



## Article

# Grafting Compatibility and Environmental Conditions on Soilless Eggplant (*Solanum melongena*) Grown in the Mediterranean Greenhouse

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**Abstract:** Grafting techniques represent an efficient tool to enhance plant growth and development. The study aims to explore the effects of different grafting combinations on the growth of eggplants under diverse greenhouse conditions. Eggplant cultivar Black bell (Bb) was employed as scion, while the hybrid F<sub>1</sub> Beaufort (Be) and *Solanum torvum* (To) were utilized as rootstock. The hypothesis behind this study pertains to grafting incompatibility with Beaufort F<sub>1</sub>. It postulates that this incompatibility can be mitigated by manipulating soil and greenhouse temperatures. The experimental factors encompassed plant combinations (Bb, Be/Bb and To/Bb), as well greenhouse and substrate temperature (both cold or heated). The Be/Bb combination showed higher values of plant vegetative traits, but it exhibited low grafting compatibility. Additionally, physiological analysis confirmed the presence of excessive growth and vegetative disorder within the Be/Bb combination. Examination of the xylem vessels revealed notable differences between the grafting combination involving Bb and the rootstock F<sub>1</sub> hybrid Be/Bb, compared to the one with To/Bb and the non-grafted Bb. Specifically, the area, diameter and number of xylem vessels were approximately 45% higher in Be/Bb than in To/Bb and the non-grafted Bb. Furthermore, a robust linear correlation was observed between plant height with morphometric and physiological traits, except fruits sets. This study lays the basis for a novel protocol for agriculture, addressing the excessive vegetative growth in rootstocks regulating air and substrate temperatures.

**Keywords:** rootstocks; temperatures; vegetable crops; *Solanum torvum*; Beaufort F<sub>1</sub>; water resistance



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## 1. Introduction

The grafting technique for horticultural crops is widely considered a valid tool due to its effectiveness for controlling pests and disease that are otherwise difficult to manage. This approach is particularly valuable when faced with the issues of the high cost of the phytochemicals, their environmental impact, risks associated with their application and, in some instances, limitations on their usage [1,2]. Consequently, the grafted plant represents a promising eco-friendly means for the control of adverse biotic factors, enhancing the cultivation of *Solanaceae* and *Cucurbitaceae* cultivars of high value in terms of product quality but sensitive to several root system pathogens [3–6]. Furthermore, the presence of eustressors in the soil, such as high salt concentrations, can induce abiotic stress in plants and cause significant damage for the crops [7,8]. The exploitation of grafting techniques extends to the tomato landraces as well, driven by the growing demand for these varieties. These landraces are highly valued for their superior organoleptic properties in contrast to commercial F<sub>1</sub> hybrids [9].

In the last several decades, several alternative techniques have emerged as potential solutions for managing soil-borne pests. These techniques encompass the soil solarization,

the trap plants, repellents and bio fumigation, but their individual efficacy has often been limited [10,11]. Despite the fact that recently, the use of herbaceous grafting has gained popularity in horticultural production, critical issues remain. These pertain to the selection of suitable rootstocks, the interactions between rootstock and scion, the development of protocols managing the grafted plant cultivation from the seedling stage in nurseries (topping and breeding with two or more branches) as well as the determination of the optimal plant density in relation to the increased vigour expressed during production phases. Moreover, the optimization of fertigation and water uptake represents an important concern, particularly within the context where water resource management assumes paramount importance for the future of agriculture [12–17]. The use of the grafting technique contributes to the development of sustainable agricultural practices. This contribution allows for the accumulation of knowledge useful for organic farming, thereby ensuring food production and safety, while preserving invaluable natural resources [18–22]. Combined with the grafting technique, the adoption of a soilless cultivation system represents a sustainable approach due to the significant reduction in arable land, urbanization, water scarcity, and climate changes [23]. Soilless cultivation presents several advantages to eggplant greenhouse cultivation, including the notably enhanced regulation of nutrients within the solution medium, thus improving plant nutrient uptake [24]. As it is widely recognized, beyond the influence of the solution medium, the air temperature and substrate conditions also play crucial roles in a soilless eggplant cultivation system [25].

The selection of suitable rootstocks for commercial use presents an ongoing challenge due to the uncertain performance of the genetically characterized materials in terms of their tolerance or resistance to adverse factors. Therefore, the prediction of the agronomic outcomes of the chosen plant materials becomes uncertain, encompassing factors such as the plant precocity, the production capacity, cycle length, the structure reproductive organs and the organoleptic quality [26]. Molecular markers can sometimes aid in the detection of the aforementioned traits [27–31]. Although grafting is widely used in horticultural production, the effect of this technique has received limited consideration. The attention has mainly been focused on gauging the successes or failures in terms of engraftment in relation to the ability of the operator and the conditions in which the grafting operation is carried out. Therefore, an important challenge is determined by the manipulation of the plant tissues that can either mask or enhance the characteristics of the rootstock and its interactions with the scion. Concerning the mechanisms that control the agronomic and functional performance of the grafted plants, it is important to identify and acquire an in-depth understanding on how environmental conditions impact these mechanisms during the cultivation phase. Additionally, the environmental conditions inside the greenhouse and the agronomic practices adopted can directly influence plant cultivation [32–35].

Although grafting offers several benefits for the horticultural production, it also poses technical, biological and economic implications of the production process of the grafted plants and their use. In addition to the previously cited issues regarding the rootstock development, the critical points which were most frequently reported concerns the following: a lack of graft rooting; reduced or excessive vigour; the cost of seedlings; more difficult and burdensome production protocols than those related to the production of seedlings from seeds in nurseries; excessive resistance or tolerance expressed by rootstocks; the incomplete knowledge of the behaviour and characteristics of rootstocks and the possible low affinity of rootstocks with subsequent functional imbalances. The selection of the growing substrate is a crucial point for promoting the plant growth of grafted plants. As reported by several authors, the appropriate substrate can stimulate the root system to grow deeply, which is essential for the establishment of a healthy and robust grafted plant [36,37]. Furthermore, in the case of eggplant, using interspecific rootstock or wild relatives can enhance plant vigour and promote growth [38].

