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A Modeling Approach to Studying the Influence of Grafting on the Anatomical Features and *SAUR* Gene Expression in Watermelons

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Abstract: Grafting alters the genetic and anatomical features of plants. Although grafting has been widely applied in plant propagation, the underlying processes that govern the effects of the procedure are not fully understood. Samples were collected to study the long-term influence of grafting on the leaf-shoot morphology, leaf-shoot anatomy, and genetic signature of the grafted plants. *Citrulus lanatus* (Thunb.) Matsum. & Nakai (cv. *Lady*) was used as the scion, and *Lagenaria siceraria* (Molina) Standl (cv. *Argentario*) as a rootstock. In grafted plants, leaf blades and petioles were 20.92% and 12.82% longer, respectively, while the midrib collenchyma was 35.68% thicker, and the diameter of the vessel member was 11.17% larger than in ungrafted plants. In the stem, grafting affected the arrangement and number of vascular bundles (from 1 to 2 rings). The thickness of the epidermis decreased by 69.79%, and the size of the external fascicular phloem decreased by 23.56%. The diameter of the vessel member of the grafted plants increased by 28.94%. Eight out of ten evaluated primers met the requirements (stability in both watermelons and bottle gourd, tissue-specific). In the genetic tests, we examined whether this change in the gene expression pattern is due to the grafting and, if so, to what extent. Seven out of eight tested Small Auxin Up-Regulated RNA (*SAUR*) genes were expressed in the ungrafted and grafted *C. lanatus* lines in four cases; the expression increased by more than 10% after grafting. The morpho-anatomical changes and genetic variation reported in this study for grafted lines of *C. lanatus* contribute to the understanding of the underlying mechanisms of plant growth observations resulting from grafting.

Keywords: anatomy; graft; vascular bundles; *SAUR*; scion; rootstock; watermelon

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1. Introduction

Grafting is an important sustainable agricultural technique that has been practiced in tree crops for thousands of years. Vegetable grafting on a commercial scale is relatively new and is becoming common in vegetable production worldwide [1,2]. Grafted plants, consisting of the scion (the new shoot system) and the rootstock (the new root system), survive as one chimeric individual after recovery from the grafting process. Grafting

success relies on rootstock-scion compatibility [2–5]. Grafts are used to induce resistance to extreme temperatures, enhance nutrient uptake, improve water use efficiency, increase endogenous hormones, enhance tolerance against diverse environmental conditions, and reduce the uptake of organic pollutants [6–10]. The significant effects of grafting on the quantity and quality of the crop are known [11–13]. The rootstock should be tolerant to a variety of both biotic and abiotic stresses to maximize the success of grafts [1,14,15]. Despite the advantages of the technique, the process is sometimes accompanied by additional costs, disease, and poor fruit quality [1,2]. The effect of grafting on growth, internode length, dry weight, and fruit production has been well studied [16–19], with Yetisir, among others, conducting several comprehensive studies focusing on grafted watermelon plants. Yetisir investigated the morphology of the grafted plants using several different rootstocks under the influence of cold stress and productivity in the case of *Fusarium* infection [12,20]. However, fewer studies focus on the anatomy of grafted plants in their later stages of life. Insights into the underlying processes that govern grafting and grafting success could be useful in increasing vegetable production under diverse conditions and are relevant to plant ecology as a whole. Studies that dealt with the anatomical and physiological changes of grafted plants have previously focused on the fruit, the grafting region, or vascular healing [21–23]. The plant's vascular system functions to deliver resources to the organs and serves as a long-distance communication system between the above and below-ground aspects of the individual [24]. Examination of the communication between organs is likely the key to understanding changes across the plant's lifespan.

Several studies have previously examined different aspects of vegetative organs. In Chouka's research [16], changes in the leaf surface, internode length, shoot and root dry weight of grafted *Citrulus lanatus* (Thunb.) Matsum. & Nakai (watermelon) were examined. Also investigated were flowering time and changes in the yield. Chouka [16] worked with different rootstocks of *C. lanatus* and found significant differences in leaf size, dry weight, and productivity. As previously discussed, research on internal tissue changes in grafted plants has focused on the grafting region and wound healing. During the regeneration, tissues stick, cells begin to divide, cell differentiation proceeds and vascular systems regenerate.

Plant hormones such as cytokinins, gibberellins or auxins play an important role during grafting. Sharma and Zhang's review article provides great insight into how phytohormones help plants survive grafting and healing [25]. Cytokinins have a positive effect on wound healing and graft union after grafting [26,27]. Gibberellins regulate important biological processes such as cell elongation and cell division [28]. Gibberellins can move across the graft union [29] and hold a significant role in the joining of scion rootstock and the normal vascular development of plants [30].

Auxins are important phytohormones, and they are vital in plants during the process of grafting. They are part of the development of xylem tissues [31]. Together with cytokinin, auxin supports vascular differentiation and increases the phloem/xylem ratio [32]. The auxin response increased with graft adherence during the healing process, and this response was specific to vascular tissues [33,34]. Some proteins, mRNAs, and small RNA graft-transducable signals are now emerging as novel mechanisms that regulate developmental root/shoot relationships and may play a key role in understanding the later life of the grafted plant. We focused on auxin and one gene family regulated by the auxin pathway.

Auxin regulates many aspects of plant development, and in grafted plants, auxin plays an important role in wound healing and tissue regeneration [35–37]. Three known gene families induced by auxin are *GH3* (Gretchen Hagen 3), *Aux/IAA* (*Auxin/Indoleacetic Acid*), and *SAUR*. *SAUR*s (*Small Auxin-Up RNA*) are key effectors of hormonal, transcription factors and environmental signals that regulate plant development and dynamic, adaptive (auxin-induced) growth [38]. *SAUR* proteins are highly conserved among higher plants and indicate functional similarity of members of the same clade in different plants [39]. Zhang investigated the proteins of *C. lanatus* (watermelon), *Arabidopsis* (rockcress), and *Cucumis sativus* (cucumber) to investigate their evolution patterns. The examined genes

could share similar functions with other *SAUR* genes from other higher plants, such as *Arabidopsis thaliana* (thale cress), *Zea mays* (maize), or *Oryza sativa* (rice).

C. lanatus is a popular, sweet fruit that is rich in vitamins and minerals, which is grown on more than 2800 hectares in Hungary alone (link 1). Among the most commonly used rootstocks, we chose *Lagenaria siceraria* (Molina) Standl (bottle gourd) because it has a 95% grafting success rate and provides one of the highest production yields [11].

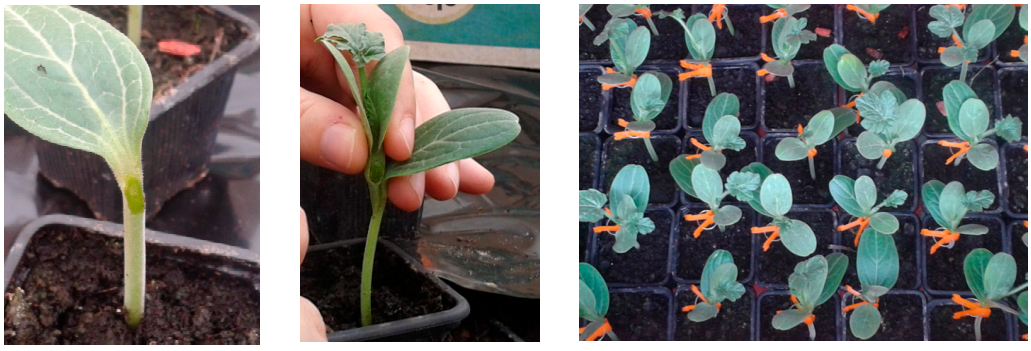
To better understand the potential changes, we tested three hypotheses using ungrafted and grafted lines of *C. lanatus*: (1) The quantitative and qualitative properties of the leaf are intensified by grafting, (2) Grafting causes both quantitative and qualitative changes to the vascular systems, and (3) There is a difference in the *SAUR* transcript abundance of grafted and ungrafted plants.

