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Root transcriptome reveals responses to plastic film mulching and grass cover in wine grape 'Cabernet Sauvignon' root and berry

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Summary

This study was designed to investigate the influence of grass and film mulching on grape (*V. vinifera*) fruit quality and root transcriptome. The groundcovers (plastic film and seeds of herba portulacae and *E. humifusa* Willd) were set or sawn on the ground under wine grape plants ('Cabernet Sauvignon'). Test plots in the control group were treated with clean tillage. Properties of plant biochemicals under covers and grape berry quality were determined after two years. RNA-seq was analyzed for grape roots under different treatments. *E. humifusa* Willd cover increased plant total N and P; and film mulching increased plant total P and decreased plant total K. Herba portulacae cover decreased berry tannin and increased anthocyanin, soluble solid and titratable acid; *E. humifusa* Willd cover increased anthocyanin, soluble solid and titratable acid. Film mulching increased the contents of berry total phenols, soluble solid, and decreased titratable acid significantly in comparison with clean tillage. Root RNA-seq showed that there were 1, 0, and 42 differently expressed genes (DEGs) in Herba portulacae, *E. humifusa* Willd and film cover, compared with clean tillage, respectively. Film mulching increased the expression of root high-affinity nitrate transporter 2.1 (NRT2.1), NRT2.4 and glutamine synthetase, which associated with 'Nitrogen metabolism', and decreased the expression of root small class I/II heat shock proteins. Plastic film mulching increased grapple quality properties via activating N metabolism. Film mulching was a more excellent mulching for improving grape quality than grass cover in Ningxia, China.

K e y w o r d s : grass cover; plastic film mulching; nitrogen metabolism; RNA-seq.

Introduction

Agricultural practices improve soil biochemical properties, nitrogen (N) utilization, soil fungal diversity as well as crop yield and quality (Hansen *et al.* 2015, Köhl *et al.* 2016, Taheri *et al.* 2016, Verzeaux *et al.* 2017). Mineral fertilization is a regular agricultural practice and eventually it will damage the ecosystem and lead to cumulative soil problems, while it increases the yield and quality of crop (Gao *et al.* 2015, Francioli *et al.* 2016, Kok and Bal 2017). Application of various agricultural practices has been paid attention to in recent years with the attention to soil environment and the sustainable development of land resources.

Tillage, cover crops, grass cover and rotation combined with fertilization are alternative agricultural practices. It has been reported that application of cover crops promote ecological intensification as it increased the soil ecosystem diversity as well as soil fertility (Mbuthia *et al.* 2015, Kader *et al.* 2017, Wittwer *et al.* 2017, Bavougian and Read 2018, Daane *et al.* 2018). Tillage reduced soil microbial biomass carbon and N contents relative to vetch cover (Mbuthia *et al.* 2015). Tillage, grass cover and fertilization affected soil metabolic capacity by influencing the activity of soil β-glucosidase, β-glucosaminidase, cellobiohydrolase and phosphodiesterase (Mbuthia *et al.* 2015, Zheng *et al.* 2018). Mulching with grass or crops minimizes water evaporation, increases soil temperature and moisture as well as improves crop water utilization efficiency, and may be an efficient management for climate change adaptation of crops (Mamkagh 2009, Mbuthia *et al.* 2015, Kader *et al.* 2017, Bavougian and Read 2018, Daane *et al.* 2018, Helder and Santos 2018). Mulching materials and methods influence soil hydrothermal environment and control crop yield and productivity as well as water use efficiency (Kader *et al.* 2017). Mulching and ground cover have been promoted vigorously for the management of orchards and vineyard sworldwide (Bavougian and Read 2018, Daane *et al.* 2018, Zheng *et al.* 2018).