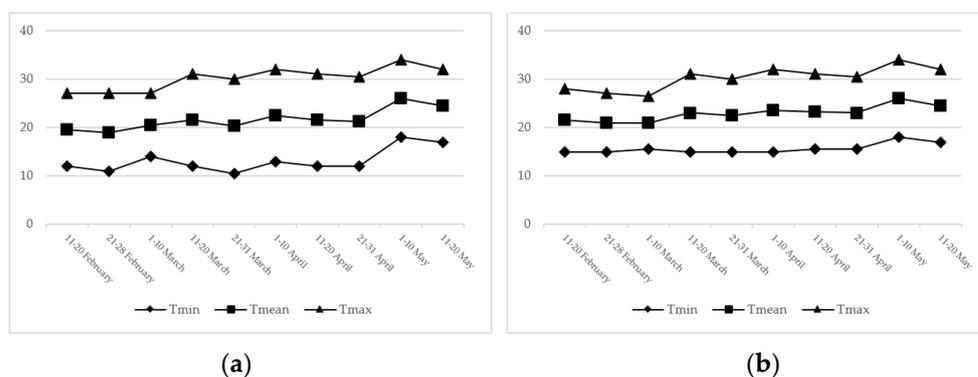
In this framework, the aim of the present work is the evaluation of different environmental conditions, represented by the greenhouse and the soil substrate temperatures, in different grafting combinations of the eggplant cultivar, Black Bell, used as scion. This

cultivar was grafted with the interspecific *S. lycopersicum* F1 hybrid Beaufort, and with the well-known *S. torvum*. The environmental conditions tested included the cold and heated greenhouse and the application of refrigerated or heated soil. The investigation performed was supported by a morphometric evaluation of the vegetative plant traits followed by a physiological analysis related to water uptake. Furthermore, a histological analysis of the xylem vessels was also performed. The study aims to lay the basis for the development of new sustainable protocols of cultivation in the perspective of the organic farming for the future of agriculture.

## 2. Materials and Methods

### 2.1. Plant Material and Experimental Design

The experimental trial was carried out during the winter season in the experimental greenhouse belonging to the Department of Agriculture, Food and Environment and (Di3A) of the University of Catania (UNICT). The greenhouse was situated 50 m above the sea level, in Catania (37°24'33" N, 15°03'32" E). The plant material consisted of the eggplant cv Black Bell (Bb), which was used as scion. It was grafted with the interspecific tomato hybrid F1 Beaufort (Be), and *Solanum torvum* (To), which were used as rootstock (Figure 1).



**Figure 1.** Temperatures recorded inside the cold and in the heated greenhouses ((a,b), respectively). Tmin: minimum temperature; Tmean: mean temperature; Tmax: maximum temperature.

The non-grafted counterpart of the Black Bell cultivar was employed as the control for the trial. The first experimental factor was the genotype (GE), represented by the different plant combinations tested, which were Bb, Be/Bb and To/Bb. The second factor was the greenhouse temperature (GT), while the third one was the substrate temperature (ST) used, which was heated or not. The experimental design employed was split plot arrangement with randomized blocks of each of the two plants, with four repetitions.

For the soilless cultivation, growing containers with a dimension of 30 cm depth and 40 cm width were used, spaced 1.0 m apart. Within each container, plants were positioned 45 cm apart, resulting in a plant density of 2.3 plants  $m^{-2}$ .

The sowing was performed in cellular trays in December 2020 while the grafting was carried out in the seedlings, 30 days after the sowing. The transplanting was carried out in the middle of February 2021 when the plantlets reached the phenological stage of 3–4 true leaves. The trial was stopped on May 25, at the beginning of the fruiting phase, after 90 days from transplantation. Throughout the growth cycle, essential cultivation practices were implemented in accordance with organic nutrition protocols. Specifically, each plant received 36 L of water and nutrients, excluding microelements. Over the growth period, a total of approximately 6 g of nitrogen (N), 2 g of phosphorus (P) and 9 g of potassium (K) were supplied per plant.

### 2.2. Greenhouse Temperature Set Up

The second experimental factor under examination was the greenhouse temperature (GT), categorized into two conditions: cold (CG) and heated greenhouse (WG). The latter

maintained a minimum air temperature above 15 °C. Inside the cold greenhouse (CG), the mean temperature varied from 19 °C to 26 °C for the third week of February and the first one of May, respectively (Figure 1a). On the other hand, the mean temperature recorded in the heated greenhouse (WG) varied from 21 °C for the third week of February and the first one of March to 26 °C, which were registered in the first week of May (Figure 1b). The thermal air and substrate conditions resulted in average air/substrate temperatures of 18/22 °C (CG/CS), 15/20 °C (CG/WG), 20/20 °C (WG/CS) and 20/24 °C (WG/WS). The minimum, maximum and mean temperatures of the thermal air inside the cold and heated greenhouse were recorded using the USB data logger (Testo, 174-T, Lenzkirch, Germany) as shown in Figure 1.

### 2.3. Substrate Temperature Set Up

The third experimental factor was the substrate temperature (ST). In both greenhouses, plants were cultivated using soilless culture techniques, utilizing specially designed growing containers. These containers were equipped with a pipe system that circulated water at room temperature (CS) or heated to 40 °C (WS). The growing medium consisted of a combination of peat and sand, both authorized for the organic farming, mixed in a 1:1 volume ratio. To ensure the water and nutritional elements saturation in the substrate, a localized fertigation system was employed, delivering the nutrient solution of 4 L h<sup>-1</sup> per plant.

### 2.4. Plant Morphometric Characterization

The growth and development processes of the plants were assessed using the international IBPGR descriptors for eggplant. Special emphasis was given to traits that are directly influenced by the grafting such as the ratio between the diameter of the graft and that of the rootstock, which serves as an index of affinity. The descriptors adopted are listed in Table 1.

