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Effects of *Cerasus humilis* (*Bge*). *Sok.* Rootstock on Peach Growth, Development, and Expression of Growth-Related Genes

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Abstract: Peach trees play an essential role as an economic crop in China. However, the increasing cost of labor has led to a decline in the benefits of peach cultivation. The use of dwarfing rootstock technology can increase planting density, reduce tree height, decrease labor requirements, and reduce production costs. The *Cerasus humilis* (*Bge*). *Sok.* is a promising dwarfing rootstock for peaches owing to its small tree size, abundant resources, strong resistance, and adaptability. In this study, we investigated the effect of *Cerasus humilis* rootstocks on peach growth and development, and related gene expression. We used Ruipan 4/*Cerasus humilis* and Ruipan 4/*Amygdalus persica* L. as experimental materials to measure the growth and fruiting characteristics of two-year-old *Cerasus humilis* rootstocks. In addition, we used bioinformatic methods to explore the effect of *Cerasus humilis* rootstock on peach growth gene expression. Our results showed that *Cerasus humilis* rootstocks can dwarf peach trees, reduce branches, increase pollen count and stigma receptivity, shorten spore development, and promote protein accumulation in the late stage of fruit maturity. The *Cerasus humilis* rootstock reduced the growth hormone content in peach trees while upregulating the expression of growth-related *PpYucca5* and *PpYucca2* genes. *PpYucca6* expression was downregulated in the early stage of shoot growth and upregulated in the middle stage. By reducing the content of growth hormones, peach trees can be dwarfed, but their impact on fruit quality is minimal. These results indicate that *Cerasus humilis* is a suitable peach dwarfing rootstock and can provide a theoretical reference for the future breeding of peach dwarfing rootstocks.

Keywords: auxin; *Cerasus humilis*; dwarfing rootstock; fruit quality; YUCCA gene

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1. Introduction

China is a major producer of peaches and occupies an important position in the field of economic crops [1,2]. Currently, grafted seedlings are mostly used in peach production, with *Amygdalus persica* L. and *Amygdalus davidiana* (*Carr.*) *Yu* peach seedlings being the main rootstocks [3,4]. However, with the establishment of modern standardized orchards, dwarf and high-density planting are widely used in orchard production because of their advantages of early, stable, and high-yielding production, as well as high-quality and labor-saving efficiency [5,6]. Peach trees are characterized by vigorous growth, multiple branching levels, and fast early growth, which require significant management and labor costs. Therefore, to adapt to the trend of modern orchard development, breeding dwarf rootstocks, controlling tree vigor, and adopting labor-saving cultivation techniques such as dwarf and high-density planting, have become one of the main goals of peach breeding [7]. Although research on dwarf peach rootstock breeding began early, few suitable dwarf rootstocks have been selected for production [8].

The development of dwarf peach rootstocks mainly comes from stone fruit trees such as apricots and plums [6,9]. Ben Yahmed et al. [10] studied the physiological behavior of different plum rootstocks and selected the most suitable rootstock under the Mediterranean climate. The *Cerasus humilis* is a shrub in the Rosaceae cherry genus with strong stress resistance, and is widely distributed in the northern regions of China. Previous studies have reported that this species has a diploid genome with 16 chromosomes (2n = 16). Some scholars believe that the *Cerasus humilis* can be used as a dwarf rootstock for peaches, but there is a lack of both systematic experimental evidence and a theoretical basis [11].

Indicators such as tree height, number of branches, and length and thickness of branches can reflect the degree of plant dwarfing [12]. The more obvious the dwarfing effect, the newer the shoots it produces [13,14]. The dwarfing of intermediate rootstocks reduces tree height, new shoot growth, and branch thickness, and the cessation time of new shoot growth is earlier [15]. The weight and size of a single fruit are important indicators of its quality. Fruit hardness and sugar and acid content affect fruit taste and flavor. Different rootstocks have different effects on fruit quality [16]. Dwarf apple rootstocks can improve fruit color, significantly increase single-fruit weight and first-grade fruit yield, increase fruit hardness, and improve intrinsic fruit quality [17,18]. Dwarf citrus rootstocks can maintain tree-vigor stability and have no significant effect on nutrient content [19]. Yakushiji et al. [20] also found that the difference in fruit quality between dwarf rootstocks and standard rootstocks of persimmons was not significant.

Plant endogenous hormones have a significant effect on the expression of plant characteristics, whereas rootstocks affect the endogenous hormone metabolism level of grafted cultivars, thereby affecting plant growth and development [4]. Auxin was the first plant hormone to be identified, and it plays an important regulatory role in plant growth and development, such as cell division and differentiation, mainly relying on the tryptophan pathway to generate auxin [21]. Tryptophan is mainly catalyzed by two enzymes to produce indole-3-acetic acid (IAA), tryptophan aminotransferase 1 (TAA1), and flavin monooxygenase encoded by the YUCCA gene [22]. Tao et al. [23] found that plants overexpressing the TAA gene did not show significant phenotypic changes caused by changes in auxin content, and the free auxin content in their body did not significantly increase. Therefore, TAA1 is not the limiting factor in the synthesis process, whereas the YUCCA gene encoding flavin monooxygenase is the main limiting enzyme in IAA biosynthesis. Overexpressing YUCCA plants showed increased auxin content, increased plant height, decreased branching number, and increased plant resistance. Le et al. [24,25] found that overexpressing *YUCCA7* improved plant drought resistance. Kim [26] also found that potato overexpressing the *Arabidopsis thaliana AtYUCCA6* gene showed drought resistance, but also showed reduced yield. Overexpression of YUCCA genes can increase plant auxin content and delay leaf senescence by regulating the expression of the senescence-related genes SAGl2, NACl, and NAC6 [27].

In our previous work, we focused on the anatomy and physiology of the graft compatibility of *Cerasus humilis* with peaches, with a focus on rootstock propagation and cultivation. Building on this work, the present study aimed to explore the impact of *Cerasus humilis* on peach tree growth and fruiting habits, as well as the expression of genes related to auxin synthesis. Specifically, we investigated the effect of *Cerasus humilis* on peach tree growth and development, fruit quality, and dwarfing mechanisms. Our ultimate goal is to provide a theoretical reference for the selection and cultivation of dwarfing rootstocks for peach trees, which is of great importance for the sustainable development of the peach industry.

2. Materials and Methods

2.1. Plant Materials

The experiment was conducted in Luoyang City, Henan Province, China (34°37′ N, 112°38′ E). The experimental field was located in a temperate continental climate zone in a warm-temperate monsoon region. The altitude of the study area ranged from 120 to 300 m. The annual average temperature is 14.2 °C, the minimum temperature is −8.1 °C, and the

average frost-free period is 211 days. The annual average rainfall is 500–600 mm, and the total annual sunshine hours are 2248.3 h.

For this study, R/O (Ruipan 4/*Cerasus humilis* (*Bge.*) *Sok.*) and R/M (Ruipan 4/*Amygdalus persica* L.) were selected as the experimental materials. Peach trees were planted in a field trial, and traditional field management practices were employed. Flood irrigation was used before budbreak in spring and after leaf fall in autumn, while other irrigation instances were adjusted based on weather conditions. At the time of planting, each tree received a base fertilizer of 5 kg of organic fertilizer with an organic matter content of 45%. Subsequently, each tree was given the same amount of organic fertilizer annually in autumn using the strip-trench fertilization method. Before budbreak in spring, 0.5 kg of urea was applied to each tree. Pest and disease control measures were taken after the trees blossomed in April, using imidacloprid and thiophanate-methyl as necessary. We adopted a completely randomized block experimental design. R/M was the control treatment. Four trees were selected as plots for each grafting combination, and the experiment was repeated thrice.

2.2. Determinations of the Growth Habits of Peach/Cerasus humilis

The tree height and branch length of the grafted peach combination were measured using a steel ruler, and the stem thickness and branch thickness were measured using a Vernier caliper. During the dormant period of 1-year-old (2017) and 2-year-old (2018) peach trees, tree height, trunk thickness, branch length, and thickness were determined. Before the buds of 3-year-old trees (2019) sprouted, 12 branches with consistent growth were selected from the upper, middle, and lower parts and the east, west, south, and north directions of each tree. One shoot with consistent growth and angle was selected and labeled from the upper part of each branch at the same position. From April to June, the length and thickness of each marked shoot were measured every two days to observe the growth dynamics of the shoots.