The grape berry quality biochemical compounds are easily influenced by soil biochemical properties, weather elements (including temperature and sunshine), variety, disease infection as well as postharvest technologies (Leeuw *et al.* 2014, Urcan *et al.* 2016, Kok and Bal 2017). Mulching with different materials changed the grape berry quality *via* changing soil properties (Jiang *et al.* 2015, Zhang *et al.* 2016a and b). Zhang *et al*. showed the plastic mulching increased grape size and content of soluble sol-

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id and Vc (ZHANG *et al.* 2016b). Mulch crops and materials have been widely applied in China orchards (HE *et al.* 2015, TIAN *et al.* 2015, ZHENG *et al.* 2017 and 2018). With attention to the mulching advances, KADER *et al.* reviewed recent advances in mulching materials and methods and suggested that plastic mulching materials have greater potential in increasing crop yield than organic ones (KADER *et al.* 2017), suggesting the different effect of these mulching methods.

There is increasing evidence showing that the root genomic profile is altered by soil stress and nutrients (SINGH *et al.* 2016, ZHOU *et al.* 2016, DOSSA *et al.* 2017, WANG *et al.* 2018). Analysis of root transcriptome reveals the interaction between plant development and soil environment (ESTÍBALIZ *et al.* 2015, KUMAR *et al.* 2016), as well as the response to soil stress (GUIMARAES *et al.* 2015, XU *et al.* 2015). Altered root genetic profiles have been shown in response to mineral stress (YAO *et al.* 2015). However, there was little knowledge about the influence of different mulching practices on the root transcriptome in fruit. Our present study was to investigate the influence of different mulching methods on the root transcriptome of wine grape (*V. vinifera* L. 'Cabernet Sauvignon)'. Grapevines were mulched with herba portulacae, *Euphorbia humifusa* Willd and plastic film with regular fertilization and irrigation. The influence of different mulching methods on soil biochemical and grape berry quality properties were investigated. RNA-seq was performed for root transcriptome. The present study revealed the influence of grass and plastic film mulching on grape root genetic profiles.

Material and Methods

Field location: We carried out our field experiment during June 2017 and Sep 2018 at Yuanshi chateau, the eastern foot of Helan Mountain, Ningxia province, China. This site (latitude 37° ~ 39°N, longitude 106°E, at 1160 m above sea level) is at a temperate arid and monsoon climate zone, with loamy-sand (sierozem) soil. The annual rainfall ranges from 99.7 ~ 233.1 mm and annual evaporation is ~ 1583.2 mm, 6.8-fold of rainfall. This site is characterized by a short frost-free period ranging from 129 d to 177 d, with 146 Kcal·cm^2 annual total solar radiation and 3039.6 h annual sunshine time.

Experimental materials and design: Grape plants (4-year old) were used as the material for our experiments. Twenty test plots were randomly assigned into four groups (n = 5 four each group) according to the single-factor, randomized-block design. Each plot contained 60 plants in 3 rows in north-south direction, with spacing of 0.6 m between plants and 5.0 m spacing between rows. Test plots in four groups were treated with grass cover methods (herba portulacae; and *E.humifusa* Willd), film mulching and clean tillage (control group). For grass cover, seeds of herba portulacae and *Euphorbia humifusa* Willd were sawn into the soil surface under grapevines as wide as 80 cm, 40 cm from grapevine main stem on each side (ridge cover). Grass height was kept between 5 ~ 10 cm by timely mowing. Black plastic films were used for film mulching, with the same width as grass cover. Grapevines were regularly drip irrigated with dropper facilities and conventionally fertilized.

Plant biochemical parameters: Three plant samples (mature leaf) were collected from three randomly selected plants in each test plot. Samples were dried, ground into powder, filtered and dissolved using distilled water with 1: 3 ratio (sample/water). Organic matter (organic carbon) was determined using $K_2Cr_2O_7$ digestion. Total N, P and K content was determined using H_2SO_4-H_2O_2 digestion-Nessler's reagent, H_2SO_4-H_2O_2 digestion-colorimetry, and H_2SO_4-H_2O_2 digestion-flame photometry, respectively. All detections were performed following the described methods by BAO *et al.* (BAOS.D, 2000) and LI *et al.* (LI *et al.* 2000). All experiments were performed in three replications randomly selected from 5 plots within one group.