**Table 1.** Descriptors analysed for the trial with their relative code and unit of measure.

Code	Descriptor
PH	Plant height (cm)
PSD	Stem diameter (mm)
PLN	Number of leaves (n)
PFN	Number of flowers (n)
PLW	Weight of leaves (g)
PLD	Leaves dry matter (%)
PSW	Stem weight (g)
PSDR	Stem dry matter (%)
FS	Number of fruit set (n)
FSW	Weight of fruit set (n)
WP	Water potential (-MPa)
RWC	Relative water content (%)
AXVs	Area of the xylem vessels (µm <sup>2</sup> )
DXVs	Diameter of the xylem vessels (µm)
NXVs	Number of the xylem vessels (vessels mm <sup>-2</sup> )

### 2.5. Physiological Analysis

During the final phase of the trial, a physiological analysis was conducted. This analysis involved the calculation of key parameters, specifically the foliar water potential (WP), which is expressed in -MPa, and the relative water content (RWC), expressed as a percentage. The determination of the first parameter (WP) was carried out throughout the Scholander pressure chamber (PMS Instrument Company, PMS-600, Albany, OR, USA). A transversal leaf slice was collected and subsequently introduced into the pressure chamber. The end of the petiole was left outside the chamber. Nitrogen gas was then blown inside the pressure chamber for stimulating the movement of the free water present in the leaf tissue

along the ribs. Once the pressure (bar) built up, it determined the appearance of water drop in the cutting surface of the petiole. Conversely, the relative water content was detected on a leaf sample at the complete growth stage (10°–12° leaf starting from the bottom). For each leaf, the fresh weight (Fw) was registered at the time of sampling, while the weight at the maximum turgor (Mt) achieved via the immersion of the petiole in water was also detected. The hydrated leaf was then placed to dry in the oven at a temperature of 70 °C in order to determine the dry weight (Dw). The data were used according to the following formula developed by Turner [39], for calculating the relative water content (RWC):

$$\text{RWC} = ((\text{Fw} - \text{Dw}) / (\text{Mt} - \text{Dw}) \times 100)$$

The index represents the water content in the leaf at the time of sampling as a percentage of the water contained in the fully hydrated tissues. Both the WP and RWC measurements were registered at 12.00 a.m. and in three moments corresponding to the time in which irrigation finished (T0), in addition to 20 and 40 h after the irrigation (T20 and T40, respectively). During the whole growing cycle (from transplanting to harvesting the first fruit), the thermal values of the air and the substrate were continuously registered.

### 2.6. Histological Analysis

The sections of the samples for the histological analysis were obtained using a manual microtome (Leica, RM2125RTS, Wetzlar, Germany) and they were adjusted at a thickness of about 20 µm. The colouring phase was conducted through the immersion of the tissue samples in the safranin dye for two seconds. Afterwards, the tissues were immersed in 100% ethanol and then in the fast green dye for 5 s. The fast green staining was repeated two times. The use of the safranin dye was to highlight the xylem vessels, while the fast green dye targeted the parenchymatic tissues. All the grafting combinations were observed through a compound optical microscope (Nikon DM500, Japan) equipped with a camera at a scale of 30 and 10 µm, respectively, to assess the distribution of the xylem vessels. The area and the diameter of the xylem vessels were registered using the software ImageJ (National Institute of Health, Bethesda, MD, USA).

### 2.7. Data Analysis

Data were statistically processed by calculating the analysis of variance (ANOVA) with the stepwise multiple comparison procedures of Student–Newman–Keuls, with different replicates. Three-way ANOVA was performed to assess the variance of the morphometric data, while the four-way ANOVA was applied for the physiological data. ANOVA was followed by Tukey’s multiple comparisons test. The software adopted for the previously mentioned statistical tests was CoStat version 6.4 (CoHort software, Birmingham, UK). The statistical significance was assigned for *p*-values < 0.05. Subsequently, the means of the different rootstock–scion combinations with different greenhouse and substrate temperatures were used for the Pearson’s correlation and for the Principal Component Analysis (PCA) to assess the sample distribution in relation to the GT and ST adopted. The software used for the Pearson’s correlation and for the PCA was IBM SPSS version 27 (IBM, Armonk, NY, USA).

## 3. Results

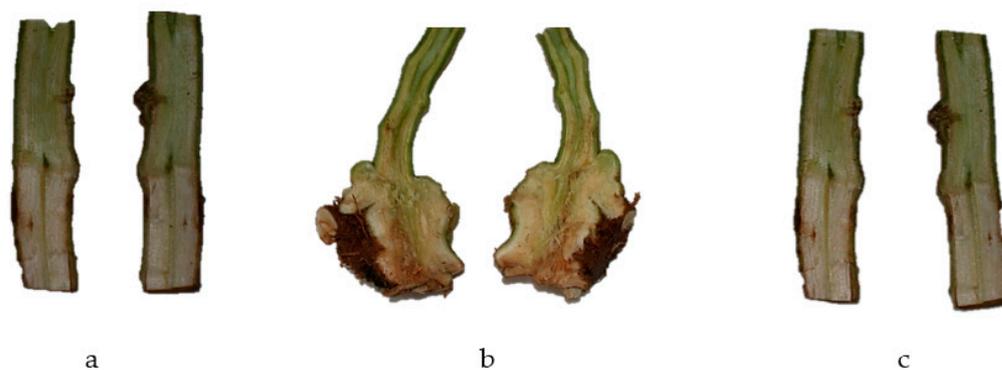
### 3.1. Temperature Recorded

The temperatures, as shown in Figure 1, impacted the growth and development of the various rootstock–scion combinations tested. This effect was expressed by the different minimum temperatures observed in the cold and heated greenhouses (CG and WG, respectively).

### 3.2. Morphometric Characterization

Regarding the morphometric traits examined, for the plant height (PH), we ascertained a significant variation among the tested genotypes, ranging from 57.83 cm for Bb, to

79.67 cm for Be/Bb. On the other hand, the plant stem diameter (PSD) varied significantly among the genotypes and its values ranged from 13.63 mm for Bb, to 16.76 mm for Be/Bb. Conversely, for the affinity index (AI) between the rootstocks and the scion adopted, we observed a significant interaction between the genotype and the soil temperature used ( $GE \times ST$ ), and its values varied from 0.44 to 0.96, for Be/Bb and To/Bb, respectively, cultivated both in WG with WS (Table 2; Figure 2).