2.3. Determinations of the Fruiting Habits of Peach/Cerasus humilis

After the peach trees entered hibernation in 2017 and 2018, the number of flower buds and the ratio of single to multiple flower buds of the selected plants were investigated. Before the flowers bloomed in 2019, 10 flowers were selected from each tree, and 10 anthers were collected from each flower to prepare a pollen suspension. One drop of the pollen suspension was placed on a hemocytometer (XB-K-25, Qiujing Biochemical Reagent Instrument Co., Ltd., Shanghai, China), and the pollen count was determined using an ordinary microscope (10× to 40× magnification). During the flowering period in 2019, 25 flowers were randomly selected, and the fertility of the stigma was determined using the diphenylamine peroxide method, 2 mm below the stigma. Sampling began on 4 March 2019, and the samples were fixed in FAA solution for sectioning and determination of spore development using a Leica microscope (SK150; MOTIC, Xiamen, China). The growth dynamics of the fruit were observed from 9 April 2019, and the longitudinal and transverse diameters of the fruit were measured once a week until maturation. From the fruit ripening period (29 May), the fruits were picked every 3 days, for a total of four periods (I period is 29 May, II period is 1 June, III period is 4 June, IV period is 7 June). Twenty fruits were randomly selected from each cultivar to determine fruit size and for observing fruit shape and color. The fruit hardness was measured using a GY-4 fruit hardness tester (GY-4; Top Instrument Co., Ltd., Hangzhou, China). Details of the method can be found in the citation. The soluble sugar content was determined by the anthrone method [28]. Starch content was determined by the iodometric method [29]. Coomassie brilliant blue method was used to determine the protein content [30,31]. The amino acid content was determined using the ninhydrin method [32]. The total soluble solids (TSS) was measured using a refractometer (PAL-1, ATAGO, Tokyo, Japan). The vitamin C content was measured using the 2,6-dichlorophenol-indoxyl method [33].

2.4. Determination and Bioinformatic Analysis of Auxin-Related Genes

2.4.1. Determination of Auxin Content

During the period of new shoot growth, healthy and disease-free new shoots from the east, west, south, north, inner, and outer parts of each peach tree were recorded. The fourth to sixth leaves below the growth point were collected in the morning from each shoot during the vigorous growth (I), slow growth (II), and growth stop (III) periods. The auxin content was determined using HPLC [34].

2.4.2. Identification of the YUCCA Gene Family

The YUCCA gene sequence of *Arabidopsis thaliana* was downloaded from TAIR (http://www.arabidopsis.org/index.jsp) as a reference sequence and compared with the reference genome of peach to identify members of the peach–YUCCA gene family. Protein composition and physicochemical properties were analyzed online using ExPaSy (http://www.expasy.org/) (Accessed: 17 June 2019). The YUCCA genes of *Arabidopsis thaliana* and peach were used to construct a phylogenetic tree (neighbor-joining) using MEGA 7.0, and the exons, introns, and conserved domains were predicted using GSDS (http://gsds.cbi.pku.edu.cn/) (Accessed: 21 July 2019). and MEME (http://meme-suite.org/tools/meme/) (Accessed: 13 July 2019).

2.4.3. Real-Time Fluorescent Quantitative PCR (qPCR)

The qPCR method [35] was used to determine gene expression levels. Primers were designed using Primer 5.0, as shown in Table 1, and the reference primer (actin) was adopted from Brandi et al. [36]. The qPCR reaction system is shown in Table 2.

Table 1. Primer sequences and target genes for qPCR analysis.

Gene	Forward Primer	Reverse Primer
PpYucca6	5′AGTTCTCCGTCCGTCCAT 3′	5′TCTTCTCGGCAACACCTC 3′
PpYucca5	5′AGGAGTGCCCTTTGTGGT 3′	5′TGGCGTATGATTCAAGGTAG 3′
PpYucca2	5′TTTTGAAGCGAAGATGGC 3′	5′CAGCAGCTAAACCAGAAGG 3′
Actin	5′GATTCCGGTGCCCAGAAGT 3′	5′CCAGCAGCTTCCATTCCAA 3′

Table 2. qPCR reaction system.

Reagent	25 μL System
2 × SYBR Green PCR mix	12.5 μL
Forward Primer (10 μM)	0.5 μL
Reverse primer (10 μM)	0.5 μL
Template DNA	5 μL
ddH_2O	6.5 μL
Total volume	25 μL

2.5. Statistical Analysis

Microsoft Office Excel 2019 and Origin 2022, DNAman, MEGA7, and Primer were used for data analysis and plotting. Significance testing was performed using SPSS software, and the data are presented as mean ± standard deviation.

3. Results

3.1. Effects of Cerasus humilis Rootstocks on Peach Tree Vigor

The effects of the *Cerasus humilis* rootstocks on peach tree vigor are shown in Table 3 and Figure 1. As shown in Table 3, compared with the control group, the height and secondary branch number of the one-year-old trees decreased by 1.14% and 3.79%, respectively. All other indicators increased, and the tertiary branch number and tertiary branch thickness increased the most, by 122.22% and 26.86%, respectively. Compared to R/M, there was

no growth in tertiary branches for R/O after two years, and the primary branch number and secondary branch thickness increased by 32.33% and 10.69%, respectively. All other indicators decreased, and tree height and secondary branch number decreased the most, by 16.35% and 34.94%, respectively. The number, length, and thickness of the branches decreased to varying degrees, which was consistent with the growth rate and volume of new shoots (Figure 1). The recorded phenology is presented in Table A1.

Table 3. Effects of *Cerasus humilis* rootstocks on peach tree growth parameters.

	Index	R/O	R/M
One-year-old	Height of tree (mm)	134.45 ± 2.55 a	136.00 ± 1.78 a
	Trunk thickness (mm)	20.13 ± 1.08 a	18.53 ± 1.23 a
	Number of primary branches	17.00 ± 0.71 a	17.67 ± 1.08 a
	Number of secondary branches	10.00 ± 0.82 a	4.50 ± 0.41 b
	Primary branch length (mm)	39.10 ± 3.58 a	38.46 ± 0.61 a
	Primary branch diameter (mm)	5.37 ± 0.46 a	4.72 ± 0.36 a
	Secondary branch length (mm)	16.58 ± 0.38 a	15.80 ± 0.65 a
	Secondary branch diameter (mm)	3.40 ± 0.70 a	2.68 ± 0.56 b
Two-year-old	Height of tree (mm)	238.67 ± 12.46 b	285.33 ± 6.79 a
	Trunk thickness (mm)	30.53 ± 1.79 a	35.49 ± 0.78 a
	Number of primary branches	43.67 ± 0.41 a	33.00 ± 0.82 a
	Number of secondary branches	43.33 ± 0.41 b	69.67 ± 2.68 a
	Number of tertiary branches	0.00 ± 0.00 b	21.00 ± 0.12 a
	Primary branch length (mm)	52.75 ± 0.55 b	54.04 ± 1.08 b
	Primary branch diameter (mm)	6.04 ± 0.16 a	6.34 ± 0.45 a
	Secondary branch length (mm)	25.01 ± 0.42 a	26.12 ± 0.86 a
	Secondary branch diameter (mm)	3.83 ± 0.15 a	3.46 ± 0.31 a
	Tertiary branch length (mm)	0.00 ± 0.00 b	8.25 ± 0.12 a
	Tertiary branch diameter (mm)	0.00 ± 0.00 b	1.39 ± 0.03 a

Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.; Means are separated by Duncan's multiple range test; different letters following the data stand for significant differences at $p = 0.05$.

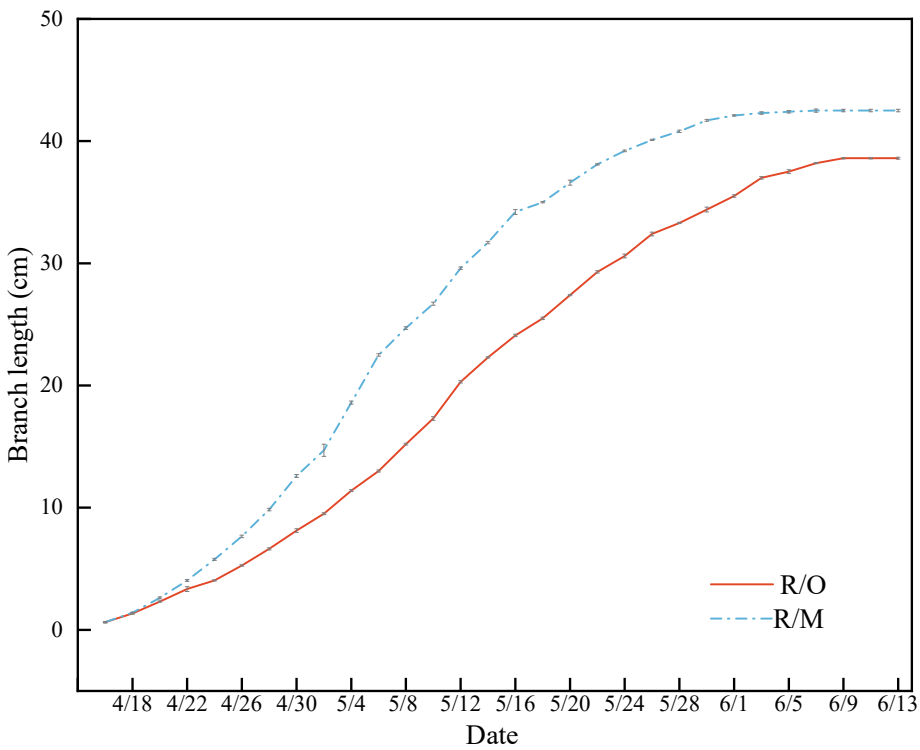

Figure 1. Dynamic determination of new shoot growth.

3.2. Result Characteristics

The effects of the *Cerasus humilis* rootstocks on flower development in peach trees are shown in Table 4. Compared to the control group, the number of flower buds in one-year-old trees increased by 100.31%, and the ratio of single flower buds to compound flower buds decreased by 2.04%. In two-year-old trees, the number of flower buds and the ratio of single flower buds to compound flower buds increased by 2.45% and 4.14%, respectively.