Grape berry quality properties: 'Cabernet Sauvignon' grape berries were harvested on Sep 25, 2018, and were hand squeezed into juice immediately. A MISCO Palm Abbe™ handheld digital refractometer (MISCO PA201, Misco, Solon, OH, USA) was used for determination of soluble solid content in grape juice. Tannin, total phenols, and titratable acid content was detected using Folin-Denis assay, Folin-Ciocalteu methods and NaOH titration methods, respectively (LI *et al.*,2000). Three replications were detected in each plot.

RNA extraction and RNA-seq: Three test plots in each group were randomly selected for RNA sequencing, 5 root samples were randomly selected from each enrolled test plot. Total RNAs were extracted from grapevine roots using TRIzol (Invitrogen, Carlsbad, CA USA), and then digested using RNase-free DNase I (Takara, Japan). RNA quantification was performed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Then, equal amount of 5 RNA samples in one plot were pooled, enriched, fragmented and then synthetized into the first strand cDNA using random hexamers, followed with synthesis of double strands DNA. DNA samples were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA). Purified cDNA samples were amplified and then subjected to sequence library construction using a Lybay Library Construction Kit (Lybaybio, Tianjin, China). Library quality was determined using Qubit 2.0 Fluorometer (Agilent Technologies, Palo Alto, CA, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies). Ultimately, all libraries were subjected to the Illumina Hiseq pair-end 150 platform.

Data processing and gene expression analysis: FASTQ files were firstly processed using FastQC (v1.11.5; http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Standard data evaluation was performed by removing adapters and reads with low quality. The short reads aligned to ribosome RNA (rRNA) were removed from high quality clean reads, with the tolerance of 5 mismatch bases. Then, clean reads without rRNA sequences were aligned to reference genome (*V. vinifera*) us-

ing TopHat2 software (Kim *et al.* 2013). Gene FPKM values (mean fragments per kilobase of transcript per million mapped reads) was calculated by using Cufflinks software (Trapnell *et al.* 2014). Total gene numbers was calculated. Principal component analysis (PCA) and correlation of samples were performed according to the FPKM value of each gene. Differentially expressed genes (DEGs) between groups were identified by pairwise comparison, with the criteria of FDR < 0.05 and |log2FC| ≥1.

Gene enrichment analysis: KEGG pathway and GO category enrichment analysis was performed by querying KEGG database (Sept. 2016; https://www.genome.jp/kegg/) and GO (June 2016; http://www.geneontology.org/), respectively. *P* value ≤ 0.05 was set as cut off value for significant item.

Statistical analysis: All data of plant biochemical parameters and grape berry quality properties were expressed as mean ± standard deviation. Differences in these parameters between groups were analyzed using t-test in GraphPad Prism 6. Difference at $p < 0.05$ was considered as significant.

Quantitative real-time PCR validation: Total RNA (1 μg) of each sample was purified and reversely transcribed into cDNA using an Invitrogen SuperScript IV RT kit (ThermoFisher, USA). Primers of selected DEGs were designed using Primer5 software. The template cDNAs were diluted 10-fold, and of this, 2 μL were added to the 25 μL reaction mixture. qRT-PCR was performed on the StepOne Plus thermocycler (Applied Biosystems) with SYBR Premix Ex-Taq II under the following thermal cycle: 95 °C for 30 s, 40 cycles at 94 °C for 5 s, 60 °C for 30 s. *GAPDH* was used as the internal reference genes. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Results

Parameters of plant and grape berry quality: In comparison with clean tillage, portulacae mulching did not change leaf total Nitrogen, phosphorus and potassium (NPK) content (Fig. 1). *E. humifusa* Willd mulching increased leaf total N and *P* ($p < 0.01$), film mulching increased leaf total *P* content ($p < 0.01$) and decreased leaf total K content ($p < 0.05$). Grape berry tannin content was significantly reduced by portulacae relative to clean tillage (13.60 ± 0.88 mg·g^{-1} *versus* 18.16 ± 1.35 mg·g^{-1}, $p < 0.01$. Tab. 1). Berry anthocyanin content was significantly increased by portulacae (5.16 ± 0.10 mg·g^{-1}), *E. humifusa* (5.46 ± 0.03 mg·g^{-1}), and film mulching (6.28 ± 0.07 mg·g^{-1}) technology, compared with clean tillage (3.84 ± 0.17 mg·g^{-1}, $p < 0.05$ for all). Herba portulacae and *E. humifusa* mulching, especially the former, increased berry soluble solid and titratable acid compared to clean tillage significantly ($p < 0.01$). Film mulching increased the contents of berry total phenols (4.90 ± 0.66 mg·g^{-1}), soluble solid (26.70 ± 0.36 %), and decreased titratable acid (0.44 ± 0.01 %) in comparison with clean tillage.

