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 P and K, 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.

Key words: 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, BANOGUAN 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, celllobiohydrolase 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 (MAMKAGI 2009, MBUTHIA et al. 2015, KADER et al. 2017, BANOGUAN 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 worldwide (BANOGUAN 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 (JANG et al. 2015, ZHANG et al. 2016a and b). ZHANG et al. showed the plastic mulching increased grape size and content of soluble solu-
id and Ve (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 (Estibaliz 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 Yuanishi 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·cm²·year 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 sown 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 dropped 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 K2Cr2O7 digestion. Total N, P and K content was determined using H2SO4-HClO4 digestion-Nessler's reagent, H2SO4-H2O2 digestion-colormetry, and H2SO4-H2O2 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-
Root transcriptome reveals responses to plastic film mulching

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Clean tillage</th>
<th>Portulacae</th>
<th><em>E. humifusa</em></th>
<th>Plastic film</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin (mg·g⁻¹)</td>
<td>18.16 ± 1.35</td>
<td>13.60 ± 0.88**</td>
<td>17.16 ± 0.84**</td>
<td>16.82 ± 1.50</td>
</tr>
<tr>
<td>Anthocyanin (mg·g⁻¹)</td>
<td>3.84 ± 0.17</td>
<td>5.16 ± 0.10**</td>
<td>5.46 ± 0.03**</td>
<td>6.28 ± 0.07**</td>
</tr>
<tr>
<td>Total phenols (mg·g⁻¹)</td>
<td>2.50 ± 0.32</td>
<td>2.26 ± 0.14</td>
<td>1.66 ± 0.61</td>
<td>4.90 ± 0.66**</td>
</tr>
<tr>
<td>Soluble solid (%)</td>
<td>24.07 ± 0.12</td>
<td>28.20 ± 0.10**</td>
<td>24.80 ± 0.00**</td>
<td>26.70 ± 0.36**</td>
</tr>
<tr>
<td>Titratable acid (%)</td>
<td>0.46 ± 0.00</td>
<td>0.70 ± 0.02**</td>
<td>0.53 ± 0.02**</td>
<td>0.44 ± 0.01**</td>
</tr>
</tbody>
</table>

Data was expressed as the mean ± standard deviation from 3 replicated samples. * and **, p < 0.05 and p < 0.01 vs. Clean tillage, respectively. &&, p < 0.01 vs. Portulacae. &&, p < 0.01 vs. *E. humifusa*. All differences were analyzed using 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* and *NRT2.4*). 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-

![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.](image)

<table>
<thead>
<tr>
<th>Pathway</th>
<th>ID</th>
<th>No.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Film versus Clean tillage (all terms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein processing in endoplasmic reticulum</td>
<td>ko04141</td>
<td>5</td>
<td>2.23E-05</td>
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<td>Nitrogen metabolism</td>
<td>ko00910</td>
<td>2</td>
<td>0.001936</td>
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<td>Sulfur metabolism</td>
<td>ko00920</td>
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<td>0.064302</td>
</tr>
<tr>
<td>Pentose and glucuronic interconversions</td>
<td>ko00040</td>
<td>1</td>
<td>0.162801</td>
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<tr>
<td>Starch and sucrose metabolism</td>
<td>ko00500</td>
<td>1</td>
<td>0.332601</td>
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<td>Film versus <em>E. humifusa</em> Wild (top 10 terms)</td>
<td></td>
<td></td>
<td></td>
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<td>Phenylpropanoid biosynthesis</td>
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<td>11</td>
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<td>Phenylalanine metabolism</td>
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<td>6</td>
<td>7.37E-05</td>
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<td>Protein processing in endoplasmic reticulum</td>
<td>ko00141</td>
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<td>0.000835</td>
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<td>Flavonoid biosynthesis</td>
<td>ko00941</td>
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<td>0.00213</td>
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<td>Circadian rhythm - plant</td>
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<td>0.006406</td>
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<tr>
<td>Stillbenoid, diaryleptanoid and gingersol biosynthesis</td>
<td>ko00945</td>
<td>2</td>
<td>0.026111</td>
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<td>Ether lipid metabolism</td>
<td>ko00565</td>
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<td>0.028389</td>
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<td>Galactose metabolism</td>
<td>ko00052</td>
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<td>0.032772</td>
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<td>Pentose and glucuronic interconversions</td>
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<tr>
<td>Film versus Portulacae (top 10 terms)</td>
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<tr>
<td>Protein processing in endoplasmic reticulum</td>
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<td>0.00283515</td>
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<tr>
<td>Plant hormone signal transduction</td>
<td>ko04075</td>
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<td>0.03575017</td>
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<td>Plant-pathogen interaction</td>
<td>ko04626</td>
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<td>Linoleic acid metabolism</td>
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<td>0.08864021</td>
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<td>Diterpenoid biosynthesis</td>
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<td>Monoterpenoid biosynthesis</td>
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<td>Sulfur metabolism</td>
<td>ko00920</td>
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<td>0.1501442</td>
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<tr>
<td>Biosynthesis of unsaturated fatty acids</td>
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<tr>
<td>Phenylalanine, tyrosine and tryptophan biosynthesis</td>
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<td>α-Linolenic acid metabolism</td>
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<td>1</td>
<td>0.2718079</td>
</tr>
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tin S-receptor-like serine/threonine-protein kinase (GaSsRK) 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 tyrosine phosphatase 4, putative (PTP4) 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 'Antioxidant activity' [including one down-regulated DEG encoding probable disease resistance protein CML31].

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].

**Discussion**

The quality of wine is mainly determined by the contents of total soluble solids, phenol compounds, tannins, titratable acids and sugar-acidity ratio (YuVuen 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 (portulaca), 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 portulaca 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 of ANS, ANR and LAR in different groups was 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 plastic film mulching. 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.](image-url)
pared with clean tillage and grass cover. In comparison with tillage, portulacaee and E. humifusa Wildl, 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 (Li 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 thermostolerance 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 NO3− 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. Danne 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 (Danne et al. 2018). In biochemical features, grass cover decreased the N level in vine petioles, suggesting the soil N competition between grass and grapevine (Danne 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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