Table 4. Number of flowers and the ratio of single-flower buds to compound-flower buds.

Grafting Combinations	One-Year-Old		Two-Year-Old	
	Flower Buds	Single Bud/Compound-Flower Buds	Flower Buds	Single Bud/Compound-Flower Buds
R/O	111.33 ± 1.43 a	1.44 ± 0.01 a	979.00 ± 2.55 a	1.51 ± 0.01 a
R/M	55.58 ± 0.86 b	1.47 ± 0.01 a	955.58 ± 0.83 a	1.45 ± 0.01 a

Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.; Means are separated by Duncan's multiple range test; different letters following the data stand for significant differences at $p = 0.05$.

During the sampling period, most spores in the peach combinations were in the tetrad stage. As the flower buds grew and developed, the developmental progress of spores in the grafted peach combinations varied. The developmental progress of R/O compared to R/M was one period ahead. R/O entered the early uninucleate stage on 9 March and the binucleate stage on 24 March, whereas R/M only entered the early uninucleate stage on 14 March and the marginal uninucleate stage on 24 March (Table 5 and Figure 2).

Table 5. Developmental progress of spores in different peach combinations.

Grafting Combinations	4 March	9 March	14 March	19 March	24 March
R/O	Tetrad stage	Early-uninucleate stage	Mid-uninucleate stage	Late-uninucleate stage	Binucleate stage
R/M	Tetrad stage	Tetrad stage	Early-uninucleate stage	Mid-uninucleate stage	Late-uninucleate stage

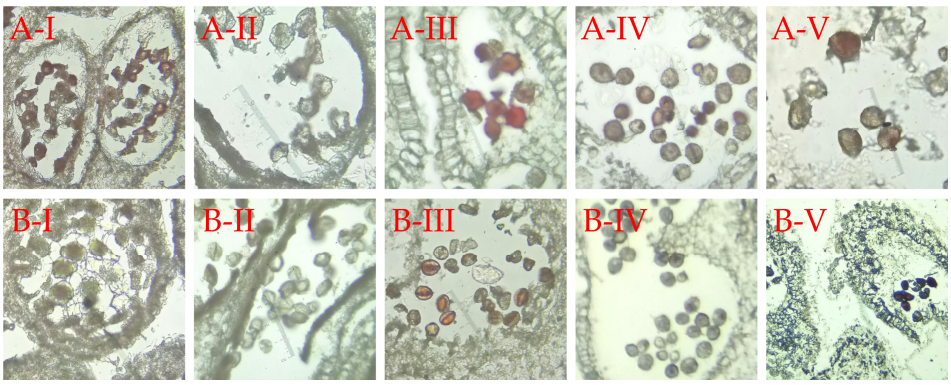

Figure 2. Development of spores in different peach combinations. Note: (**I**): 4 March, (**II**): 9 March, (**III**): 14 March, (**IV**): 19 March, (**V**): 24 March, (**A**): R/O, (**B**): R/M.

Compared to R/M, the pollen quantity of R/O significantly increased by 28.10%, whereas the difference in stigma receptivity, although increasing by 2.71%, was not significant (Table 6).

Table 6. Stigma receptivity and pollen quantity in different grafted peach combinations.

Grafting Combinations	Stigma Receptivity	Pollen Quantity
R/O	86.77 ± 0.24 a	2370.00 ± 17.49 a
R/M	84.42 ± 0.38 a	1704.00 ± 8.74 b

Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.; Means are separated by Duncan's multiple range test; different letters following the data stand for significant differences at $p = 0.05$.

The effects of the *Cerasus humilis* rootstocks on peach fruit growth dynamics are shown in Figure 3. During the fruit growth period, the longitudinal and transverse diameters of the grafted peach combinations showed a continuously increasing trend. Compared to R/M, the longitudinal and transverse diameters of R/O were smaller from 30 April to 21 May, but the growth rates of both diameters increased from 14 May to 21 May.

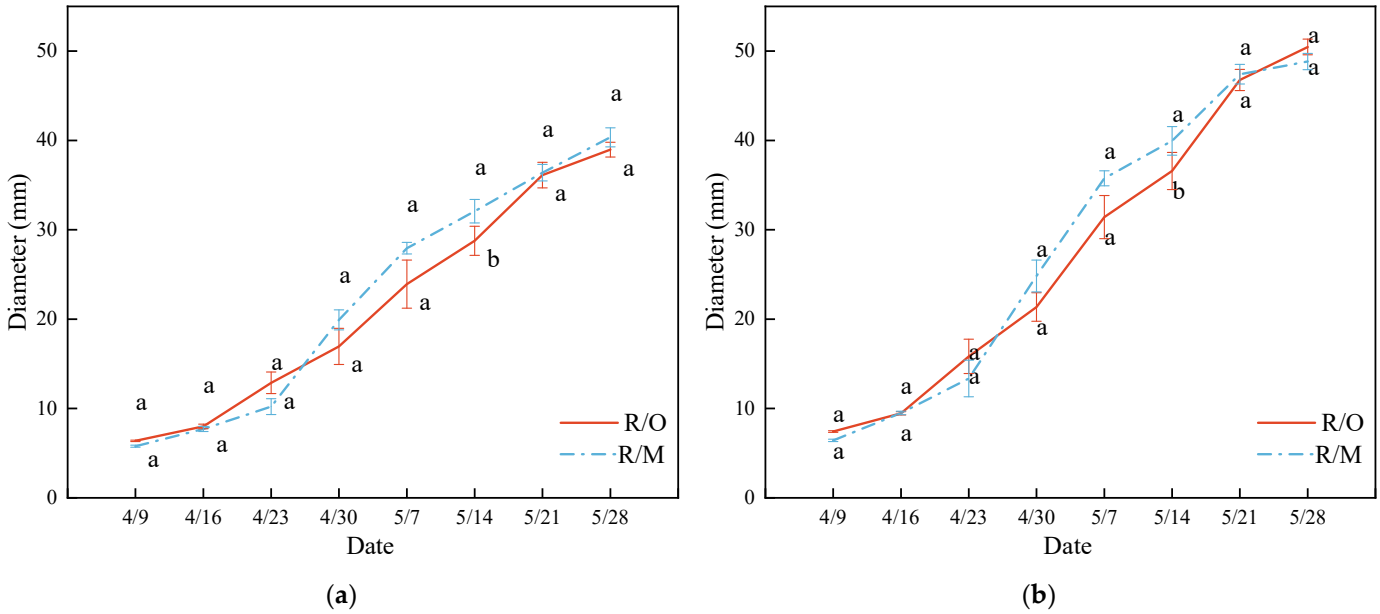

Figure 3. Comparison of longitudinal and transverse diameters of fruit during the fruit growth period for grafted peach combinations. (**a**) Longitudinal diameter. (**b**) Transverse diameter. Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.; Means are separated by Duncan's multiple range test; different letters following the data stand for significant differences at $p = 0.05$.

As shown in Figure 4, the Ruipan 4 peach continued to grow during fruit ripening, and the transverse diameter was greater than the longitudinal diameter. Compared to R/M, the longitudinal and transverse diameters of R/O decreased in all four stages, with a decrease in longitudinal diameters by 2.42%, 1.02%, 0.00%, and 1.04%, respectively, and transverse diameters by 1.52%, 1.95%, 1.87%, and 1.88%, respectively.

As shown in Figure 5, the single fruit weights of R/O and R/M decreased by 16.03%, 8.93%, 8.68%, and 8.68%, respectively, during the four stages, but the differences were not significant. The hardness of the fruits at different ripening stages varied. The hardness of the grafted fruit combinations initially increased and then decreased. Compared to R/M, the fruit hardness of R/O increased significantly by 32.44%, 30.14%, 48.00%, and 54.66% in the four stages, indicating that the *Cerasus humilis* rootstock was effective. The TSS of the grafted fruit combinations showed an increasing trend, followed by a decreasing trend. Compared to R/M, the TSS of R/O decreased by 17.60% in stage I and increased by 5.20%, 12.14%, and 2.05% in the other three stages.

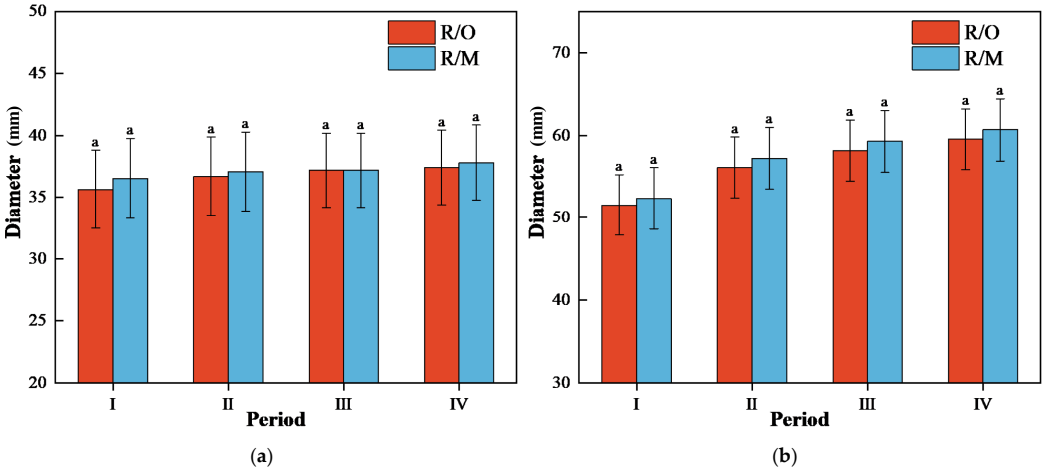

Figure 4. Comparison of different fruit-ripening stages for the grafted peach combinations. (**a**) Longitudinal diameter. (**b**) Transverse diameter. Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.; Means are separated by Duncan's multiple range test; different letters following the data stand for significant differences at $p = 0.05$; I period is 29 May, II period is 1 June, III period is 4 June, IV period is 7 June.