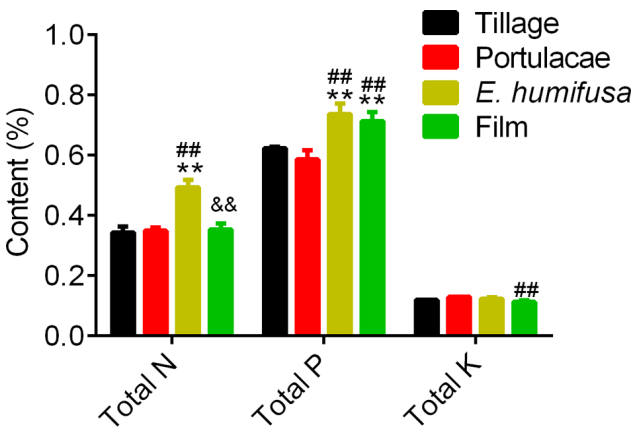

Fig. 1: Plant biochemical parameters after being treated with different mulching methods. **, $p < 0.01$ *vs.* Clean tillage. ##, $p < 0.01$ *vs.* portulacae. &&, $p < 0.01$ *vs. E. humifusa*. All differences were analyzed using t-test.

Sequencing data summary: Illumina sequencing generated a total of 701.2 M raw reads and 102.82 G raw data (Tab. S1). After qualification, there were 696.16 M clean reads and 101.91 G clean data with an averaged 90.93 % Q30 value and 43.64 % GC content. A total of 1.17 M rRNA reads (averaged ratio 0.17 %) were removed from the clean data and 698.8 M statistical reads were obtained finally. Ultimately, an averaged 75.30 % sequencing data were mapped to the reference *V. vinifera* genome (Tab. S1), these suggested the high quality of our sequencing data. All the statistical reads without rRNA reads were assembled using Cufflinks, and the number of known and novel genes were calculated (Tab. S1). The number of genes in samples ranged from 19,781 to 22,656, with

Table 1

The grape berry quality indicators from the test plots under different treatments

Parameters	Clean tillage	Portulacae	*E. humifusa*	Plastic film
Tannin (mg·g^{-1})	18.16 ± 1.35	13.60 ± 0.88**	17.16 ± 0.84$^{\#\#}$	16.82 ± 1.50$^{\#}$
Anthocyanin (mg·g^{-1})	3.84 ± 0.17	5.16 ± 0.10**	5.46 ± 0.03**$^{,\#\#}$	6.28 ± 0.07**$^{,\#\#,\&\&}$
Total phenols (mg·g^{-1})	2.50 ± 0.32	2.26 ± 0.14	1.66 ± 0.61	4.90 ± 0.66**$^{,\#\#,\&\&}$
Soluble solid (%)	24.07 ± 0.12	28.20 ± 0.10**	24.80 ± 0.00**$^{,\#\#}$	26.70 ± 0.36**$^{,\#\#,\&\&}$
Titratable acid (%)	0.46 ± 0.00	0.70 ± 0.02**	0.53 ± 0.02**$^{,\#\#}$	0.44 ± 0.01*$^{,\#\#,\&\&}$

Data was expressed as the mean ± standard deviation from 3 replicated samples. * and **, $p < 0.05$ and $p < 0.01$ *vs.* Clean tillage, respectively. $^{\#}$ and $^{\#\#}$, $p < 0.05$ and $p < 0.01$ vs. portulacae, respectively. $^{\&}$ and $^{\&\&}$, $p < 0.05$ and $p < 0.01$ *vs. E. humifusa*, respectively. All differences were analyzed by t-test.

