhanced the relative content of CK, ABA and IAA in storage roots significantly. Transcriptional profiling indicated the largest fraction of significantly DEGs and pathways was involved in starch biosynthesis and sucrose metabolism by 1 mM Gly stimulus. A few starch-related genes were proved to be significantly up-regulated with 1 mM Gly treatment by RNA-Seq and RT-qPCR. All the results revealed that 1 mM Gly application had a positive effect on storage root growth and starch biosynthesis of Xushu16.

Contribution

Chuanzhe Li and Hongbo Ma conducted experiments. Chuanzhe Li and Wenjing Yao analyzed data and wrote manuscript. Jianping Wang revised manuscript. Jidong Wang and Yuchun Ai contributed new reagents and analytical tools. Hongbo Ma and Yongchun Zhang conceived and designed research. All authors read and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.10.012.

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