Phases of experimental work were: (1) Seedling growing and grafting: *C. lanatus* (cv. *Lady*) was used as the scion and the *L. siceraria* (cv. *Argentario*) as a rootstock, (2) Morpho-anatomical (microscopical) analysis of ungrafted and grafted *C. lanatus* leaf and stem, and (3) The expression of *ClaSAUR9*, *ClaSAUR10*, *ClaSAUR11*, *ClaSAUR16*, *ClaSAUR19*, *ClaSAUR27*, *ClaSAUR32*, *ClaSAUR36*, *ClaSAUR41*, and *ClaSAUR51* was tested in the leaves of the ungrafted *C. lanatus*, grafted *C. lanatus*, and *L. siceraria* using the PCR method.

2. Materials and Methods

2.1. Plant Material and Culture Conditions

Citrulus lanatus (cv. *Lady*)—watermelon—was used in the grafting as the scion, and *Lagenaria siceraria* (Molina) Standl (cv. *Argentario*)—bottle gourd—as rootstock (Figure 1). During the experiment, the seeds from the same F1 hybrid were used via KITE zrt. (Kukorica- és Iparinövény-termesztési Együttműködés zrt., Nádudvar, Hungary), *C. lanatus* seeds came from Nunhems Netherlands BV, Nunhem, The Netherlands and *L. siceraria* from Syngenta Crop Protection AG, Basel, Switzerland. The seeds of *C. lanatus* and *L. siceraria* were planted in a commercial peat pot (6 cm × 6 cm × 6 cm seedlings) (Kekkilä BVB, Vantaa, Finland). The plants were grafted after three weeks and were then kept in a greenhouse for another three weeks, under a 13/11 h day/night period, at an average temperature of 27 °C ± 2 °C, with 70–80% relative humidity. The grafting took place in growth phase 1 according to the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale. Each plant was occasionally watered with 100 mL of water. YaraLive Calcinit fertilizer was added to the irrigation water in the greenhouse life stage. Except during sowing and planting (~six-week-old plants), when we watered all plants 100 mL supplemented with Ferticare Starter 15-30-15 fertilizer, each time based on the distribution company's recipe. Seed sowing, seedling cultivation and grafting began in April, and morphological sample collection took place in July of the same year. Voucher specimens of the seeds labeled with a unique code were deposited at the Institute of Biology, Faculty of Sciences, University of Pécs, Hungary (Voucher code: CL, LS).



(**a**) (**b**) (**c**)

Figure 1. Rootstock with one cotyledon and growing point cut off (**a**), grafting (**b**) and after grafting (**c**).

2.2. The Applied Grafting Method and Field Work

Grafting was performed as previously described [40]. One cotyledon grafting method was used. Rootstock seeds were sown five days before scion seeds due to the different germination rates and probability. At the time of grafting, the rootstock plants still had their cotyledons and had one or two true leaves. *Citrulus lanatus* (cv. *Lady*) was grafted once the first true leaf appeared, but before it reached full development both in the scion and rootstock seedlings. One cotyledon and the first true leaf were cut with a razor blade following the angle of the leaf petiole. The hypocotyl of the scion was cut at a 35–45° angle on one side. The two surfaces were matched and held together with a grafting clip.

After grafting, the plants were placed in a healing chamber at constant humidity (80–90% humidity, 26–28 °C) and controlled light conditions (in the first 48 h the healing chamber was covered with black plastic). After three days, the chamber was ventilated. After 4–8 days, we increased the ventilation for longer and longer periods and slowly increased the number of illuminated hours. After nine days, the plants were placed under a 13/11 day/night period. The plant's water resources were consistently replenished by the high humidity, as watering is not possible due to the graft wound. When the plants reached the three-to-five-leaf stage, they were removed from the healing chamber and transplanted into plastic pots (8143 cm^3) containing a growing medium mix (Kekkilä peat) and arid sand and occasionally wetted with 100 mL of water. At approximately six weeks old, the seedlings were planted in an experimental field in Cece, Hungary (46.75°; 18.65°), where they matured from May to July. Average temperatures were 15.89 °C, 29.51 °C, and 23.37 °C, while monthly precipitation was 61.00 mm, 32.50 mm, and 64.50 mm in May, June, and July, respectively.

2.3. Morpho-Anatomical Examinations

Only mature plants were included in the morpho-anatomical analysis. For morphometric examinations, the leaf length, leaf width, and petiole length of 20 grafted and 20 ungrafted leaves from 10 different plants of each type were measured. The fourth and fifth leaves on the main stem were measured, and only healthy, well-developed leaves were included.

For anatomical examinations, 15 leaf samples and 15 stem samples were collected from both grafted and ungrafted *C. lanatus*. Stem samples were collected 30–35 cm above the graft region on the main shoot. The samples were dehydrated at increasing ethanol concentrations (50%, 70%, 90%), infiltrated with Technovit 7100 solution (Kulzer GmbH, Wehrheim, Germany), and placed into the hydroxyethyl methacrylate-based resin. The blocks were then sectioned with a rotary microtome (Anglia Scientific, Cambridge, UK) and 10 µm cross-sections stained with toluidine blue. Cross sections were examined with a light microscope (NIKON ECLIPSE 80i, Tokyo, Japan) with magnification 10 × 40, and micrographs were taken with the software Spot Basic 4.0.

Quantitative histological traits, including the thickness of the epidermis, palisade, spongy parenchyma, midrib collenchyma, diameter of the xylem, vessel member, phloem, and vascular bundles of stems and leaves, were measured with Image Tool 3.0 (UTHSCSA, San Antonio, TX, USA).

Linear models were created and analyzed using R ver. 3.6.2 [41]. In the models, the measured traits were the dependent variable, and the graft status was the independent variable. The graphical evaluation of the models (that is, normality check) was performed according to Crawley [42]. The function drop term with the F-test was used for statistical significance testing [43].

2.4. Quantitative RT-PCR Analysis

The fourth mature leaf away from the branching or grafting zone from 10 plants of each treatment group was collected for testing (grafted, ungrafted, bottle gourd). RNA isolation, cDNA synthesis from reserves, and quantitative RT-PCR analysis were performed as previously described [44]. Total RNA was purified with Direct-zolTM RNA MiniPrep Plus according to the protocol provided by the manufacturer. Purified RNA samples were sub-

jected to cDNA synthesis using the SuperScript™ IV Reverse Transcriptase (Invitrogen™, Thermo Fisher Scientific Inc., Waltham, MA, USA) system with random hexamers. Purified RNA samples were subjected to cDNA synthesis using the SuperScript™ IV Reverse Transcriptase (Invitrogen™) system with random hexamers based on the instructions from the manufacturer. Quantitative RealTime PCR analysis of cDNA samples was performed on the CFX Opus 96 Real-Time PCR System (Bio-Rad Laboratories, Vienna, Austria) using GoTaq® qPCR Master Mix (Promega Corporation, Madison, WIS, USA) with the following cycling conditions: polymerase activation at 95 °C for 2 min, then 45 cycles of denaturation at 95 °C for 3 s, annealing and denaturation at 60 °C for 30 s—used plate: Multiplate® PCR PlatesTM 96-well, clear. Directly after PCR amplification, the amplicons were subjected to melting curve analyses in which samples were ramped from 65 °C, increasing the temperature in 0.5 °C increments up to 95 °C with fluorescence acquisition after 5 s incremental holding periods. After data acquisition, the melting curves of the amplicons were visually inspected in the CFX Maestro 2.3 software (Bio-Rad). The *C. lanatus* clathrin adaptor complex subunit (*ClCAC*, Table S1) gene was used as the endogenous control [45].