19,061 (73.78 %) ~ 21,787 (84.33 %) known genes. PCA and Pearson's correlation analysis showed there were various correlations among samples (Suppl. Figure).

Overall analysis of DEGs: Compared with clean tillage treatment, herba portulacae, *E. humifusa* and plastic film mulching induced 1 (up-regulated), 0 and 41 DEGs (16 down- and 25 up-regulated) in grape berry root (Fig. 2A). Herba portulacae mulching decreased 1 DEG in grape berry root in comparison with *E. humifusa* Willd mulching. Plastic film mulching induced 192 DEGs (42 down- and 150 up-regulated DEGs) and 119 DEGs (16 down- and 103 up-regulated DEGs) compared with *E. humifusa* Willd mulching and herba portulacae mulching, respectively. A total of 312 DEGs were identified after removing the overlapping genes (Fig. 2B).

DEGs induced by plastic film mulching: Among the 42 DEGs induced by film mulching in comparison with clean tillage, some genes were enriched into GO biological processes including 'response to stimulus' [including up-regulated DEGs encoding SPX domain-containing protein 1 (*SPX1*), high-affinity nitrate transporter 2.1 (*NRT2.1*) and *NRT2.4*, and down-regulated EID1-like F-box protein 3 (*EDL3*) gene] and 'reproduction' (down-regulated *EDL3*); biological processes including 'transporter activity' (including up-regulated *NRT1, NRT2.1* and *NRT2.4*) and 'transcription factor activity, protein binding' (down-regulated transcription factor *TT2*); and cellular component of 'membrane' and 'cell part' (*NRT2.1* and *NRT2.4*) (suppl. Tab. S2). These genes associated with 'Protein processing in endoplasmic reticulum' [including down-regulated DEGs encoding heat shock proteins (*HSPs*)] and 'Nitrogen metabolism' (including *NRT2.1*

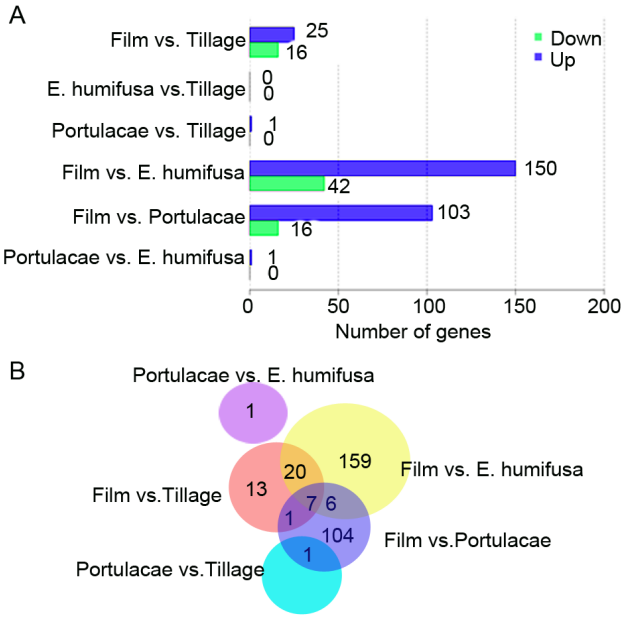

Fig. 2: The number of differentially expressed genes (DEGs) induced by different mulching technologies. **A**, diagram of DEGs number by pairwise comparison. Blue and green bar notes up- and down-regulated DEGs, respectively. **B**, the Venn figure of DEGs and the overlapping numbers.

and *NRT2.4*; Tab. 2). Film mulching induced 192 DEGs relative to *E. humifusa* Willd mulching associated with biological processes including 'response to stimulus' [including down-regulated DEGs encoding *HSPs*, dehydrin and *EDL3*], 'reproductive process' [including up-regulated transcription factor TCP4 gene and G-type lec-