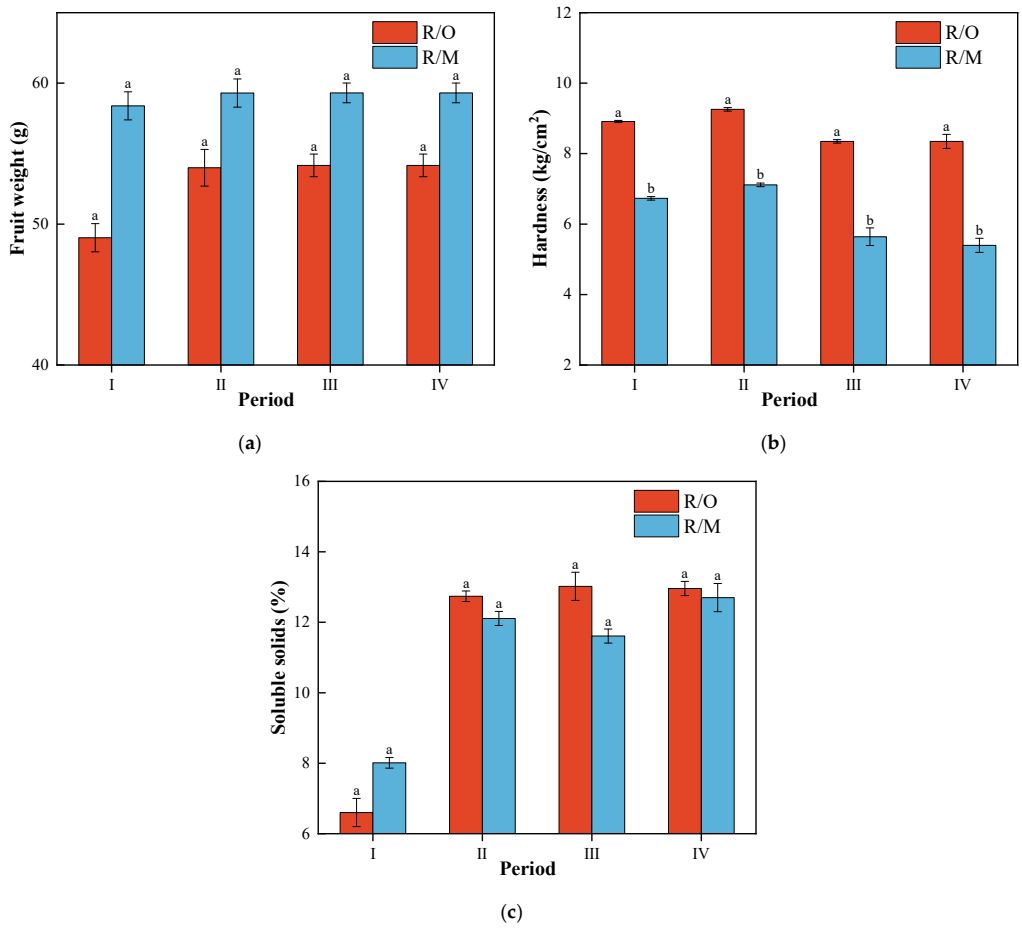

Figure 5. Comparison of single-fruit weight (**a**), hardness (**b**), and soluble solids (**c**) of fruits at different maturity stages. Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.; Means are separated by Duncan's multiple range test; different letters following the data stand for significant differences at $p = 0.05$; I period is 29 May, II period is 1 June, III period is 4 June, IV period is 7 June.

As shown in Figure 6, the trends in soluble sugar and starch contents during fruit ripening were the opposite. Compared to R/M, the soluble sugar content of R/O decreased significantly by 21.29% in the first stage and by 7.92%, 1.93%, and 3.11% in the remaining three stages. The starch content increased significantly by 121.54%, 52.93%, 221.10%, and 205.23% in the four stages. The soluble protein content decreased by 13.97% and 15.89% in stage I and II, respectively, and increased by 97.22% and 14.81% in stage III and IV, respectively. When the rootstock was *Cerasus humilis*, the vitamin C content increased and then decreased. Compared to R/M, the vitamin C content of R/O increased significantly by 6.14% in stage III and decreased by 3.74%, 4.38%, and 13.40% in stages I, II, and IV, respectively (Figure 6d).

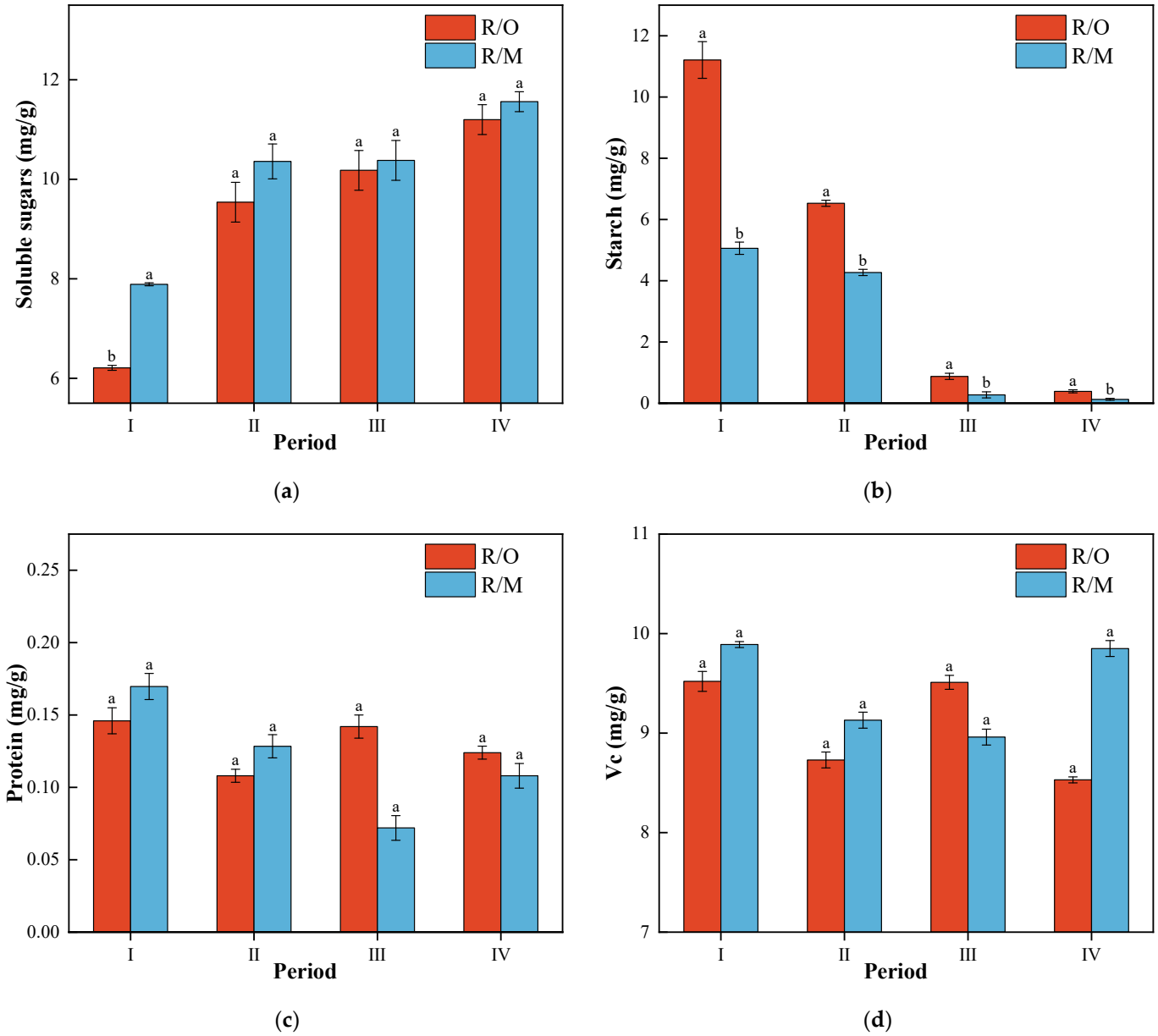

Figure 6. Comparison of soluble sugars (**a**), starch (**b**), protein (**c**), and vitamin C (**d**) in fruits of different maturity levels. Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.; Means are separated by Duncan's multiple range test; different letters following the data stand for significant differences at $p = 0.05$; I period is 29 May, II period is 1 June, III period is 4 June, IV period is 7 June.