We randomly chose 10 different *SAUR* genes for the comparative study of the expression of *SAUR* genes on grafted and ungrafted *C. lanatus* and *L. siceraria*, which we used as rootstock. *ClaSAUR* gene sequences from the *C. lanatus* genome database (http://cucurbitgenomics.org/v2/ (accessed on 3 July 2024)), were queried, the BLAST search in *L. siceraria* and primers were tested in both lines.

2.5. Statistical Analysis

The normality of residuals was proven with the Kolmogorov–Smirnov test in *SAUR* research, and the homogeneity of scatter was also proven with Levene's test. For the analysis of the data and correlation, a two-factor ANOVA model was applied.

Data are means ± standard deviations of five independent determinations (n = 5); data in the same column with different superscripted symbols (*, **, ***) indicate significant differences among grafted and ungrafted plant materials: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Results

3.1. Leaf Morpho-Anatomical Changes

The leaf morphometric analysis showed that the length and width of the blade, as well as the length of the petiole, were significantly influenced by grafting (Figure 2). The grafted leaves were, on average, 3.83 cm longer than the leaves from the ungrafted plants (df = 1; F = 74.21; $p < 0.001$). The shortest (7.00 cm) measured leaf occurred in ungrafted *C. lanatus* (df = 1; F = 36.54; $p < 0.001$). The grafted plants produced longer petioles (average 12.40 cm) than ungrafted individuals (average 10.81 cm) (df = 1; F = 26.88; $p < 0.001$).

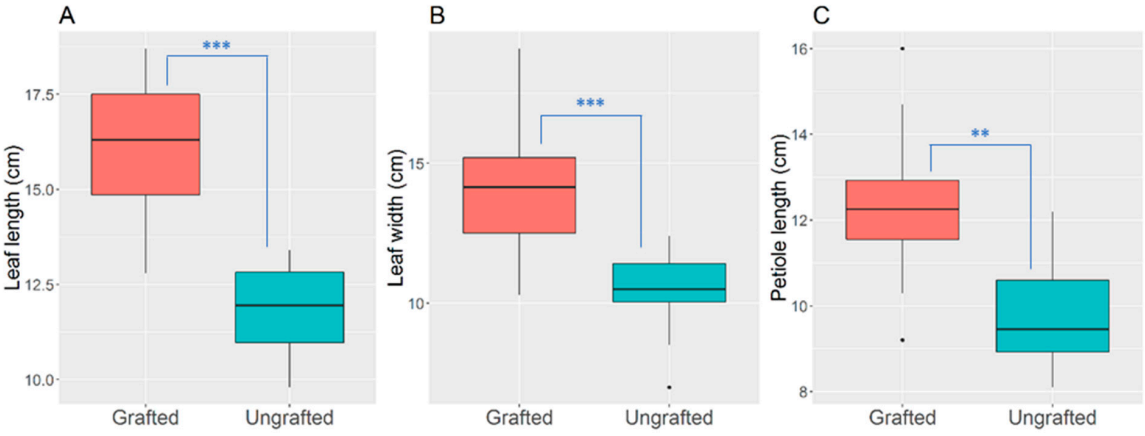
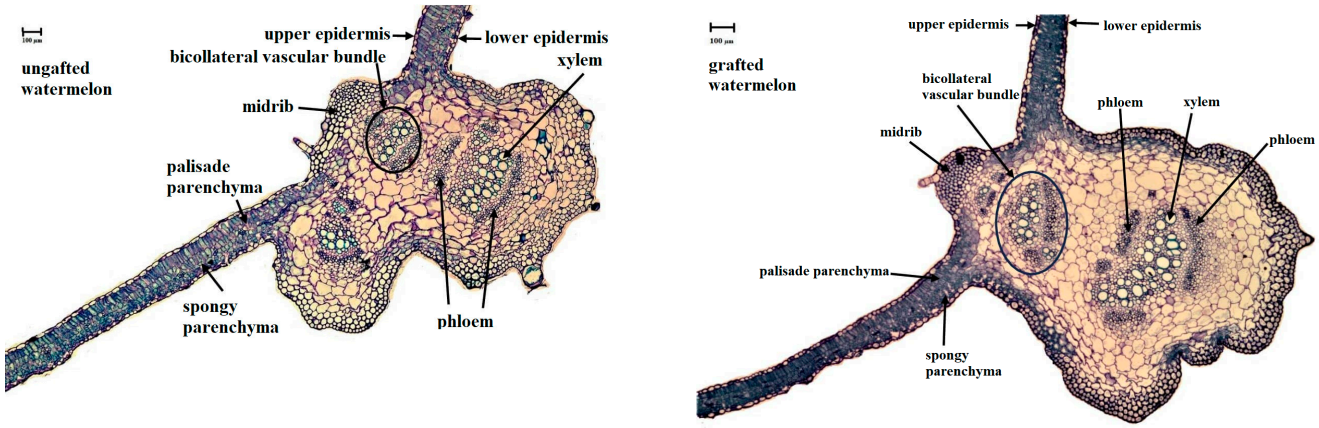


Figure 2. Morphometrical analysis of leaf blades and petioles: (**A**) leaf blade length, (**B**) leaf blade width, and (**C**) length of the petiole. Data are means ± standard deviations of five independent measurements (n = 5); ** $p < 0.01$, *** $p < 0.001$.

An anatomical examination of the leaf cross-sections revealed that vascular bundles were bicollateral (Figure 3). There were no significant differences in the measured anatomical parameters, only in the thickness of the midrib collenchyma, which was 89.98 µm ± 21.05 µm vs. 139.90 µm ± 3.32 µm in ungrafted vs. grafted *C. lanatus* plants, respectively (df = 1; F = 11.442; $p = 0.003$).

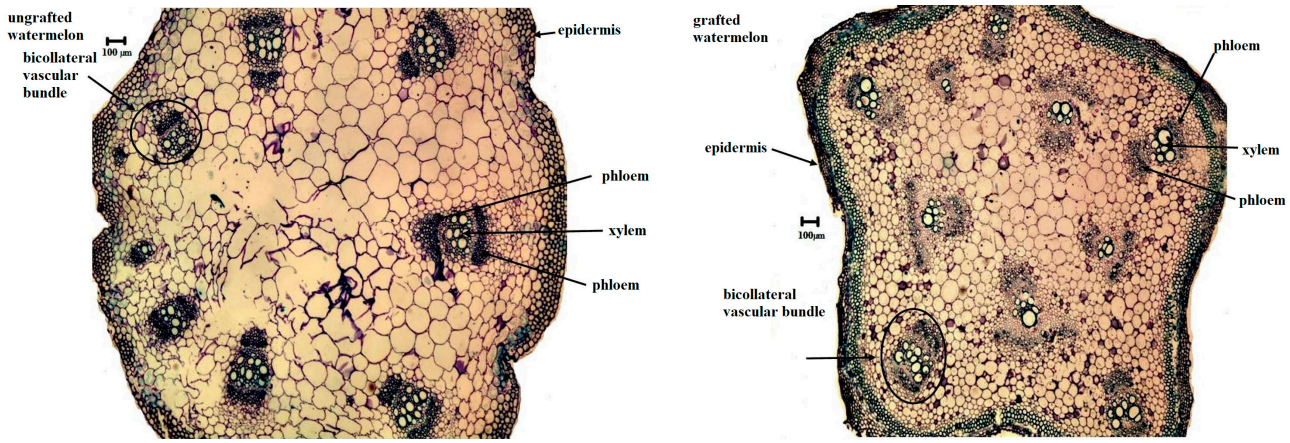


(**a**) (**b**)

Figure 3. Cross-sectional anatomy of (**a**) ungrafted and (**b**) grafted *C. lanatus* leaf. Significant differences were found in the thickness of the midrib collenchyma at $p < 0.05$.