Table 2

Pathway analysis of the differentially expressed genes

Pathway	ID	No	*P* value
Film versus Clean tillage (all terms)			
Protein processing in endoplasmic reticulum	ko04141	5	2.23E-05
Nitrogen metabolism	ko00910	2	0.001936
Sulfur metabolism	ko00920	1	0.064302
Pentose and glucuronate interconversions	ko00040	1	0.162801
Starch and sucrose metabolism	ko00500	1	0.332601
Film *versus E. humifusa* Wild (top 10 terms)			
Phenylpropanoid biosynthesis	ko00940	11	8.70E-06
Phenylalanine metabolism	ko00360	6	7.37E-05
Nitrogen metabolism	ko00910	4	0.000714
Protein processing in endoplasmic reticulum	ko04141	9	0.000835
Flavonoid biosynthesis	ko00941	5	0.00213
Circadian rhythm - plant	ko04712	4	0.006406
Stilbenoid, diarylheptanoid and gingerol biosynthesis	ko00945	2	0.026111
Ether lipid metabolism	ko00565	2	0.028389
Galactose metabolism	ko00052	3	0.032772
Pentose and glucuronate interconversions	ko00040	3	0.091578
Film *versus* Portulacae (top 10 terms)			
Protein processing in endoplasmic reticulum	ko04141	5	0.00283515
Plant hormone signal transduction	ko04075	4	0.03575017
Plant-pathogen interaction	ko04626	3	0.08020063
Linoleic acid metabolism	ko00591	1	0.08864021
Diterpenoid biosynthesis	ko00904	1	0.1054443
Monoterpenoid biosynthesis	ko00902	1	0.113735
Sulfur metabolism	ko00920	1	0.1501442
Biosynthesis of unsaturated fatty acids	ko01040	1	0.1541003
Phenylalanine, tyrosine and tryptophan biosynthesis	ko00400	1	0.2039572
α-Linolenic acid metabolism	ko00592	1	0.2718079

tin S-receptor-like serine/threonine-protein kinase (*GsSRK*) encoding gene, and down-regulated *EDL3*], and 'signaling' [up-regulated DEGs encoding probable disease resistance proteins (*DRPs*) and down-regulated *EDL3*]; molecular function of 'transcription factor activity, protein binding' [including up-regulated DEGs encoding protein TIFY 9 and myb-related protein Myb4, and down-regulated *TT2* gene] and 'antioxidant activity' (including up-regulated peroxidase 73 gene]; and cellular component categories including 'membrane' [including up-regulated *NRT2.1* and *NRT 2.4*, and down-regulated DEGs encoding cytochrome P450 82C4 (*CYP82C4*), bidirectional sugar transporter *SWEET14* and *HSPs*], and 'cell part' (including up-regulated *NRT2.1* and *NRT 2.4* and down-regulated *HSPs*) (suppl. Tab. S2). Pathway enrichment analysis showed these genes associated with 'Phenylpropanoid biosynthesis' [including up-regulated DEGs encoding peroxidases (*PRXs*) and phenylalanine ammonia-lyase (*PAL*)], 'Nitrogen metabolism' [including up-regulated DEGs encoding *NRT2.1* and *NRT 2.4* and glutamine synthetase (*GS*)], 'Flavonoid biosynthesis' [up-regulated DEGs encoding flavonoid 3',5'-hydroxylase (F3'5'H)-2 and stilbene synthases (*STSs*)], and 'Stilbenoid, diarylheptanoid and gingerol biosynthesis' [including up-regulated DEGs encoding STSs] (Tab. 2).

In comparison with herba portulacae mulching, film mulching induced DEGs involved in biological processes including 'reproduction' [including up-regulated DEG encoding *LECRK4*], 'growth' [one up-regulated COBRA-like protein 7 encoding gene], 'response to stimulus' [up-regulated DEGs encoding protein *TIFY 10A* and probable *DRPs*] and 'signaling' [including up-regulated DEGs encoding *DRPs*]; molecular functions including 'transcription factor activity, protein binding' [including down-regulated DEG encoding *TT2*, and up-regulated DEGs encoding protein *TIFY 10* and transcription factor *MYB114*] and 'antioxidant activity' [including one down-regulated DEG encoding peroxidase 27]; cellular component categories including 'membrane' and 'cell part' (including down-regulated DEGs encoding *PRXs, TT2* and *HSPs*) (suppl. Tab. S4). KEGG pathway enrichment analysis showed these genes associated with 'Plant hormone signal transduction' [including down-regulated DEGs encoding *HSPs*] and 'Plant-pathogen interaction' [including up-regulated DEGs encoding probable calcium-binding protein CML31].

qRT-PCR validation of RNA-seq: To verify the RNA-seq data, 6 DEGs were randomly selected. After correlation analysis, we found that all the fold changes of these 6 genes highly corresponded with the FPKM value of RNA-seq (Fig. 3).