3.3. Effects of Cerasus humilis Rootstock on Peach Auxin-Related Synthesis Genes

3.3.1. Identification and Structural Analysis of the YUCCA Gene Family Related to Auxin Synthesis

As shown in Table 7, eight YUCCA genes were identified in peaches. The numbers of amino acids in these eight YUCCA genes range from 71 to 421, the molecular weight ranges from 7.79 kDa to 46.29 kDa, the theoretical isoelectric point ranges from 5.18 to 10.10, and the protein hydrophobicity ranges from −0.476 to 0.068.

Table 7. Basic information on the YUCCA gene families.

Reference Genome ID	Gene Name	Number of Amino Acids/Number	Molecular	Theoretical Isoelectric Point	Protein Hydrophobicity
C12342.graph-c0	*PpYucca6*	421	46.29	9.12	−0.135
C4270.graph-c0	*PpYucca3a*	144	15.93	10.10	−0.476
C264.graph-c0	*PpYucca4*	170	18.75	8.62	0.068
C264.graph-c1	*PpYucca1*	71	7.79	5.18	−0.342
C14832.graph-co	*PpYucca3b*	216	24.58	5.54	−0.246
C23148.graph-c0	*PpYucca10*	372	40.25	8.47	−0.363
C14564.graph-co	*PpYucca5*	400	44.50	8.68	−0.083
C18467.graph-c0	*PpYucca2*	400	44.77	8.87	−0.038

Cluster analysis of eight Yucca genes and eleven Yucca genes in *Arabidopsis thaliana* (Figure 7) showed that these genes can be divided into two main groups: *AtYucca10*, *AtYucca11*, and *PpYucca10* belong to one group, whereas the other genes belong to the other group. Analysis of the exon and intron structures of the eight *PpYucca* genes using the online GSDS tool (Figure 8) revealed that *PpYucca1*, *PpYucca3*, *PpYucca4*, *PpYucca5*, and *PpYucca6* have two exons, whereas *PpYucca10*, *PpYucca2*, and *PpYucca3b* have one exon. *PpYucca1* contained only one intron.

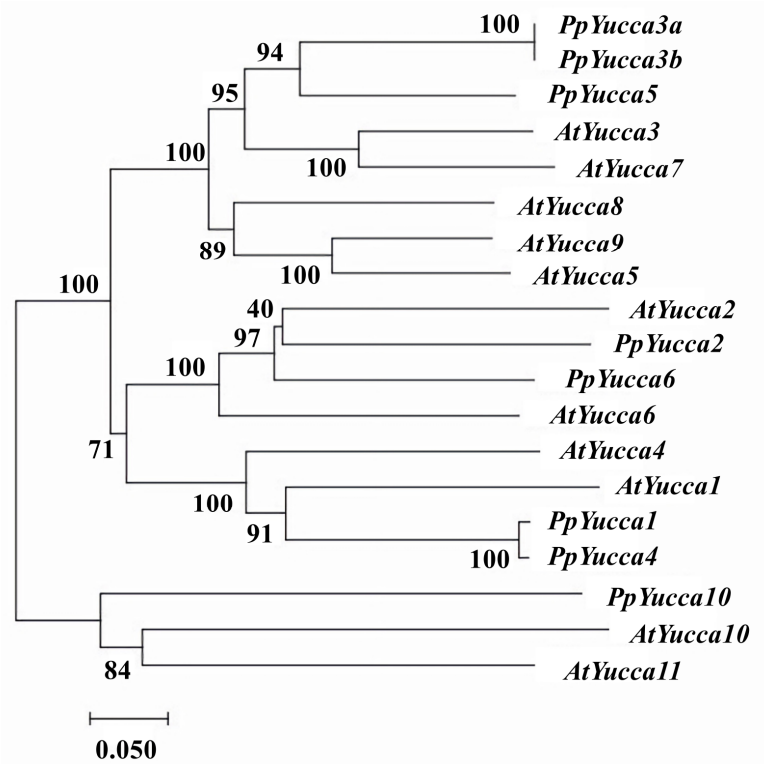

Figure 7. Phylogenetic trees of eight Yucca genes in *Cerasus humilis* and eleven Yucca genes in *Arabidopsis thaliana*.

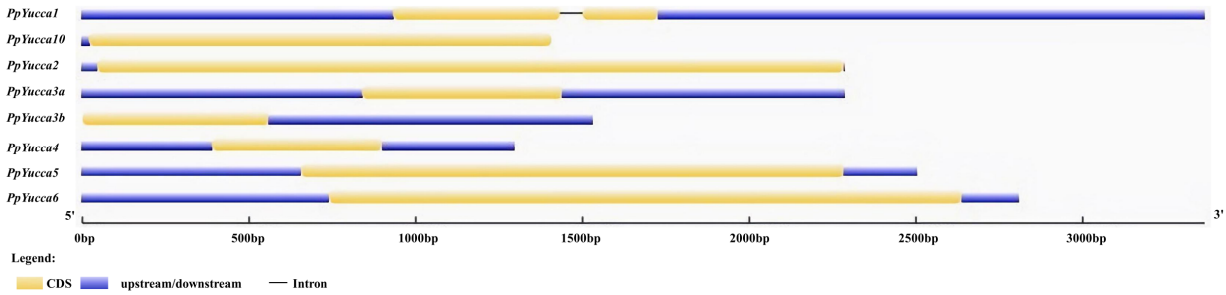

Figure 8. Exon and intron structure of the YUCCA gene family in each peach grafting combination.

Ten conserved motifs in the R/O Yucca protein were predicted using the MEME website, and the amino acid sequences of these motifs are shown in Table 8. Figure 9 shows the distribution of these ten conserved motifs in the eight Yucca genes.

Table 8. Amino acid sequences of ten conserved motifs in R/O YUCCA proteins.

Order Number	Motif of Conserved Proteins
1	ERANCIASLWQKKTYDRLKLHLPKQFCZLPLMPFPEDFPEYPTKQQFIDY
2	EVEYICRWLIVATGENAEPVVPEFEGLEEFGGPILHTSSYKSG
3	FRGKKVLVVGCGNSGMEVSLDLCNHNASPSLVVRDSVHVLPREMFGKSTF
4	DFFSKDGLPKKPFPNGWKGECGLYAVGFTRRGLLGASLDAM
5	KIKSGDIKVVPGIKRFKHGAVEFIDGKTLDFDAIILATGYRSNVPSWLKE
6	LKWLPIRLVDKLLLLVSRLILGNTEQLGLNRPKVGPLELKNMTGKTPVLD
7	RRCIFVPGPVIVGAGPSGLATAACLKEKGVPFVIL
8	YAEHFDIKPKFNETVQSARYDETFGFWRV
9	YNGHCVENIPQM
10	DIEKCWKEEAKQC

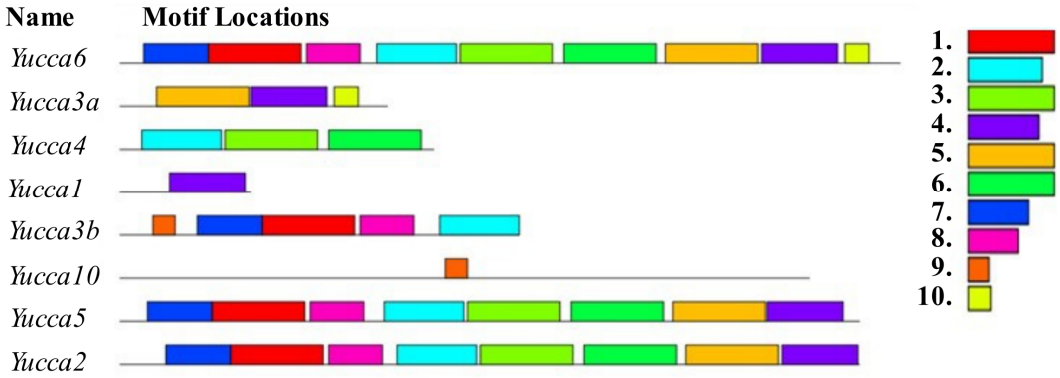

Figure 9. Conserved motif distribution of the R/O YUCCA protein.

3.3.2. Effect of *Cerasus humilis* Rootstock on Differential Gene Expression in Peach at Different Stages

The growth hormone content of the two grafted peach combinations showed a consistent decreasing trend during these three periods, with the highest hormone content observed during the vigorous growth period of the new shoots. Compared to *Amygdalus persica* L., *Cerasus humilis* rootstock significantly reduced IAA content by 9.55%, 19.44%, and 32.35%, respectively (Figure 10a).

Transcriptome sequencing results indicated that only three genes in the YUCCA gene family, *Yucca2*, *Yucca5*, and *Yucca6*, were differentially expressed in Phase I. Among them, *Yucca5* and *Yucca6* were upregulated and *Yucca2* was downregulated. As a result, these three genes were selected for qPCR analysis. Regardless of the use of peach or *Cerasus humilis* rootstocks, both graft combinations showed a trend of initially increasing and then decreasing *PpYucca6* expression, and the expression level reached its maximum in Phase II.