**Figure 2.** The different genotypes tested, which were (a) Bb, (b) Be/Bb and (c) To/Bb. Noteworthy, in (b): the grafting disaffinity between the rootstock Beaufort (Be) and the scion Black Bell (Bb).

Concerning the plant leaves number (PLN), we observed a significant difference among the genotypes, varying from 17.88 to 26.83 leaves for Bb and Be/Bb, respectively (Table 2). Additionally, we registered a significant interaction between the second and the third experimental factors ( $GT \times ST$ ), and its value varied from 14.33 leaves for Bb grown in WG adopting the CS, to 33.83 leaves for Be/Bb in the CG grown with the CS (Table 2). Values higher than 20.0 leaves were registered for Bb only grown by WG/WS, and for both Be/Bb and To/Bb cultivated by CG with all the ST, and by WG only with the WS.

Regarding the number of flowers per plant (PFN), its value exhibited variation across the different genotypes, ranging from 7.21 to 10.44 flowers for Bb and To/Bb, respectively. Concerning the plant leaf weight (PLW), we detected a significant interaction between the first and the second experimental factors ( $GE \times GT$ ), and its values varied, in relation to the means of the genotypes, from 229.14 g for To/Bb to 415.61 g for Be/Bb. PLW varied significantly in relation to the ST, ranging from 254.5 g to 348.9 g for the CS and WS, respectively. For the plant leaves dry matter (PLD), we observed a significant interaction between the genotype and the soil temperature ( $GE \times ST$ ). The PSD values varied among the means of genotypes from 14.57% to 19.41% for Be/Bb and To/Bb, respectively. PLD values higher than 18% were registered for Bb and To/Bb grown in CG with all the ST, and in WG, only adopting the CS. Additionally, we registered values lower than 15.0 for Be/Bb cultivated by CG with all the ST tested, and in the WG, only using the WS.

With reference to the plant stem weight (PSW), a significant interaction of  $GE \times GT$  was detected. PSW values showed a significant interaction of  $GE \times GT$ , varying from 106.7 g to 281.7 g for Bb and Be/Bb, both cultivated in the CG, respectively. In relation to the means of the genotypes, PSW varied from 111.03 g for Bb to 242.37 g for Be/Bb. PSW values higher than 200.0 g were registered for Be/Bb grown in CG in each ST, in addition to the WG, only for the WS conditions. Also, for To/Bb, we observed PSW values higher than 200.0 g but only in CG with the WS, and in WG, adopting the CS. Contrarily, PSW values lower than 200.0 g were registered for Bb grown in each GT and for each ST, in addition to Be/Bb grown by WG, adopting the CS, and for To/Bb grown in CG by CS, and in WG with WS. With regard to plant stem dry matter, we detected a significant variation among the genotypes, varying from 16.06% for Be/Bb to 20.92% for To/Bb (Table 2). Concerning the number of fruits set (FS), it showed not significantly variation among genotypes and its value ranged from 1.25 to 1.96 fruits per plant (Table 2).

**Table 2.** Variation of the morphometric traits in relation to the greenhouse temperatures (GTs) cold and hot (CG and WG), in addition to the soil ones (STs), cold or hot (CS and WS).

Trait	Bb					Be/Bb					To/Bb					Mean			
	CG		WG		Mean	CG		WG		Mean	CG		WG		Mean	CG	WG	CS	WS
	CS	WS	CS	WS		CS	WS	CS	WS		CS	WS	CS	WS					
PH	58.50	54.17	52.83	65.83	57.83	81.83	86.00	72.50	78.33	79.67	60.33	63.00	58.50	65.77	61.90	67.31	65.63	64.08	68.85
PSD	13.63	13.34	13.88	13.67	13.63	16.77	18.38	16.15	15.73	16.76	16.19	13.18	16.42	15.75	15.39	15.25	15.27	15.51	15.01
AI	1	1	1	1	1.00	0.54	0.45	0.57	0.44	0.50	0.90	0.92	0.95	0.96	0.93	0.80	0.82	0.83	0.80
PLN	17.17	18.67	14.33	21.33	17.88	33.83	28.33	19.50	25.67	26.83	23.00	21.00	18.77	21.77	21.14	23.67	20.23	21.10	22.80
PFN	7.83	8.50	6.00	6.50	7.21	10.50	12.17	9.00	9.33	10.25	11.17	9.33	11.27	10.00	10.44	9.92	8.68	9.30	9.31
PLW	190.00	201.8	164.2	485.2	260.30	453.8	547.18	333.7	327.67	415.61	193.83	241.0	191.27	290.3	229.14	304.63	298.7	254.5	348.9
PLD	18.19	18.54	18.61	17.05	18.10	13.99	14.57	15.71	14.01	14.57	19.09	20.51	20.37	17.68	19.41	17.48	17.24	17.66	17.06
PSW	100.95	112.5	101.5	129.2	111.03	257.9	305.42	194.5	211.67	242.37	134.77	257.9	305.42	194.5	223.14	194.90	189.5	182.50	201.8
PSDR	18.58	22.03	21.21	19.33	20.29	16.31	17.14	16.49	14.29	16.06	22.00	21.72	21.02	18.92	20.92	19.63	18.54	19.27	18.91
FS	0.00	2.33	1.00	1.67	1.25	1.67	2.67	1.50	2.00	1.96	3.67	0.00	2.33	1.00	1.75	1.72	1.58	1.70	1.61
FSW	0.00	73.27	36.17	47.33	39.19	68.45	165.30	458.2	430.00	280.48	114.77	0.00	73.27	36.17	56.05	70.30	180.2	125.14	125.3

Significancy of the differences								
Trait	GE	GT	ST	GE × GT	GE × ST	GT × ST	GE × GT × ST	
PH	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
PSD	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
AI	***	n.s.	*	n.s.	**	n.s.	n.s.	
PLN	***	*	n.s.	n.s.	n.s.	*	n.s.	
PFN	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
PLW	**	n.s.	*	*	n.s.	n.s.	n.s.	
PLD	***	n.s.	n.s.	n.s.	**	n.s.	n.s.	
PSW	***	n.s.	n.s.	*	n.s.	n.s.	n.s.	
PSDR	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
FS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
FSW	**	Ns	n.s.	n.s.	n.s.	*	n.s.	