Discussion

The quality of wine is mainly determined by the contents of total soluble solids, phenol compounds, tannins, titratable acids and sugar-acidity ratio (Yuyuen *et al.* 2015, Mencarelli and Bellincontro 2018). Among these factors, excessive accumulation of sugar and polyphenols, which has positive effect on human health, influence the flavor and aroma of wine (Urcan *et al.* 2016). Our present study showed that grass and plastic film mulching altered wine grape quality differently.

In comparison with clean tillage, grass cover increased grape berry soluble solid (both), decreased tannin (portulacae), and increased titratable acid (both) and anthocyanin (both) (Tab. 1). These results suggested that grass cover changed wine flavor and aroma by decreasing tannin and increased titratable acid content. Mulching with plastic film significantly increased anthocyanin, total phenols and soluble solid, and decreased titratable acid ($p < 0.05$) and tannin ($p > 0.05$), suggesting plastic film mulching improved the flavor and quality of grape and wine. These results suggested that plastic film had higher efficiency in improving grape berry quality than mulching with grasses (both portulacae and *E.humifusa* Willd). This result was consistent with the reported facts that plastic mulching materials showed higher efficiency in improving crop yield and quality (Kader *et al.* 2017). In addition, previous studies showed that *ANS*, *ANR* and *LAR* act as regulators in tannin metabolism of plant (Peng *et al.* 2012, Damiani 2007). The expression level os *ANS*, *ANR* and *LAR* in different groups were not statistically significant (p value > 0.05), but trend to be correlated to tannic acid content, so we speculate that *ANS*, *ANR* and *LAR* mediated the change of tannic acid content.

It has been shown that plastic mulching materials showed higher efficiency in modulating soil environment than cover grass and crops (Kader *et al.* 2017). Kader and Senge reviewed the influence of plastic mulching materials on soil and showed the reported higher soil moisture content under plastic mulching materials compared with grass cover (Kader *et al.* 2017). Plastic materials mulching absorbs solar radiation and reduces heat loss from soil and therefore there is an increase in temperature (Wang *et al.* 2015b, Kader *et al.* 2017). In addition, plastic material mulching conserves soil water and moisture, which enhances soil hydrothermal environment and improves plant growth and crop production (Kader *et al.* 2017, Zheng *et al.* 2017). In addition, the increased soil ecosystem diversity by both plastic film and grass cover leads to soil ecological intensification (Daane *et al.* 2018). Our present study showed that the quality of grape berry was increased by plastic film mulching in comparison with grass cover. This suggested the higher efficiency of using plastic film mulching for grape berry management. Our RNA-seq analysis showed that plastic film mulching altered root transcriptome com-

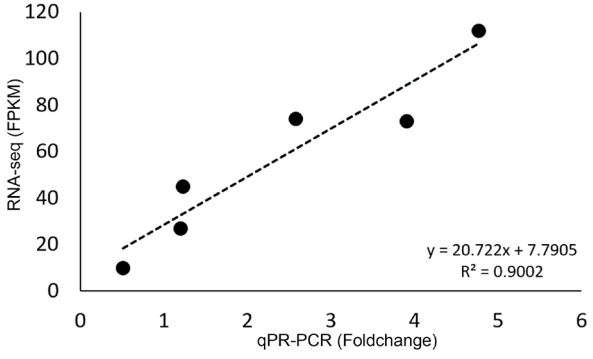

Fig. 3: qRT-PCR validation of RNA-seq.