3.2. Stem Anatomical Changes

The stem epidermis consisted of a single layer of isodiametric cells. The vascular bundles were bicollateral (Figure 4). The structural and size differences were revealed in the vascular tissue system between the grafted and ungrafted plants (Figures 4 and 5). In the case of ungrafted *C. lanatus*, eight bicollateral vascular bundles were found in one ring, while the grafted plants had ten bicollateral vascular bundles in two rings (Figure 4). Significant differences were found in epidermis thickness (Figures 4 and 5a), the thickness of the external fascicular phloem bundle (Figures 5b and 6), and the vessel member diameter (Figures 4 and 5d) between the two lines. The epidermis layer was 22.362 µm ± 4.63 µm vs. 13.17 µm ± 6.71 µm thick (df = 1; F = 11.442; $p = 0.003$); the diameter of the members of the vessel was 38.86 µm ± 10.98 µm vs. 54.69 µm ± 18.54 µm (df = 1; F = 8.374; $p = 0.008$); while the thickness of external fascicular phloem was 81.81 µm ± 19.16 µm vs. 66.21 µm ± 10.80 µm (df = 1; F = 5.745; $p = 0.024$) in ungrafted and grafted *C. lanatus* (Figure 5).



(**a**) (**b**)

Figure 4. Cross-sectional anatomy of the stem of (**a**) ungrafted and (**b**) grafted *Citrullus lanatus.* After grafting, the number of vascular bundles increased from 8 to 10, and their structure changed.

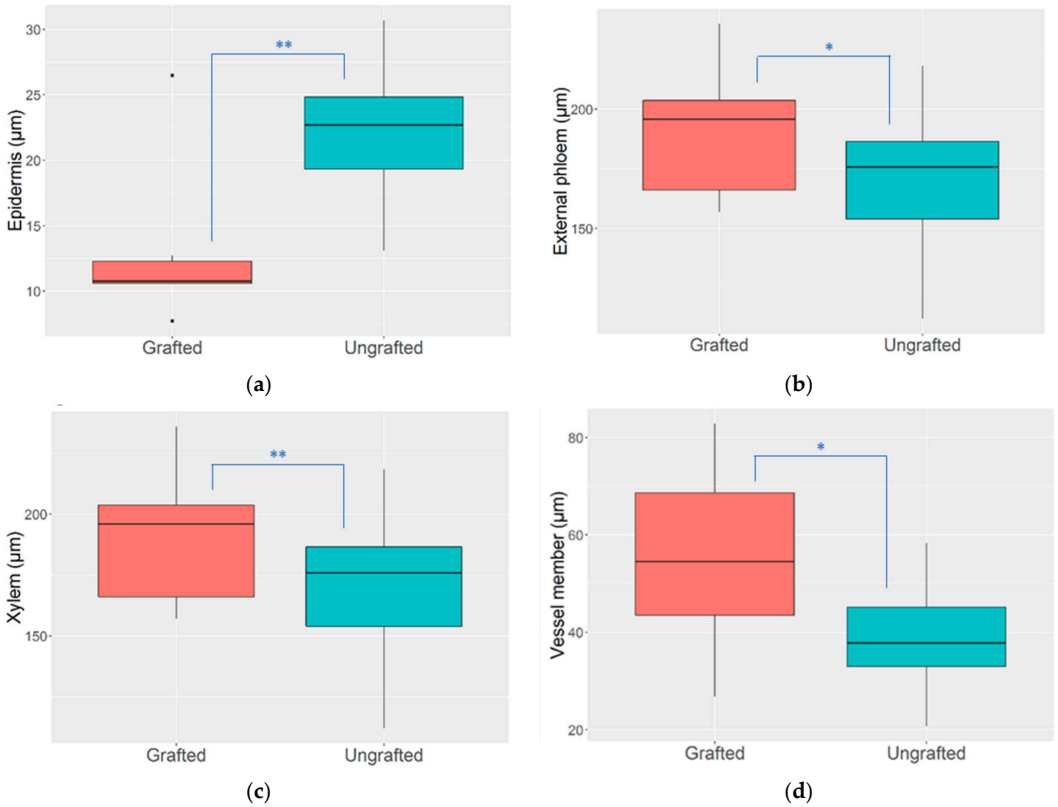


Figure 5. Effect of grafting on the anatomy of the stem: (**a**) thickness of epidermis, (**b**) thickness of external fascicular phloem, (**c**) diameter of the xylem, (**d**) diameter of vessel member. Significant differences in the thickness of the epidermis, external fascicular phloem, and the diameter of the vessel member were found. Data are means ± standard deviations of five independent determinations (n = 5); * $p < 0.05$, ** $p < 0.01$.

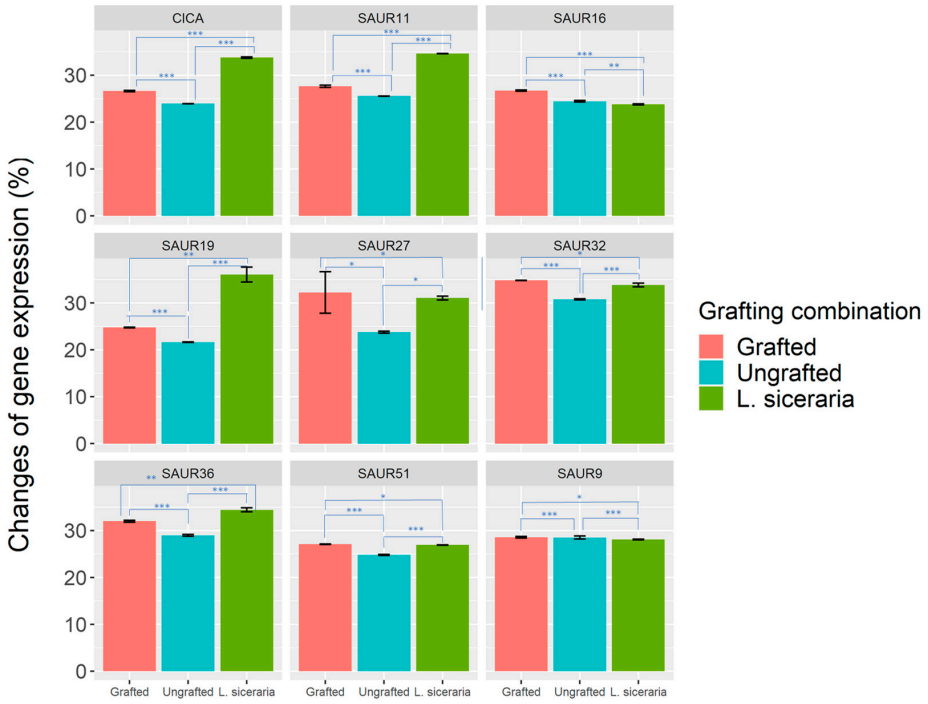


Figure 6. Changes in gene expression (%) in the plant types. We used the clathrin adaptor complex subunit (*ClCAC*) as a reference gene. There was a significant difference in the gene expression of seven

genes between the grafted and ungrafted groups, of which it was more than 10% in the case of four (*ClaSAUR9*, *ClaSAUR27* and *ClaSAUR19*). Data are means ± standard deviations of five independent determinations (n = 5); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.3. Expression Analysis of Grafted *C. lanatus* SAUR Genes

The expression of *ClaSAUR9*, *ClaSAUR10*, *ClaSAUR11*, *ClaSAUR16*, *ClaSAUR19*, *ClaSAUR27*, *ClaSAUR32*, *ClaSAUR36*, *ClaSAUR41*, and *ClaSAUR51* was tested in the leaves of the three groups (ungrafted *C. lanatus*, grafted *C. lanatus*, and *L. siceraria*) using PCR (PTC-200 Peltier Thermal Cycler, Sigma-Aldrich, Saint Louis, MO, USA). Eight *ClaSAUR* primer sets produced stable and strong signals in all three groups (Table 1), and their expression patterns were examined by qRT-PCR analysis.