PpYucca6 in the *Cerasus humilis* rootstock increased by 6.48% in Phase II, decreased by 0.99% in Phase I, and decreased by 1.28% in Phase III (Figure 10b). The expression level of the *PpYucca5* gene in both graft combinations showed an initial increasing trend followed by a decreasing trend. Compared to the peach rootstock, the expression level of *PpYucca5* in the *Cerasus humilis* rootstock significantly increased by 98.51% and 99.05% in Phases I and II, respectively, and by 22.92% in Phase III (Figure 10c). The expression level of the *PpYucca2* gene in both graft combinations showed a trend of initially decreasing and then increasing. Compared to the peach rootstock, the expression level of *PpYucca2* in the *Cerasus humilis* rootstock significantly increased by 87.26%, 73.92%, and 83.83% in Phases I, II, and III, respectively (Figure 10d).

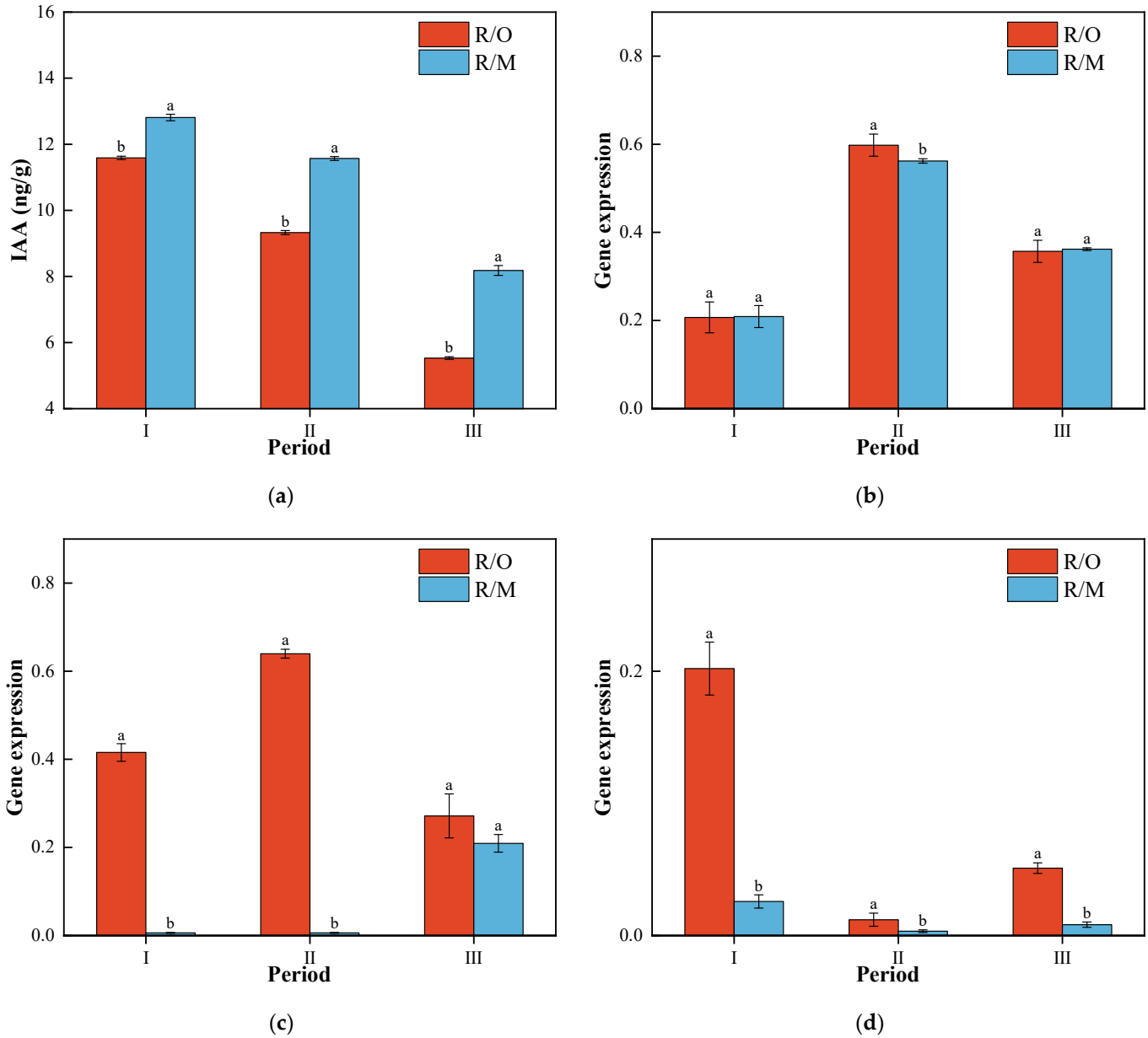

Figure 10. Comparison of IAA production and differential gene expression of Yucca in the grafted peach combination. (**a**) IAA production. (**b**) Expression of *PpYucca6* gene. (**c**) Expression of *PpYucca5* gene. (**d**) Expression of *PpYucca2* gene. Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.; Means are separated by Duncan's multiple range test; different letters following the data stand for significant differences at $p = 0.05$; I period is 29 May, II period is 1 June, III period is 4 June, IV period is 7 June.

4. Discussion

Dwarfing rootstocks can increase productivity and improve fruit quality by reducing tree vigor [37,38]. This study aimed to investigate the effects of *Cerasus humilis* rootstocks on the growth, development, and fruit quality of peach trees, as well as to explore the underlying mechanism of tree dwarfing. The results showed that indicators such as shoot growth, branch length, and trunk thickness can be used to reflect the effects of tree dwarfing [39]. Rootstock dwarfing significantly reduces fruit tree height and diameter [40]. The experiment measured various indicators of tree growth, including tree height, trunk thickness, number of branches, length and thickness of branches, and changes in shoot length and thickness during the new shoot growth period. The results revealed that the use of *Cerasus humilis* rootstocks caused dwarfing of peach trees, with reduced branch and shoot growth, as well as shortened shoot length. Moreover, these effects were found to be more pronounced with increasing tree age and a decrease in the degree of branching. Dziedzic et al. [41] assessed the quality and quantity of pollen grains affected by different sweet cherry rootstocks, and the effects were inseparable from the rootstock. Compared to R/M, the pollen amount of R/O increased significantly, while the stigma receptivity increased, but the difference was not significant. Tosun and Koyuncu [42] also came to a similar conclusion by observing the pollination characteristics of eight cherry rootstocks.

The growth and development of fruit both have a direct impact on its quality, as reflected in changes in volume and weight [43]. Key indicators of fruit quality include its appearance, which is closely tied to its shape and weight, as represented by longitudinal and transverse diameters as well as the weight of individual fruit. Additionally, fruit hardness is an important factor in determining its storability, while the nutritional quality of the fruit is largely determined by its content of soluble sugars and vitamin C [44]. Palliotti et al. [45] examined how the implementation of new rootstocks (5489, 5512, and 6262) from the Merbein series affected the production of Australian Shiraz grapes. The study revealed that the utilization of these rootstocks brought about a decreased pruning weight and reduced levels of pH and TSS in the berry juice in comparison with traditional rootstocks. The choice of rootstock can significantly affect the vigor, yield, and nutritional quality of the fruit in crops [46]. In this study, changes in the longitudinal and transverse diameters of the fruit during growth and development were measured, along with fruit quality at different stages of maturity. The results indicate that the use of *Cerasus humilis* rootstocks did not significantly reduce the longitudinal and transverse diameters or weight of the fruit. Moreover, there was no significant effect on the nutritional quality of the fruit or its longitudinal and transverse diameters at different stages of maturity. However, in the later stages of fruit maturity, the *Cerasus humilis* rootstock was found to promote protein accumulation and result in significantly higher fruit hardness than the peach rootstock, which could improve fruit storage and transportation [47].

Auxin plays an important role in the growth and development of plants and is closely related to the dwarfing effect and auxin content of rootstocks [48,49]. Soumelidou et al. [50] found that the polar transport of auxin in dwarfing rootstocks was weaker than that in non-dwarfing rootstocks. Sorce et al. [4] reported that the IAA content of the root exudates of non-grafted trees was higher than that of the dwarfing rootstock. In this experiment, it was found that the IAA content was highest during the period of vigorous growth of new shoots and lowest during the period of cessation of the growth of new shoots. In this study, the IAA content of the *Cerasus humilis* rootstock was compared to that of the peach rootstock during three different periods of growth and development. While no significant differences were observed between the two rootstocks, the content of IAA in the *Cerasus humilis* rootstock decreased to varying degrees, indicating that its use resulted in a reduction in auxin content and subsequent dwarfing of the tree. The tryptophan pathway is the primary pathway for auxin synthesis, with YUCCA serving as the rate-limiting enzyme in this process. Previous studies have demonstrated that plants overexpressing YUCCA exhibit increased auxin content, greater plant height, and reduced branching [51,52]. By better understanding the underlying mechanisms of dwarfing, researchers can develop new

strategies for improving fruit production and enhancing the sustainability of horticultural systems. Zhang et al. [53] studied the function of the YUCCA gene family in weeping peach trees and found that nine *PpYUCCAs* were expressed in weeping peach trees, which may lead to auxin accumulation. However, the functions of these genes require further investigation. In this study, we found that the *Cerasus humilis* rootstock reduced the auxin content of peach trees. Among them, the *PpYucca1* gene had the smallest number of amino acids, the lowest molecular weight, and the lowest theoretical isoelectric point (71, 7.79 kDa, and 5.18, respectively). The *PpYucca6* gene has the largest number of amino acids (421) and the highest molecular weight (46.29 kDa). The theoretical isoelectric point of the *PpYucca3a* gene was the highest, at 10.10, and only the protein hydrophobicity of the *PpYucca4* gene was greater than 0, whereas the hydrophobicity of the other genes was less than 0. Subcellular localization prediction showed that YUCCA genes were located in the cell nucleus. *PpYucca6* had the most conserved motifs (nine), whereas *PpYucca10* and *PpYucca1* had the least. The specific biological function of these motifs requires further investigation. In comparison to the peach rootstock, the expression levels of growth-related genes *PpYucca5* and *PpYucca2* were found to be upregulated in the *Cerasus humilis* rootstock, while the expression level of *PpYucca6* was initially downregulated during early shoot growth and then upregulated during middle-stage shoot growth. These results suggest that the *Cerasus humilis* rootstock regulates multiple genes to induce dwarfing in the tree, underscoring the complex interplay between gene expression and tree growth.