pared with clean tillage and grass cover. In comparison with tillage, portulacae and *E.humifusa* Willd, film mulching induced 42 DEGs, 119 and 192 DEGs, respectively. Film mulching decreased the expression of PRXs, class I (18.1 kDa) and II (17.3 kDa) HSPs and transcription factor *TT2* encoding genes in grapevine root, compared with clean tillage and grass cover. *TT2* is a R2R3 type MYB in Arabidopsis, which correlates with the accumulation of PA in the seeds and flowers of transgenic cacao (LIU *et al.* 2015), tobacco (PÉREZ-DÍAZ *et al.* 2016) and cotton (NAN *et al.* 2017). Plant PRXs and small HSPs play important roles in protecting cells from stresses and infections as well as plant growth and development (BELA *et al.* 2015, PARK and SEO 2015, MCLOUGHLIN *et al.* 2016, PANDEY *et al.* 2017). Small HSPs regulate cellular proteostasis (TREWEEK *et al.* 2015). It has been reported that the overexpression of a small HSP (18.6 kDa) gene enhanced thermotolerance and abiotic stresses (including heat, drought, salt and cold) in rice (WANG *et al.* 2015a). SUN *et al.* transgened a HSP17 gene from *Agrostis stolonifera* into *Arabidopsis* and found the overexpressed HSP17 enhanced *Arabidopsis* sensitivity to salinity and exogenous abscisic acid (SUN *et al.* 2016). The fact that these genes were down-regulated in the grape root under plastic film mulching compared with others might suggest the suppressed immunity and defense ability to stresses.

By contrast, we observed the up-regulation of *DRPs, PAL, NRT2.1/2.4* and *GS* genes in grape root treated by plastic film mulching, compared with clean tillage and grass cover. The increased expression of GS in plant indicates improved crop N use efficiency (THOMSEN *et al.* 2014). GS enzyme is crucial for ammonium assimilation and N remobilization by assimilating ammonium into the amide position of glutamine (LEA and MIFLIN 2011). Its overexpression promotes grain yield and use efficiency of soil N (TANG *et al.* 2017). Both members of *NRT2* family (*NRT2.1* and *NRT2.4*) were up-regulated in root by plastic film mulching. *NRT2* family is known to control N uptake and transport (KANT 2017). LI *et al*. showed that *NRT2.1*, a plasma membrane intrinsic protein, was a high-affinity NO_3^- transporter associated with the root water transport capacity and shoot to root signaling in Arabidopsis (LI *et al.* 2016). GU *et al.* showed that the transfer of chrysanthemum *NRT2.1* gene to *Arabidopsis thaliana* plants enhanced nitrogen uptake rate, suggesting it was a high affinity root *NRT* (GU *et al.* 2016). Our present study showed high affinity *NRT2.1* and *NRT2.4* were enriched into 'membrane' and 'nitrogen metabolism' KEGG pathways, suggesting they are located at plasma membrane and functions in N uptake and transportation. This was consistent with the report from WANG *et al.* showing that plastic film mulching increased use efficiency of soil nitrate-N and crop yield (WANG and XING 2016).

In comparison with film mulching, there is another restricting factor of grass cover in addition to lower soil moisture and temperature which is the soil N competition between grass and crops. DAANE *et al.* showed that the native grass ground cover on the soil surface of vineyards decreased leafhopper pests partially while increasing parasitism rates of pests (DAANE *et al.* 2018). In biochemical features, grass cover decreased the N level in vine petioles, suggesting the soil N competition between grass and grapevine (DAANE *et al.* 2018). Taken together, these results suggested that plastic film mulching might result in higher grape yield via promoting N uptake, compared with tillage and grass cover.

Conclusions

Plastic film mulching improved the quality of grape berry, whereas the influence of mulching with grass on grape quality remains to be explored. Plastic film mulching increased the expression of root genes related to N metabolism and transport (including *NRT2.1* and *NRT2.4*), while decreased the expression of root genes associated with plant defense and stress response (including small HSP and PRX encoding genes). More experiments with comprehensive molecular research might uncover this contradiction. We concluded that film mulching was an efficiency method for improving grape quality.

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