Table 1. Expression of *SAUR* genes in ungrafted *C. lanatus*, grafted *C. lanatus*, and *L. siceraria* leaves.

Gene	Ungrafted *C. lanatus*	Grafted *C. lanatus*	*L. siceraria*
ClCAC	1	1	1
ClaSAUR9	1	1	1
ClaSAUR10	1	0	0
ClaSAUR11	1	1	1
ClaSAUR16	1	1	1
CLaSAUR19	1	1	1
ClaSAUR27	1	1	1
ClaSAUR32	1	1	1
ClaSAUR36	1	1	1
ClaSAUR41	1	0	0
ClaSAUR51	1	1	1

Notes: 1—stable, strong signal. 0—no signal or non-specifically displayed signal.

Seven out of eight genes showed significantly different expression between the ungrafted and grafted *C. lanatus* (watermelon) plants: *ClaSAUR11* (df = 2, F = 1639.30, $p < 0.001$), *ClaSAUR16* (df = 2, F = 382.89, $p < 0.001$), *ClaSAUR19* (df = 2, F = 258.25, $p = 0.007$), *ClaSAUR27* (df = 2, F = 9.53, $p = 0.016$), *ClaSAUR32* (df = 2, F = 170.68, $p < 0.001$), *ClaSAUR36* (df = 2, F = 250.60, $p < 0.001$), *ClaSAUR51* (df = 2, F = 895.67, $p < 0.001$) (Figure 6). For all genes examined, gene expression increased in grafted watermelons compared to ungrafted watermelons. In four cases, the level of gene expression increased by more than 10% after grafting (*ClaSAUR27*, *ClaSAUR19*, *ClaSAUR32*, *ClaSAUR36*).

4. Discussion

4.1. Leaf Morpho-Anatomical Changes

Morphological examination of the leaves leads to the conclusion that grafting increases the size of the leaf and petiole in grafted individuals. These results are similar to the findings of several previous studies on grafted plants and confirm changes in the size of the grafted plant under the influence of different subjects [46–48]. An increase in leaf size was also found after grafting, which is consistent with Chouka and Jaberi [16]. Navas and Garnier [49] and Yetisir and Sari [11,20] explained this effect by changes in nutrient and water uptake, which substantially alter leaf plasticity. The improved ability of grafted plants to take up water and nutrients from the soil may help explain the results of this study as the grafted and ungrafted plants were grown in the same soil, at the same time, and the same irrigation and fertilization regimes, suggesting that the source limitations were similar. For the anatomical sampling, however, we had to damage the plant. Single samples were taken from mature plants to avoid possible effects from anatomical sampling.

Anatomical examination of the leaf did not reveal any structural differentiation, and only one parameter showed a significant difference between the groups (thickness of the midrib collenchyma). Other studies have suggested that the more extensive mechanical tissues supported the increase in leaf size. In grafted plants, the size of the midrib collenchyma was significantly larger, similar to the findings of Lawson and Morison [50], Read and Stokes, and da Silva et al. [51,52], who claimed that the increase in the number of mechanical tissues supported the increased leaf size. The larger proportion of supporting ground tissues is probably accompanied by increased photosynthetic performance and water transport capacity [53]. Buckley et al. [54] supported this conclusion through experimental work suggesting that sub-epidermal mechanical tissues can play a key role in stomatal responses to water status.

4.2. Stem Anatomy Changes

Grafting significantly decreased the thickness of the stem epidermis in the tested individuals. The thickness of the epidermis can be affected by external influences (UV radiation, infection, environment) or internal influences such as enzyme changes [55–58]. In our case, finding the underlying mechanism for the change requires further investigation, but it is most likely an effect of grafting since the plants were exposed to the same environmental conditions.

Ungrafted samples of *C. lanatus* had eight vascular bundles in one ring in the stem, which differs from the typical ten vascular bundles in a varied arrangement in the Cucurbitaceae family [59]. In the case of grafted *C. lanatus*, the bicollateral vascular system with ten vascular bundles was re-established, which is specific in the Cucurbitaceae family and can be found in *L. siceraria* [60]. Plant hormones, such as auxin, can regulate the development of the vascular system [61,62]; this information can be exchanged between the rootstock and the scion [63]. According to Michurin's graft hybrid concept [64], grafting involves the transfer of genetic material, leading to heritable changes in the scion [2]. The results of this study support this hypothesis, but further experiments are necessary to fully explore this possibility.

The impact of grafting on the vascular system was evident in the increased number and arrangement of vascular bundles, as well as the diameter of the vessel members. Previous studies have suggested that grafting increases stem length and that vessel diameter reflects the length of the conductive path of water along the stems [65]. Grafting also increased the thickness of the external fascicular phloem (EFP) significantly. Zhang et al. [66] demonstrated for both *C. lanatus* and *L. siceraria* that the phloem sap was mainly derived from the fascicular phloem rather than from the extra fascicular phloem, the volume issuing from the internal fascicular phloem being higher than from the EFP. Gaupels and Ghirardo [67] demonstrated the protective role of the EFP system against herbivory and subsequent microbial infection, which may explain better defense mechanisms of grafted plants. The grafted *C. lanatus* plants had a larger, more complex, and more developed vascular system than the members of the ungrafted plants. A more developed vascular system can provide better nutrition and water [68,69], which in turn can lead to increased leaf biomass.

4.3. Differentially Expressed ClaSAUR Genes

The first goal of our research was to find *SAUR* genes whose sequence was identified in *C. lanatus* as well as in *L. siceraria*. These genes have an influence on plant growth and tissue arrangement. We could easily achieve this with the help of the literature [39,70]. In recent years, there has been an increase in studies related to the importance of *SAUR* genes in the regulation of growth. *SAUR* genes are important elements of auxin-induced development but can also act independently of auxin. The second goal was to ensure that there was a flow of information between the rootstock and scion after grafting [63].

Examination of the chosen *SAUR* gene expression of grafted and ungrafted plants revealed several changes. In grafted plants, the expression of seven genes increased when compared to that of ungrafted watermelon. Four of these seven genes, *ClaSAUR19*, *ClaSAUR32*, *ClaSAUR27* and *ClaSAUR36I*, showed an increase of more than 10% in gene expression. All four genes were highly expressed in leaf tissue, and all of them showed some degree of reaction under *IAA* treatment, where the expression of *ClaSAUR19* reduced more than threefold [39]. In addition, the sequences of all four genes show high homology with functionally well-described *SAUR* genes identified in other plants. Therefore, it can be assumed that since the *SAUR* genes are highly conserved sequences, the function of the genes we examined and the genes or gene families described in the majority of *Arabidopsis* are very similar or even the same.