\*\*\*, \*\* and \* indicate  $p \leq 0.001$ ,  $p \leq 0.01$  and  $p \leq 0.05$ , respectively. The analysed traits were the plant height (PH), the stem diameter (PSD), the affinity index (AI), the leaves and flower number (PLN and PFN, respectively), the plant leaves' weight and dry matter (PLW and PLD, respectively), the stem weight and dry matter (PSW and PSDM, respectively) and the number and the weight of fruits set (FS and FSW, respectively). The first experimental factor was the genotype (GE), the second one was the greenhouse temperature (GT), while the third one was the substrate temperature (ST).

Finally, for the weight of the fruits set (FSW), we observed a significant interaction between the second and the third experimental factors (GT × ST), registering values higher than 400.0 g only for Be/Bb grown in WG, adopting both the ST. Moreover, FSW values lower than 100.0 g were registered for Bb in both the GT and for each ST, as well as for Be/Bb cultivated in CG adopting the CS, in addition to To/Bb grown in CG using the WS and in the CG for each ST. We ascertained a significant variation of the FSW value in relation to the genotypes, which ranged from 36.19 g to 280.0 g for Bb and Be/Bb, respectively (Table 2).

### 3.3. Physiological Evaluation

For the water potential (WP), we observed a significant interaction among all the experimental factors tested, which were the genotype, the greenhouse, the soil temperature and the time after irrigation (GE × GT × ST × TAI). WP value varied from −0.55 MPa for Be/Bb grown in CG with the CS at T0, to −20.9 MPa for Bb cultivated in CG with the WS in T40 (Table 3). WP values higher than −15.0 MPa were observed for Bb grown in CG adopting the CS, at T40, as well as for To/Bb cultivated by CG using the WS, at T40. Instead, WP values lower than −2 MPa were registered for Be/Bb grown by CG with both the STs (CS and WS) for all the TAI detected, as well for the same genotype cultivated in the WG, under both the STs, but only at T0 and T20 (Table 3).

**Table 3.** Variation of the water potential (-MP) in relation to the different greenhouse and soil temperatures (GTs and STs).

		T0	T20	T40	Mean
CG CS	Bb	2.35 ± (1.25)	5 ± (1.8)	16.55 ± (0.35)	7.97 ± (7.55)
	Be/Bb	0.55 ± (0.15)	0.95 ± (0.05)	1.65 ± (0.05)	1.05 ± (0.56)
	To/Bb	2.65 ± (0.05)	3.5 ± (0.6)	13.5 ± (0.80)	6.55 ± (6.03)
<b>Mean</b>		1.85 ± (1.14)	3.15 ± (2.05)	10.57 ± (7.87)	5.19 ± (3.65)
CG WS	Bb	14.05 ± (0.55)	14.85 ± (1.35)	20.9 ± (1.20)	16.60 ± (3.75)
	Be/Bb	1.1 ± (0.1)	1.35 ± (0.65)	1.85 ± (0.15)	1.43 ± (0.38)
	To/Bb	2.8 ± (0.10)	14.4 ± (0.9)	19.75 ± (1.55)	12.32 ± (8.66)
<b>Mean</b>		6.13 ± (7.29)	10.2 ± (7.67)	14.167 ± (10.68)	10.12 ± (7.82)
WG CS	Bb	2.5 ± (0.10)	3.5 ± (1.0)	6.4 ± (0.2)	4.13 ± (2.03)
	Be/Bb	0.65 ± (0)	1.4 ± (0.20)	2.85 ± (0.75)	1.63 ± (1.12)
	To/Bb	11.05 ± (0.55)	11.8 ± (0)	12.55 ± (0.25)	11.80 ± (0.75)
<b>Mean</b>		4.88 ± (5.80)	5.57 ± (5.50)	7.25 ± (4.88)	5.85 ± (5.30)
WG WS	Bb	3.05 ± (0.15)	13 ± (0.5)	13.7 ± (0.10)	9.92 ± (5.96)
	Be/Bb	1.95 ± (0.05)	2 ± (0)	2.1 ± (1)	2.02 ± (0.08)
	To/Bb	3.8 ± (0)	8.45 ± (0.55)	13.1 ± (1.1)	8.45 ± (8.66)
<b>Mean</b>		2.93 ± (0.93)	7.82 ± (5.53)	9.63 ± (6.53)	6.80 ± (4.20)
<b>Mean per genotype</b>					
		<b>T0</b>	<b>T1</b>	<b>T2</b>	
<b>Bb</b>		5.60 ± (5.94)	9.09 ± (6.67)	14.39 ± (6.09)	
<b>Be/Bb</b>		1.06 ± (0.64)	1.43 ± (0.43)	2.11 ± (0.53)	
<b>To/Bb</b>		5.19 ± (4.23)	9.54 ± (4.70)	14.71 ± (3.38)	

Table 3. Cont.

Significancy of the differences	
GE	***
GT	***
GE × GT	***
ST	***
GE × ST	***
GT × ST	***
GE × GT × ST	***
TAI	***
GE × TAI	***
GT × TAI	***
GE × GT × TAI	***
ST × TAI	***
GE × ST × TAI	***
GT × ST × TAI	***
GE × GT × ST × TAI	***

\*\*\* indicates  $p \leq 0.001$ , respectively; 0 (T0), 20 (T20) and 40 (T40) represents the time after irrigation in hours. Numbers in brackets indicate the standard deviation.

On the other hand, for the relative water content (RWC), the interaction GE × GT × ST × TAI was also detected, and its value varied from 75.50% for Bb cultivated by CG, using the WS, at T40, to 99.60% for Be/Bb grown by CG with the CS, at T0 (Table 4). RWC values lower than 80.0% were observed for Bb and To/Bb grown by CG applying the WS, at T40, as well as for To/Bb cultivated by WG, adopting both the STs, at T40 (Table 4).

Table 4. Variation of the relative water content (%) in relation to the different greenhouse and soil temperatures (GTs and STs).