5. Conclusions

The *Cerasus humilis* rootstock had a dwarfing effect and a minor impact on fruit quality. By regulating multiple genes to reduce auxin content, *Cerasus humilis* rootstocks have the potential to be a useful dwarfing rootstock for peach trees. This study explored the dwarfing mechanism of peach/*Cerasus humilis* in terms of growth habits and synthesis-related genes of auxin. However, the relationship between the entire YUCCA gene family of auxin synthesis-related genes and dwarfing has not been studied thoroughly. In future studies, we plan to validate the function of related genes and explore the role of other hormones in the dwarfing mechanism of peach/*Cerasus humilis* rootstocks. We also aim to investigate the potential of the *Cerasus humilis* rootstock for commercial peach production through field trials to evaluate its performance under different environmental conditions and its impact on fruit yield and quality.

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Appendix A

Table A1. Determinations on two grafted peach compounds.

Index	R/O	R/M
The initiation period/(month/day)	7 March	7 March
Dew red phase/(month/day)	19 March	13 March
At the beginning of flowering/(month/day)	25 March	21 March
Summer season/(month/day)	27 March	23 March
Late flowering/(month/day)	31 March	28 March
Leaf spreading period/(month/day)	28 March	24 March
Long-term shoot growth/(month/day)	10 April–24 July	10 April–7 August
The period of fruit enlargement/(month/day)	16 April–28 May	16 April–28 May
Fructescence/(month/day)	29 May–9 June	29 May–7 June
Leaf fall period/(month/day)	28 October	28 October
Period of dormancy/(month/day)	28 November	28 November

Note: R/O: Ruipan 4/*Cerasus humilis*; RM: Ruipan 4/*Amygdalus persica* L.

References

1. Li, A.; Zhao, J.; Xi, J.; Yang, X.; Jin, X.; Chen, Q.; Pan, L. Geographical authentication of peach in China based on stable isotope combined with multielement analysis of peach juice. *Food Control* **2021**, *127*, 108126. [CrossRef]
2. Jiao, W.; Shu, C.; Li, X.; Cao, J.; Fan, X.; Jiang, W. Preparation of a chitosan-chlorogenic acid conjugate and its application as edible coating in postharvest preservation of peach fruit. *Postharvest Biol. Technol.* **2019**, *154*, 129–136. [CrossRef]
3. Conejero, A.; Romero, C.; Cunill, M.; Mestre, M.A.; Martínez-Calvo, J.; Badenes, M.L.; Llácer, G. In vitro shoot-tip grafting for safe *Prunus* budwood exchange. *Sci. Hortic.* **2013**, *150*, 365–370. [CrossRef]
4. Sorce, C.; Massai, R.; Picciarelli, P.; Lorenzi, R. Hormonal relationships in xylem sap of grafted and ungrafted *Prunus* rootstocks. *Sci. Hortic.* **2002**, *93*, 333–342. [CrossRef]
5. Menzel, C.M.; Le Lagadec, M.D. Increasing the productivity of avocado orchards using high-density plantings: A review. *Sci. Hortic.* **2014**, *177*, 21–36. [CrossRef]
6. Manganaris, G.A.; Minas, I.; Cirilli, M.; Torres, R.; Bassi, D.; Costa, G. Peach for the future: A specialty crop revisited. *Sci. Hortic.* **2022**, *305*, 111390. [CrossRef]
7. Gjamovski, V.; Kiprijanovski, M. Influence of nine dwarfing apple rootstocks on vigour and productivity of apple cultivar 'Granny Smith'. *Sci. Hortic.* **2011**, *129*, 742–746. [CrossRef]
8. Anthony, B.M.; Minas, I.S. Optimizing Peach Tree Canopy Architecture for Efficient Light Use, Increased Productivity and Improved Fruit Quality. *Agronomy* **2021**, *11*, 1961. [CrossRef]
9. Anthony, B.M.; Minas, I.S. Redefining the impact of preharvest factors on peach fruit quality development and metabolism: A review. *Sci. Hortic.* **2022**, *297*, 110919. [CrossRef]
10. Ben Yahmed, J.; Ghrab, M.; Moreno, M.Á.; Pinochet, J.; Ben Mimoun, M. Leaf mineral nutrition and tree vigor of 'Subirana' flat peach cultivar grafted on different *Prunus* rootstocks in a warm Mediterranean area. *J. Plant Nutr.* **2020**, *43*, 811–822. [CrossRef]
11. Zhang, Q.; Gu, D. Genetic Relationships among 10 *Prunus* Rootstock Species from China, Based on Simple Sequence Repeat Markers. *J. Am. Soc. Hortic. Sci.* **2016**, *141*, 520–526. [CrossRef]
12. Fang, S.; Yao, J.; Li, Y.; Zhu, S.; Pan, J.; Li, Q.; Wang, W.; Kong, J.; He, L.; Zhang, Y.; et al. Fine mapping and characterization of the *Crinkled Dwarf* gene in cotton. *Ind. Crops Prod.* **2022**, *184*, 115034. [CrossRef]
13. Noda, K.; Okuda, H.; Iwagaki, I. Indole acetic acid and abscisic acid levels in new shoots and fibrous roots of citrus scion-rootstock combinations. *Sci. Hortic.* **2000**, *84*, 245–254. [CrossRef]
14. Artlip, T.S.; Wisniewski, M.E.; Arora, R.; Norelli, J.L. An apple rootstock overexpressing a peach CBF gene alters growth and flowering in the scion but does not impact cold hardiness or dormancy. *Hortic. Res.* **2016**, *3*, 16006. [CrossRef]
15. Basile, B.; Marsal, J.; DeJong, T.M. Daily shoot extension growth of peach trees growing on rootstocks that reduce scion growth is related to daily dynamics of stem water potential. *Tree Physiol.* **2003**, *23*, 695–704. [CrossRef]
16. Continella, A.; Pannitteri, C.; La Malfa, S.; Legua, P.; Distefano, G.; Nicolosi, E.; Gentile, A. Influence of different rootstocks on yield precocity and fruit quality of 'Tarocco Scirè' pigmented sweet orange. *Sci. Hortic.* **2018**, *230*, 62–67. [CrossRef]
17. Sirgedaitė-Šėžienė, V.; Laužikė, K.; Uselis, N.; Samuolienė, G. Metabolic Response of *Malus domestica* Borkh cv. Rubin Apple to Canopy Training Treatments in Intensive Orchards. *Horticulturae* **2022**, *8*, 300. [CrossRef]
18. Hayat, F.; Li, J.; Iqbal, S.; Khan, U.; Ali, N.A.; Peng, Y.; Hong, L.; Asghar, S.; Javed, H.U.; Li, C.; et al. Hormonal Interactions Underlying Rootstock-Induced Vigor Control in Horticultural Crops. *Appl. Sci.* **2023**, *13*, 1237. [CrossRef]
19. Hong, H.R.; Oh, E.U.; Han, S.G.; Yun, S.H.; Kim, H.B.; Song, K.J. Characterization of Soluble Sugar Content, Related Enzyme Activity and Gene Expression in the Fruits of 'Minihyang' Mandarin on Different Rootstocks. *Horticulturae* **2022**, *8*, 47. [CrossRef]