ClaSAUR27 gene expression was significantly higher in the grafted plants compared to the other two types of plants [39]. The *ClaSAUR27* gene has a high homology with the *AtSAUR16* and *AtSAUR50* genes (belonging to the *AtSAUR10* clade). The *AtSAUR10*-clade genes generally induce cell elongation but exhibit various expression patterns and different responses to hormones. With the help of these genes, the plant can fine-tune growth in a variety of tissues in response to internal and external signals [71]. The rootstock part and scion part of the grafted plant continuously communicate with each other in response to environmental stimuli [34]. *ClaSAUR27* may help the plant in this fine-tuning.

Using our own results, as well as Qui and Zhang's research, we can deduce certain relationships between *ClaSAUR19* and *AtSAUR41* subfamily members. The sequence of the *ClaSAUR19* gene is similar to the members of the *AtSAUR41* subfamily; they can be detected in large quantities in the leaf tissues and, according to Qui [72], in root tissues. Genes of the *Arabidopsis SAUR41* subfamily, and presumably the *ClaSAUR19* gene due to its high homology, can be induced by ABA to modulate cell expansion, ion homeostasis, and salt tolerance.

The homology between the *ClaSAUR32* gene and the *AtSAUR32* gene was almost 100%. *ClaSAUR32* was highly expressed in leaf, shoot and flower tissues but not in the root [39]. *AtSAUR32*, which can be found in *Arabidopsis*, regulates ABA-mediated responses [73]. The *ClaSAUR36* sequence is almost 100% identical to the *AtSAUR76* gene. Both *ClaSAUR36* and *AtSAUR76* can be detected in all organs and are less sensitive to auxin treatment. According to Markakis et al. [74], *AtSAUR76* plays a minor role in cell elongation.

Our results may be useful in designing future experiments, while we do not claim that the morphological changes we measured are the result of changes in the expression of the examined genes. Future studies are required to examine to what extent the functions of the genes we are examining are actually similar to their homologous counterparts. Knock-out experiments of the tested genes may be useful in understanding the processes of the rootstock effects in grafted plants.

5. Conclusions

Significant differences were demonstrated between grafted and ungrafted plants of *C. lanatus* regarding the morphoanatomy of both the leaf (size, midrib collenchyma) and the stem (thickness of epidermis, EFP, vessel member diameter, changes in the vascular system organization), as well as *SAUR* gene expression pattern. On the basis of the anatomical and gene expression examination of the leaf, we suggest that there is a correlation between the changes in the expression of individual *SAUR* genes and the differential leaf growth observed in *C. lanatus*. These results suggest that the rootstock has a substantial impact on various aspects of the scion. However, several questions remain regarding the functional significance and genetic regulation of these anatomical traits, which should be explored in future research projects.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14071472/s1, Table S1: Primers of *Citrullus lanatus ClCAC* gene (endogenous control) and 10 *SAUR* genes used for qRT-PCR.

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References

1. Lee, J.M.; Oda, M. Grafting of herbaceous vegetable and ornamental crops. *Hortic. Rev.* **2003**, *28*, 61–124. [CrossRef]
2. Goldschmidt, E. Plant grafting: New mechanisms, evolutionary implications. *Front Plant Sci.* **2014**, *5*, 727. [CrossRef]
3. Ren, Y.; Guo, S.R.; Shu, S.; Xu, Y.; Sun, J. Isolation and expression pattern analysis of CmRNF5 and CmNPH3L potentially involved in graft compatibility in cucumber/pumpkin graft combinations. *Sci. Hortic.* **2018**, *227*, 92–101. [CrossRef]
4. Ren, Y.; Xu, Q.; Wang, L.; Guo, S.; Shu, S.; Lu, N.; Sun, J. Involvement of metabolic, physiological and hormonal responses in the graft-compatible process of cucumber/pumpkin combinations was revealed through the integrative analysis of mRNA and miRNA expression. *Plant Physiol. Biochem.* **2018**, *129*, 368–380. [CrossRef] [PubMed]
5. Gisbert-Mullor, R.; Pascual-Seva, N.; Martínez-Gimeno, M.A.; López-Serrano, L.; Badal Marín, E.; Pérez-Pérez, J.G.; Bonet, L.; Padilla, Y.G.; Calatayud, Á.; Pascual, B.; et al. Grafting onto an Appropriate Rootstock Reduces the Impact on Yield and Quality of Controlled Deficit Irrigated Pepper Crops. *Agronomy* **2020**, *10*, 1529. [CrossRef]
6. Otani, T.; Seike, N. Comparative effects of rootstock and scion on dieldrin and endrin uptake by grafted cucumber (*Cucumis sativus*). *J. Pestic. Sci.* **2006**, *31*, 316–321. [CrossRef]
7. Dong, H.H.; Niu, Y.H.; Li, W.J.; Zhang, D.M. Effects of cotton rootstock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence. *J. Exp. Bot.* **2008**, *59*, 1295–1304. [CrossRef] [PubMed]
8. Huang, Y.; Li, J.; Hua, B.; Liu, Z.; Fan, M.; Bie, Z. Grafting onto different rootstocks as a means to improve watermelon tolerance to low potassium stress. *Sci. Hortic.* **2013**, *149*, 80–85. [CrossRef]
9. Pina, A.; Cookson, S.J.; Calatayud, A.; Errea, P. Physiological and molecular mechanisms underlying graft compatibility. In *Vegetable Grafting: Principles and Practices*, 2nd ed.; Colla, G., Pérez-Alfocea, F., Schwarz, D., Eds.; CABI: London, UK, 2017; pp. 132–154. [CrossRef]
10. Lu, K.; Sun, J.; Li, Q.; Li, X.; Jin, S. Effect of Cold Stress on Growth, Physiological Characteristics, and Clavin-Cyle-Related Gene Expression of Grafted watermelon Seedlings of Different Gourd Rootstocks. *Horticulturae* **2021**, *7*, 391. [CrossRef]
11. Yetisir, H.; Sari, N. Effect of different rootstock on plant growth, yield and quality of watermelon. *Aust. J. Exp. Agric.* **2003**, *43*, 1269–1274. [CrossRef]
12. Yetisir, H.; Sari, N.; Yücel, S. Rootstock resistance to *Fusarium* wilt and effect on watermelon fruit yield and quality. *Phytoparasitica* **2003**, *31*, 163–169T. [CrossRef]
13. Turhan, A.; Ozmen, N.; Kuscu, H.; Serbeci, M.S.; Seniz, V. Influence of rootstocks on yield and fruit characteristics and quality of watermelon. *Hortic. Environ. Biotechnol.* **2012**, *53*, 336–341. [CrossRef]
14. Nisini, P.T.; Colla, G.; Grantani, E.; Temperini, O.; Crinó, P.; Saccado, F. Rootstock resistance to fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars. *Sci. Hortic.* **2002**, *93*, 281–288. [CrossRef]
15. Ombódi, A. Az oltás elméleti és gyakorlati szerepe a dinnyetermesztésben. *Hajtásos Korai Termesztés* **2005**, *36*, 9–12.
16. Chouka, A.S.; Jaberi, H. Effect of grafting on watermelon vegetative and root development, production and fruit quality. *Acta Hortic.* **1999**, *492*, 85–94. [CrossRef]
17. Colla, G.; Rouphael, Y.; Cardarelli, M.; Salerno, A.; Rea, E. The effectiveness of grafting to improve alkalinity tolerance in watermelon. *Environ. Exp. Bot.* **2010**, *68*, 283–291. [CrossRef]
18. Mohamed, F.H.; El-Hamed, K.E.A.; Elwan, M.W.M.; Hussien, M.N.E. Impact of grafting on watermelon growth, fruit yield and quality. *Horticulturae* **2012**, *76*, 99–118. [CrossRef]
19. Farhadi, A.; Aroeii, H.; Nemati, H.; Salehi, R.; Giuffrida, F. The effectiveness of different rootstocks for improving yield and growth of cucumber cultivated hydroponically in a greenhouse. *Horticulturae* **2016**, *2*, 1. [CrossRef]