		T0	T20	T40	Mean
CG CS	Bb	92.80 ± (1.18)	88.93 ± (6.66)	87.06 ± (0.39)	89.60 ± (2.93)
	Be/Bb	99.60 ± (0.10)	96.02 ± (2.85)	95.88 ± (1.27)	97.17 ± (2.11)
	To/Bb	93.74 ± (3.86)	92.40 ± (0.91)	82.99 ± (0.65)	89.71 ± (5.86)
<b>Mean</b>		95.38 ± (6.38)	92.45 ± (3.55)	88.64 ± (6.59)	92.16 ± (4.34)
CG WS	Bb	97.27 ± (2.49)	94.81 ± (0.55)	75.50 ± (1.21)	89.19 ± (11.92)
	Be/Bb	98.57 ± (0.54)	98.53 ± (0.27)	96.69 ± (0.41)	97.93 ± (1.07)
	To/Bb	99.15 ± (0.43)	85.66 ± (0.43)	79.58 ± (9.63)	88.13 ± (10.02)
<b>Mean</b>		98.33 ± (0.96)	93.00 ± (6.62)	83.92 ± (11.24)	91.75 ± (5.38)
WG CS	Bb	82.51 ± (5.27)	81.48 ± (5.79)	77.47 ± (4.18)	80.49 ± (2.66)
	Be/Bb	92.69 ± (1.78)	92.16 ± (3.38)	90.71 ± (0.31)	91.85 ± (1.03)
	To/Bb	91.20 ± (8.01)	85.06 ± (6.71)	78.91 ± (5.41)	85.06 ± (6.15)
<b>Mean</b>		88.8 ± (5.50)	86.23 ± (5.44)	82.36 ± (7.26)	85.80 ± (5.72)
WG WS	Bb	87.99 ± (1.60)	87.33 ± (3.50)	83.66 ± (5.79)	86.33 ± (2.33)
	Be/Bb	95.47 ± (0.62)	95.03 ± (0.09)	81.83 ± (2.64)	90.78 ± (7.75)
	To/Bb	93.95 ± (0.36)	86.12 ± (0.17)	78.30 ± (0.02)	86.12 ± (7.83)
<b>Mean</b>		92.47 ± (3.95)	89.49 ± (4.83)	81.26 ± (2.72)	87.74 ± (2.63)
Mean per genotype					
		T0	T1	T2	
<b>Bb</b>		90.14 ± (6.34)	88.14 ± (5.48)	80.92 ± (5.37)	
<b>Be/Bb</b>		96.58 ± (3.13)	95.44 ± (2.63)	91.28 ± (6.83)	
<b>To/Bb</b>		94.51 ± (3.34)	87.31 ± (3.42)	79.95 ± (2.10)	

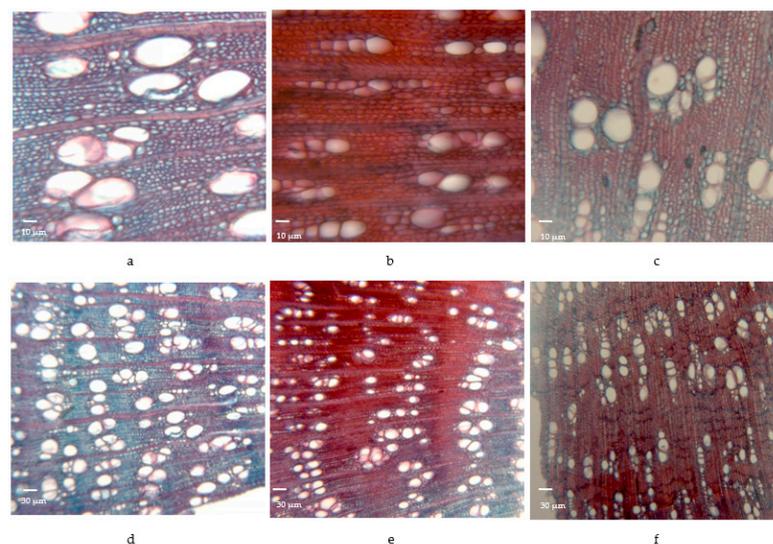
Table 4. Cont.

	Significancy of the differences
GE	***
GT	***
GE × GT	n.s.
ST	n.s.
GE × ST	n.s.
GT × ST	n.s.
GE × GT × ST	n.s.
TAI	***
GE × TAI	***
GT × TAI	n.s.
GE × GT × TAI	*
ST × TAI	***
GE × ST × TAI	n.s.
GT × ST × TAI	n.s.
GE × GT × ST × TAI	***

\*\*\* and \* indicate  $p \leq 0.001$  and  $p \leq 0.05$ , respectively; 0 (T0), 20 (T20) and 40 (T40) represents the time after irrigation in hours. The first experimental factor was the genotype (GE), while the second and the third ones were the greenhouse and substrate temperatures (GTs and STs, respectively). Numbers in brackets indicate the standard deviation.

### 3.4. Histological Analysis

With regard to the histological analysis, we ascertained a significant variation for the grafting combination in relation to the non-grafted biont (Figure 3). The area of the xylem vessels (AXVs) varied significantly among the genotypes, and its value fluctuated from  $350.17 \mu\text{m}^2$  to  $682.39 \mu\text{m}^2$  for Bb and Be/Bb, respectively (Table 5; Figure 3). With regard to the diameter of the xylem vessels (DXVs), we ascertained a variation among the genotypes, and its value ranged from  $21.98 \mu\text{m}$  for the non-grafted Bb to  $43.16 \mu\text{m}$  for Be/Bb (Table 5). Concerning the number of xylem vessels (NXVs), it varied significantly in relation to the rootstock combination, varying from  $7.17 \text{ vessels mm}^{-2}$  for To/Bb to  $11.33 \text{ vessels mm}^{-2}$  for Be/Bb (Table 5).



**Figure 3.** Transversal cross-section of the basal side of the stem for each genotype tested, which were (a,d) Bb; (b,e) Be/Bb; (c,f) To/Bb. The picture was obtained with the optical microscope equipped with a camera at the scale of 10× and 30× view, respectively.