20. Yakushiji, H.; Sugiura, H.; Yamasaki, A.; Azuma, A.; Koshita, Y. Tree growth, productivity, and fruit quality of 'Fuyu' persimmon trees onto different dwarfing rootstocks. *Sci. Hortic.* **2021**, *278*, 109869. [CrossRef]
21. Zhao, Y. Auxin Biosynthesis and Its Role in Plant Development. *Annu. Rev. Plant Biol.* **2010**, *61*, 49–64. [CrossRef]
22. Quint, M.; Gray, W.M. Auxin signaling. *Curr. Opin. Plant Biol.* **2006**, *9*, 448–453. [CrossRef]
23. Tao, Y.; Ferrer, J.-L.; Ljung, K.; Pojer, F.; Hong, F.; Long, J.A.; Li, L.; Moreno, J.E.; Bowman, M.E.; Ivans, L.J.; et al. Rapid Synthesis of Auxin via a New Tryptophan-Dependent Pathway Is Required for Shade Avoidance in Plants. *Cell* **2008**, *133*, 164–176. [CrossRef]
24. Lee, M.; Jung, J.-H.; Han, D.-Y.; Seo, P.J.; Park, W.J.; Park, C.-M. Activation of a flavin monooxygenase gene YUCCA7 enhances drought resistance in *Arabidopsis*. *Planta* **2012**, *235*, 923–938. [CrossRef]
25. Lee, H.-J.; Jung, J.-H.; Llorca, L.; Kim, S.-G.; Lee, S.; Baldwin, I.; Park, C.-M. FCA mediates thermal adaptation of stem growth by attenuating auxin action in *Arabidopsis*. *Nat. Commun.* **2014**, *5*, 5473. [CrossRef]
26. Kim, J.I.; Baek, D.; Park, H.C.; Chun, H.J.; Oh, D.-H.; Lee, M.K.; Cha, J.-Y.; Kim, W.-Y.; Kim, M.C.; Chung, W.S.; et al. Overexpression of *Arabidopsis YUCCA6* in Potato Results in High-Auxin Developmental Phenotypes and Enhanced Resistance to Water Deficit. *Mol. Plant* **2013**, *6*, 337–349. [CrossRef]
27. Knäbel, M.; Friend, A.P.; Palmer, J.W.; Diack, R.; Wiedow, C.; Alspach, P.; Deng, C.; Gardiner, S.E.; Tustin, D.S.; Schaffer, R. Genetic control of pear rootstock-induced dwarfing and precocity is linked to a chromosomal region syntenic to the apple *Dw1* loci. *BMC Plant Biol.* **2015**, *15*, 230. [CrossRef]
28. Leng, F.; Sun, S.; Jing, Y.; Wang, F.; Wei, Q.; Wang, X.; Zhu, X. A rapid and sensitive method for determination of trace amounts of glucose by anthrone-sulfuric acid method. *Bulg. Chem. Commun.* **2016**, *48*, 109–113.
29. Cole, M.; Eggleston, G.; Gilbert, A.; Rose, I.; Andrzejewski, B.; St Cyr, E.; Stewart, D. The presence and implication of soluble, swollen, and insoluble starch at the sugarcane factory and refinery. *Int. Sugar J.* **2013**, *115*, 844–851.
30. Wang, X.C.; Li, X.F.; Li, Y.X. A modified Coomassie Brilliant Blue staining method at nanogram sensitivity compatible with proteomic analysis. *Biotechnol. Lett.* **2007**, *29*, 1599–1603. [CrossRef]
31. Sharma, A.K.; Dhiman, A.; Nayak, A.K.; Mishra, R.; Agrawal, G. Environmentally benign approach for the efficient sequestration of methylene blue and coomassie brilliant blue using graphene oxide emended gelatin/κ-carrageenan hydrogels. *Int. J. Biol. Macromol.* **2022**, *219*, 353–365. [CrossRef]
32. Sun, S.-W.; Lin, Y.-C.; Weng, Y.-M.; Chen, M.-J. Efficiency improvements on ninhydrin method for amino acid quantification. *J. Food Compos. Anal.* **2006**, *19*, 112–117. [CrossRef]
33. Nielsen, S.S. Vitamin C Determination by Indophenol Method. In *Food Analysis Laboratory Manual*; Nielsen, S.S., Ed.; Springer International Publishing: Cham, Switzerland, 2017; pp. 143–146. [CrossRef]
34. Dobrev, P.I.; Havlíček, L.; Vágner, M.; Malbeck, J.; Kamínek, M. Purification and determination of plant hormones auxin and abscisic acid using solid phase extraction and two-dimensional high performance liquid chromatography. *J. Chromatogr. A* **2005**, *1075*, 159–166. [CrossRef]
35. Bai, L.; Zhu, H.; Shi, Y.; Li, Y.; Miao, Y.; Yu, X.; Zhang, Y.; Li, Y. Antisense Overexpression of Gγ Subunit CsGG3.1-2 Reduces Soluble Sugar Content and Chilling Tolerance in Cucumber. *Horticulturae* **2023**, *9*, 240. [CrossRef]
36. Brandi, F.; Bar, E.; Mourgues, F.; Horváth, G.; Turcsi, E.; Giuliano, G.; Liverani, A.; Tartarini, S.; Lewinsohn, E.; Rosati, C. Study of 'Redhaven' peach and its white-fleshed mutant suggests a key role of CCD4 carotenoid dioxygenase in carotenoid and norisoprenoid volatile metabolism. *BMC Plant Biol.* **2011**, *11*, 24. [CrossRef]
37. Bruckner, C.H.; DeJong, T.M. Proposed pre-selection method for identification of dwarfing peach rootstocks based on rapid shoot xylem vessel analysis. *Sci. Hortic.* **2014**, *165*, 404–409. [CrossRef]
38. Forner-Giner, M.A.; Rodriguez-Gamir, J.; Martinez-Alcantara, B.; Quiñones, A.; Iglesias, D.J.; Primo-Millo, E.; Forner, J. Performance of Navel orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418). *Sci. Hortic.* **2014**, *179*, 376–387. [CrossRef]
39. Jupa, R.; Mészáros, M.; Hoch, G.; Plavcová, L. Trunk radial growth, water and carbon relations of mature apple trees on two size-controlling rootstocks during severe summer drought. *Tree Physiol.* **2022**, *42*, 289–303. [CrossRef]
40. Li, Q.; Gao, Y.; Wang, K.; Feng, J.; Sun, S.; Lu, X.; Liu, Z.; Zhao, D.; Li, L.; Wang, D. Transcriptome Analysis of the Effects of Grafting Interstocks on Apple Rootstocks and Scions. *Int. J. Mol. Sci.* **2023**, *24*, 807. [CrossRef]
41. Dziedzic, E.; Bieniasz, M.; Kowalczyk, B. Morphological and physiological features of sweet cherry floral organ affecting the potential fruit crop in relation to the rootstock. *Sci. Hortic.* **2019**, *251*, 127–135. [CrossRef]
42. Tosun, F.; Koyuncu, F. Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: Pollen performance tests, germination tests, germination procedures, in vitro and in vivo pollinations. *Hortic. Sci.* **2007**, *34*, 47–53. [CrossRef]
43. Larson, J.E.; Perkins-Veazie, P.; Ma, G.; Kon, T.M. Quantification and Prediction with Near Infrared Spectroscopy of Carbohydrates throughout Apple Fruit Development. *Horticulturae* **2023**, *9*, 279. [CrossRef]
44. Gao, S.; Xu, J.-h. Hyperspectral image information fusion-based detection of soluble solids content in red globe grapes. *Comput. Electron. Agric.* **2022**, *196*, 106822. [CrossRef]
45. Palliotti, A.; Tombesi, S.; Silvestroni, O.; Lanari, V.; Gatti, M.; Poni, S. Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: A review. *Sci. Hortic.* **2014**, *178*, 43–54. [CrossRef]
46. Minas, I.S.; Anthony, B.M.; Pieper, J.R.; Sterle, D.G. Large-scale and accurate non-destructive visual to near infrared spectroscopy-based assessment of the effect of rootstock on peach fruit internal quality. *Eur. J. Agron.* **2023**, *143*, 126706. [CrossRef]

47. Luo, Z.; Zhang, J.; Li, J.; Yang, C.; Wang, T.; Ouyang, B.; Li, H.; Giovannoni, J.; Ye, Z. A STAY-GREEN protein S l SGR 1 regulates lycopene and β-carotene accumulation by interacting directly with S l PSY 1 during ripening processes in tomato. *New Phytol.* **2013**, *198*, 442–452. [CrossRef]
48. Li, H.L.; Zhang, H.; Yu, C.; Ma, L.; Wang, Y.; Zhang, X.Z.; Han, Z.H. Possible roles of auxin and zeatin for initiating the dwarfing effect of M9 used as apple rootstock or interstock. *Acta Physiol. Plant.* **2012**, *34*, 235–244. [CrossRef]
49. Song, C.; Zhang, D.; Zhang, J.; Zheng, L.; Zhao, C.; Ma, J.; An, N.; Han, M. Expression analysis of key auxin synthesis, transport, and metabolism genes in different young dwarfing apple trees. *Acta Physiol. Plant.* **2016**, *38*, 43. [CrossRef]
50. Soumelidou, K.; Morris, D.A.; Battey, N.H.; Barnett, J.R.; John, P. Auxin transport capacity in relation to the dwarfing effect of apple rootstocks. *J. Hortic. Sci.* **1994**, *69*, 719–725. [CrossRef]
51. Petrasek, J.; Friml, J. Auxin transport routes in plant development. *Development* **2009**, *136*, 2675–2688. [CrossRef]
52. Yamamoto, Y.; Kamiya, N.; Morinaka, Y.; Matsuoka, M.; Sazuka, T. Auxin biosynthesis by the YUCCA genes in rice. *Plant Physiol.* **2007**, *143*, 1362–1371. [CrossRef] [PubMed]
53. Zhang, Y.; Mao, Q.; Ma, R.; Xu, J.; Yu, M. Genome-Wide Identification and Expression Analysis of the *PpYUCCA* Gene Family in Weeping Peach Trees (*Prunus persica* 'Pendula'). *Horticulturae* **2022**, *8*, 878. [CrossRef]