20. Yetisir, H.; Sari, N. Effect of Hypocotyl Morphology on Survival Rate and Growth of Watermelon Seedlings Grafted on Rootstocks with Different Emergence Performance at Various Temperatures. *Turk. J. Agric. For.* **2004**, *28*, 231–236.
21. Colla, G.; Rouphael, Y.; Cardarelli, M.; Rea, E. Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants. *Hort. Sci.* **2006**, *41*, 622–627. [CrossRef]
22. Proietti, S.; Rouphael, Y.; Colla, G.; Cardarelli, M.; De Agazio, M.; Zacchini, M.; Rea, E.; Moscatello, S.; Battistelli, A. Fruit quality of mini-watermelon as affected by grafting and irrigation regimes. *J. Sci. Food Agric.* **2008**, *88*, 1107–1114. [CrossRef]
23. Melnyk, C.W. Plant grafting: Insights into tissue regeneration. *Regeneration* **2016**, *4*, 3–14. [CrossRef] [PubMed]
24. Lucas, W.J.; Groover, A.; Lichtenberger, R.; Furuta, K.; Yadav, S.R.; Helariutta, Y.; He, X.Q.; Fukuda, H.; Kang, J.; Brady, S.M.; et al. The plant vascular system: Evolution, development and functions. *J. Integr. Plant Biol.* **2013**, *55*, 294–388. [CrossRef] [PubMed]
25. Sharma, A.; Zheng, B. Molecular Responses during Plant Grafting and Its Regulation by Auxins, Cytokinins, and Gibberellins. *Biomolecules* **2019**, *9*, 397. [CrossRef] [PubMed]
26. Köse, C.; Güleryüz, M. Effects of auxins and cytokinins on graft union of grapevine (*Vitis vinifera*). *N. Z. J. Crop Hortic. Sci.* **2006**, *34*, 145–150. [CrossRef]
27. Mitchell, J.; Van Staden, J. Cytokinins and the wounding response in potato tissue. *Z. Pflanzenphysiol.* **1983**, *109*, 1–5. [CrossRef]
28. Hu, Y.; Zhou, L.; Huang, M.; He, X.; Yang, Y.; Liu, X.; Li, Y.; Hou, X. Gibberellins play an essential role in late embryogenesis of *Arabidopsis*. *Nat. Plants.* **2018**, *4*, 289–298. [CrossRef] [PubMed]
29. Ragni, L.; Nieminen, K.; Pacheco-Villalobos, D.; Sibout, R.; Schwechheimer, C.; Hardtke, C.S. Mobile gibberellin directly stimulates *Arabidopsis* hypocotyl xylem expansion. *Plant Cell* **2011**, *23*, 1322–1336. [CrossRef] [PubMed]
30. Regnault, T.; Daviere, J.M.; Wild, M.; Sakvarelidze-Achard, L.; Heintz, D.; Carrera Bergua, E.; Lopez Diaz, I.; Gong, F.; Hedden, P.; Achard, P. The gibberellin precursor GA12 acts as a long-distance growth signal in *Arabidopsis*. *Nat. Plants.* **2015**, *1*, 15073. [CrossRef]
31. Růžička, K.; Ursache, R.; Hejátko, J.; Helariutta, Y. Xylem development-from the cradle to the grave. *New Phytol.* **2015**, *207*, 519–535. [CrossRef]
32. Aloni, R. The role of cytokinin in organised differentiation of vascular tissues. *Funct. Plant Biol.* **1993**, *20*, 601–608. [CrossRef]
33. Yin, H.; Yan, B.; Sun, J.; Jia, P.; Zhang, Z.; Yan, X.; Chai, J.; Ren, Z.; Zheng, G.; Liu, H. Graft-union development: A delicate process that involves cell-cell communication between scion and stock for local auxin accumulation. *J. Exp. Bot.* **2012**, *11*, 4219–4232. [CrossRef]
34. Melnyk, C.W.; Schuster, C.; Leyser, O.; Meyerowitz, E.M. A development framework for graft formation and vascular reconnection in Arabidopsis thaliana. *Curr. Biol.* **2015**, *25*, 1306–1318. [CrossRef] [PubMed]
35. Aloni, B.; Karni, L.; Deventurero, G.; Levin, Z.; Cohen, R.; Katzir, N.; Lotan-Pompan, M.; Edelstein, M.; Aktas, H.; Turhan, E.; et al. Physiological and biochemical changes at the rootstock-scion interface in graft combinations between *Cucurbita* root stocks and a melon scion. *J. Hortic. Sci. Biotechnol.* **2008**, *83*, 777–783. [CrossRef]
36. Teale, W.D.; Paponov, I.A.; Palme, K. Auxin in action: Signalling, transport and the control of plant growth and development. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 847–859. [CrossRef] [PubMed]
37. Cano-Delgado, A.; Lee, J.Y.; Demura, T. Regulatory Mechanisms for specification and patterning of plant vascular tissues. *Annu. Rev. Cell Dev. Biol.* **2010**, *26*, 605–637. [CrossRef] [PubMed]
38. Ren, H.; Gray, W.M. SAUR proteinsas effectors of hormonal and environmental signals in plant growth. *Mol. Plant.* **2015**, *8*, 1153–1164. [CrossRef] [PubMed]
39. Zhang, N.; Huang, X.; Bao, Y.; Wang, B.; Zeng, H.; Cheng, W.; Tang, M.; Li, Y.; Ren, J.; Sun, Y. Genome-wide identification of SAUR genes in watermelon (*Citrullus lanatus*). *Physiol. Mol. Biol. Plants* **2017**, *23*, 619–628. [CrossRef]
40. Garner, R.J. *The Grafter's Handbook*, 6th ed.; Octopus Publishing Group: London, UK, 2013; p. 320.
41. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
42. Crawley, M.J. *Statistics: An Introduction Using R*, 2nd ed.; John Wiley and Sons: Bognor Regis, UK, 2014; p. 360.
43. Venables, W.N.; Ripley, B.D. *Modern Applied Statistics with S*, 4th ed.; Springer: New York, NY, USA, 2002; p. 497.
44. Huang, X.; Chen, J.; Bao, Y.; Liu, L.; Jiang, H.; An, X.; Dai, L.; Wang, B.; Peng, D. Transcript profiling reveals auxin and cytokinin signaling pathways and transcription regulation during in vitro organogenesis of ramie (*Boehmeria nivea* L. Gaud). *PLoS ONE* **2014**, *9*, e113768. [CrossRef]
45. Kong, Q.; Yuan, J.; Gao, L.; Zhao, S.; Jiang, W.; Huang, Y.; Bie, Z. Identification of suitable reference genes for gene expression normalization in qRT-PCR analysis in watermelon. *PLoS ONE* **2014**, *9*, e90612. [CrossRef]
46. Yetisir, H.; Caliskan, M.E.; Soylu, S.; Sakar, M. Some physiological and growth responses of watermelon (*Citrullus lanatus*) Matsum and Nakai) grafted onto *Lagenaria siceraria* to flooding. *Environ. Exp. Bot.* **2006**, *58*, 1–8. [CrossRef]
47. Sáenz-Pérez, C.A.; Osorio-Hernández, E.; Estrada-Drouaillet, B.; Castro-Nava, S.; Delgado-Martínez, R.; López-Badillo, C.M.; Rodríguez-Herrera, R. Rootstock Influence on Growth and Mineral Content of *Citrus limon* and *Citrus sinensis* cv. Valencia Inoculated with *Candidatus* Liberibacter Asiaticus. *Agronomy* **2020**, *10*, 1564. [CrossRef]
48. Rahmatian, A.; Delshad, M.; Salehi, R. Effect of grafting on growth, yield and fruit quality of single and double stemmed tomato plants grown hydroponically. *Prot. Hortic.* **2014**, *55*, 115–119. [CrossRef]
49. Navas, M.L.; Garnier, E. Plasticity of whole plant and leaf traits in *Rubia peregrine* in response to light, nutrient and water availability. *Acta Oecol.* **2002**, *23*, 375–383. [CrossRef]