**Table 5.** Variation of the xylem measurement in stem cross-sections of the genotype Bb, Be/Bb and To/Bb.

Genotype	AXVs	DXVs	NXVs
Bb	350.17 ± (32.84) ab	21.98 ± (4.61) b	8.17 ± (1.04) b
Be/Bb	682.39 ± (71.92) a	43.16 ± (1.22) a	11.33 ± (2.25) a
To/Bb	366.46 ± (34.42) b	28.61 ± (6.39) b	7.17 ± (1.26) b

Means followed by the same letter in each column are not significantly different according to the Tukey's multiple range test ( $p \leq 0.05$ ). The analysed traits were: the area of xylem vessels (AXVs) expressed in  $\mu\text{m}^2$ , their diameter (DXVs) and their number per unit of area (vessels  $\text{mm}^{-2}$ ). Number in brackets indicates the standard deviation.

### 3.5. Pearson's Correlation and PCA Analysis

The plant height (PH) showed the highest correlation among the traits, displaying a strong linear relationship with all the evaluated parameters, except for the number of fruits set (FS). All traits were positively correlated with PH, apart from AI, PLD, PSDR and WP, for which we ascertained a strong negative correlation (Table 6). On the other hand, the FS did not show any correlation. With regard to the physiological parameters, the water potential (WP) was positively correlated with the AI, the PLD and the PSDR, while it was negatively correlated with the PH, PSD, PLN and PSW (Table 6). On the other hand, the relative water content (RWC) showed a positive correlation with the PH, PSD, PFN, PLN, PLW and PSW, while it was negatively correlated with the AI, PLD and PSDR.

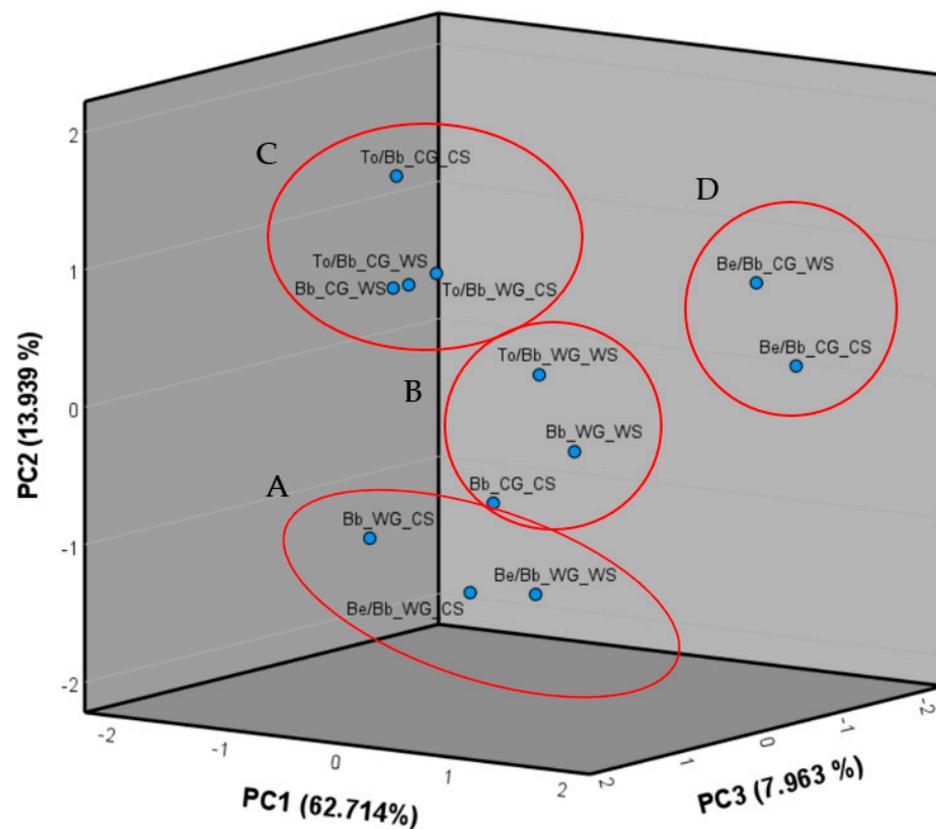
**Table 6.** Pearson's correlation among the analysed morphometric and physiological traits.

	PH	PSD	AI	PFN	PLN	PLW	PLD	PSW	PSDR	FS	FSW	WP	RWC
PH	1.000												
PSD	0.717 **	1.000											
AI	-0.915 **	-0.717 **	1.000										
PFN	0.502 *	0.802 **	-0.481	1.000									
PLN	0.856 **	0.643 *	-0.723 **	0.598 *	1.000								
PLW	0.846 **	0.501 *	-0.630 *	0.234	0.724 **	1.000							
PLD	-0.863 **	-0.542 *	0.852 **	-0.159	-0.702 **	-0.750 **	1.000						
PSW	0.940 **	0.820 **	-0.858 **	0.670 **	0.832 **	0.761 **	-0.736 **	1.000					
PSDR	-0.826 **	-0.496	0.833 **	-0.156	-0.572 *	-0.612 *	0.907 **	-0.677 **	1.000				
FS	0.251	0.262	-0.284	0.432	0.411	0.218	-0.090	0.269	0.158	1.000			
FSW	0.505 *	0.321	-0.708 **	0.162	0.186	0.212	-0.511 *	0.402	-0.625 *	0.243	1.000		
WP	-0.714 **	-0.638 *	0.779 **	-0.187	-0.505 *	-0.484	0.771 **	-0.631 *	0.755 **	-0.036	-0.491	1.000	
RWC	0.822 **	0.613 *	-0.776 **	0.600 *	0.818 **	0.658 **	-0.697 **	0.780 **	-0.589 *	0.378	0.323	-0.483	1.000

\*\* and \* indicate, respectively, differences at  $p \leq 0.05$  and  $p \leq 0.01$  probability levels.

With regard to the analysis of the principal components (PCA), we ascertained a significant positive correlation for PH, PSW, PLN, RWC, PSD and PLW with the first axe (Table 7). Conversely, the AI, PLD, PSDR and WP were negatively correlated. Furthermore, the second axe showed a significant positive correlation with the PFN and FS, while the third one was positively correlated with the FSW (Table 7).