50. Lawson, T.; Morison, J. Visualising patterns of CO_2 diffusion in leaves. *New Phyt.* **2006**, *169*, 641–643. [CrossRef] [PubMed]
51. Read, J.; Stokes, A. Plant biomechanics in an ecological context. *Am. J. Bot.* **2006**, *93*, 1546–1565. [CrossRef] [PubMed]
52. da Silva, N.R.; Florindo, J.B.; Gómez, M.C.; Rossatto, D.R.; Kolb, R.M.; Bruno, O.M. Plant Identification Based on leaf Midrib Cross-Section Images Using Fractal Descriptors. *PLoS ONE* **2015**, *10*, e0130014. [CrossRef] [PubMed]
53. Barbosa, M.A.M.; Chitwood, D.H.; Azevedo, A.A.; Araújo, W.L.; Ribeiro, D.M.; Peres, L.E.; Martins, S.C.V.; Zsögön, A. Bundle sheath extensions affect leaf structural and physiological plasticity in response to irradiance. *Plant Cell Environ.* **2019**, *42*, 1575–1589. [CrossRef] [PubMed]
54. Buckley, T.N.; Sack, L.; Gilbert, M.E. The role of bundle sheath extensions and life form in stomatal responses to leaf water status. *Plant Physiol.* **2011**, *156*, 962–973. [CrossRef]
55. Day, T.A.; Martin, G.; Vogelmann, T.C. Penetration of UV-B radiation in foliage: Evidence that the epidermis behaves as a non-uniform filter. *Plant Cell Environ.* **1993**, *16*, 735–741. [CrossRef]
56. Savaldi-Goldstein, S.; Peto, C.; Chory, J. The epidermis both drives and restricts plant shoot growth. *Nature* **2007**, *446*, 199–202. [CrossRef] [PubMed]
57. Omara, R.I.; Abdelaal, K.A.A. Biochemical, histopathological and genetic analysis associated with leaf rust infection in wheat plants (*Triticum aestivum* L.). *Physiol. Mol. Plant Pathol.* **2018**, *104*, 48–57. [CrossRef]
58. Liu, W.; Li, Z.; Qi, Z. Variation in leaf traits at different altitudes reflects the adaptive strategy of plants to environmental changes. *Ecol. Evol.* **2020**, *10*, 8166–8175. [CrossRef]
59. Oda, M.; Nagaoka, M.; Mori, T.; Sei, M. Effect of hypocotyl morphology on survival rate and growth of cucumber seedling grafted on *Cucurbita* spp. *Jpn. Agric. Res. Q.* **1993**, *26*, 259–263.
60. Makesh Kumar, B.; Veni, P.; Vijayalakshmi, M.; Selvaraj, K.; Stephan, J. Stem Anatomy of Four Genera of Family Cucurbitaceae. *Int. J. Adv. Res. Bot.* **2020**, *6*, 15–18. [CrossRef]
61. Sachs, T. *Pattern Formation in Plant Tissues*, 25th ed.; Developmental and cell Biology Series; Cambridge University Press: Cambridge, UK, 2005; 248p.
62. Floyd, S.K.; Bowman, J.L. Gene regulation: Ancient microRNA target sequences in plants. *Nature* **2004**, *428*, 485–486. [CrossRef] [PubMed]
63. Lewsey, M.G.; Hardcastle, T.J.; Melnyk, C.W.; Molnár, A.; Valli, A.; Ulrich, M.A.; Nery, J.R.; Baulcombe, D.C.; Ecker, J.R. Mobile small RNAs regulate genome-wide DNA methylation. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E801–E810. [CrossRef]
64. Michurin, I.V. *Selected Works*; Foreign Languages Publishing House: Soviet Socialist Republics, Moscow, 1949; p. 527.
65. Rosell, J.A.; Olson, M.E.; Anfodillo, T. Scaling of xylem vessel diameter with plant size: Causes, predictions, and outstanding questions. *Curr. For. Rep.* **2017**, *3*, 46–59. [CrossRef]
66. Zhang, C.; Yu, X.; Ayre, B.G.; Turgeon, R. The origin and composition of cucurbit "phloem" exudate. *Plant Physiol.* **2012**, *158*, 1873–1882. [CrossRef]
67. Gaupels, F.; Ghirardo, A. The extrafascicular phloem is made for fighting. *Front. Plant Sci.* **2013**, *4*, 187. [CrossRef]
68. De Rybel, B.; Mahönen, A.P.; Helariutta, Y.; Weijers, D. Plant vascular development: From early specification to differentiation. *Nat. Rev. Mol. Cell Biol.* **2016**, *17*, 30–40. [CrossRef] [PubMed]
69. Kawaguchi, K.; Nakaune, M.; Ma, J.F.; Kojima, M.; Takebayashi, Y.; Sakakibara, H.; Otagaki, S.; Matsumoto, S.; Shiratake, K. Plant Hormone and Inorganic Ion Concentrations in the Xylem Exudate of Grafted Plants Depend on the Scion–Rootstock Combination. *Plants* **2022**, *11*, 2594. [CrossRef] [PubMed]
70. Shireen, F.; Nawaz, M.A.; Xiong, M.; Ahmad, A.; Sohail, H.; Chen, Z.; Abouseif, Y.; Huang, Y.; Bie, Z. Pumpkin rootstock improves the growth and development of watermelon by enhancing uptake and transport of boron and regulating the gene expression. *Plant Physiol. Biochem.* **2020**, *154*, 204–218. [CrossRef] [PubMed]
71. van Mourik, H.; Dijk, A.D.J.; Stortenbeker, N.; Angenent, G.C.; Bemer, M. Divergent regulation of *Arabidopsis SAUR* genes: A focus on the SAUR10-clade. *BMC Plant Biol.* **2017**, *17*, 245. [CrossRef] [PubMed]
72. Qiu, T.; Qi, M.; Ding, X.; Zheng, Y.; Zhou, T.; Chen, Y.; Ning, H.; Zhu, M.; Bian, H.; Wang, J. The SAUR41 subfamily of SMALL AUXIN UP RNA genes is abscisic acid inducible to modulate cell expansion and salt tolerance in *Arabidopsis thaliana* seedlings. *Ann. Bot.* **2020**, *125*, 805–819. [CrossRef] [PubMed]
73. He, Y.; Liu, Y.; Li, M.; Lamin-Samu, A.T.; Yang, D.; Yu, X.; Izhar, M.; Jan, I.; Ali, M.; Lu, G. The *Arabidopsis* SMALL AUXIN UP RNA32 protein regulates ABA-mediated responses to drought stress. *Front. Plant Sci.* **2021**, *12*, 625493. [CrossRef]
74. Markakis, M.N.; Boron, A.K.; Van Loock, B.; Saini, K.; Cirera, S.; Verbelen, J.P.; Vissenberg, K. Characterization of a small auxin-up RNA (SAUR)-like gene involved in *Arabidopsis thaliana* development. *PLoS ONE* **2013**, *8*, e82596. [CrossRef]

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