The PCA plot highlighted the distribution of the genotypes in relation to the GT and ST adopted, clustering them into four different groups (A, B, C, D), as shown in Figure 4. In group A, the rootstock–scion combinations of Be/Bb, both cultivated by WG and adopting the CS and the WS, showed the highest FSW; therefore, they are close to the third axe (Figure 4). Additionally, Be/Bb grown by WG, with CS and WS, showed high values of PH and RWC and low values of AI and WP. In group B, the genotypes To/Bb grown in WG using the WS, the Bb genotype cultivated by WG with WS, and finally, the Bb grown in CG with the CS all showed the highest values of AI, high values of WP, low values of RWC, and intermediate values of PH (Figure 4). Moreover, the C group is correlated with the second axe, and the genotype To/Bb grown in the CG with the CS exhibited the highest FS value. Subsequently, in group D, the genotypes Be/Bb—both cultivated by CG using CS and WS—showed the highest PH and PSD values and therefore exhibited a strong correlation with the first axe (Figure 4).



**Figure 4.** Principal Component Analysis was employed for studying the distribution patterns in relation to the three experimental factors tested. The first one pertains to the genotype variations (GE), for which we distinguish the cultivar Black Bell in its non-grafted form (Bb), and the Bb used as scion with the interspecific hybrid Beaufort F1 (Be/Bb) in addition to *S. torvum* (To/Bb). The second experimental factor was the greenhouse temperature, which was cold and the heated (CG and WG, respectively). The third factor encompasses substrate temperatures, which was cold and hot (CS and WS, respectively). The four groups (A, B, C and D) represent the different clusters generated as a result of the correlation matrix between the examined traits and the three components extracted.

**Table 7.** Correlation matrix and percentage of variance (Var) among the analysed traits and the extracted components.

	PC1	PC2	PC3
PH	<b>0.981</b>	−0.042	−0.099
PSD	<b>0.795</b>	0.293	0.070
AI	<b>−0.951</b>	0.118	−0.221
PFN	0.566	<b>0.700</b>	0.128
PLN	<b>0.854</b>	0.277	−0.267
PLW	<b>0.777</b>	−0.046	−0.414
PLD	<b>−0.877</b>	0.371	0.103
PSW	<b>0.938</b>	0.154	−0.100
PSDR	<b>−0.817</b>	0.525	0.004
FS	0.304	<b>0.645</b>	0.375
FSW	0.545	−0.322	<b>0.725</b>
WP	<b>−0.760</b>	0.366	−0.110
RWC	<b>0.848</b>	0.227	−0.114
Var (%)	62.714	13.939	7.963

#### 4. Discussion

Our findings provide an extended comprehension of the performance of grafted eggplants under different growing conditions. The analysed traits were chosen to highlight the variations in vegetative growth among the different rootstock–scion combinations. In alignment with this context, Johnson et al. [40] assessed the growth performance of *S. melongena* scion grafted onto Beaufort F1 rootstock, which is one of the rootstocks employed in our study. They measured stem diameter and plant height after approximately 85 days from transplanting and compared the results between grafted and non-grafted plants. In this framework, our results were consistent with those achieved by Johnson et al. [40]. Moreover, similar to the previously cited study, we adopted a shorter growing cycle to facilitate a direct comparison of various environmental conditions, aiming to mitigate the effects of excessive Beaufort F1 rootstock growth. Furthermore, our study builds upon their findings by examining additional aspects of plant performance under different growing conditions.

Regarding the affinity index, it serves as a gauge of the compatibility between the scion and rootstock in a grafted plant. This index reflects the degree to which the two plant parts can establish a functional vascular connection, which is essential for the transport of water and for the nutrients uptake in the plant vascular system [41]. In this context, our manuscript delved into the analysis of the affinity index (AI) of grafted eggplants under varying greenhouse and soil temperatures. In contrast to the outcomes of Goto et al. [42], who tested the behaviour of Beaufort F1 and *S. torvum* rootstocks grafted with tomato scions, our observations regarding the AI significantly diverged from their findings. Specifically, they reported AI values close to 1.00 only for the interspecific grafting combination with the rootstock Beaufort F1, while they ascertained intermediate AI values for the grafting with *S. torvum*. On the other hand, we registered AI values close to 1.00 for the combination between *S. torvum* and Black Bell (To/Bb). This discrepancy in results can be attributed to the robust affinity between the *S. torvum* rootstock and the Black Bell scion, in contrast to the imbalance observed in the Be/Bb combination. As it shown in Figure 2, the incompatibility between Be/Bb is evidenced by the discrepant sizes of the rootstock and scion at the grafting point. The incompatibility detected at the grafting point was probably a result of the rootstock's robust root system, which had a high capacity for absorbing water and nutrients from the deeper soil layers.

Concerning the vegetative parameters that we registered after 90 days, we achieved higher values for the number of plant leaves than those obtained in the work of Poudel and Lee [43]. They reported around 13 leaves in 62 days for the ungrafted and self-grafted eggplant, along with approximately 12 leaves for various rootstock–scion combinations. Additionally, the results that we achieved are consistent to the ones in the study of Gökseven and Akbudak [44], who studied different rootstock–scion combinations in *S. melongena*. However, our study showed lower values of plant stem diameters, likely due to our 90-day evaluation period, contrasting with their 110-day analysis.

The different behaviour of Beaufort F1 and *S. torvum* rootstocks grafted with the Black Bell eggplant cultivar affects the physiological water relations of the grafted eggplant during the 40 h after irrigation. In this framework, the physiological analysis serves as a valuable tool for understanding the reasons behind the distinct responses in terms of vigour among the various grafting combinations. In this context, combinations involving Beaufort F1 (Be) rootstock showed lower water potential values and higher relative water content in leaves. This phenomenon could be attributed to the rootstock's excessive water absorption, leading to a vegetative imbalance characterized by hydropic leaf tissue and observed seepage from the leaf margins.

In our study, all the genotypes showed a significant increase in the water potential (WP) values from the time when irrigation occurred (T0) to 40 h post-irrigation (T40). RWC values were about 17 % higher on average compared to the work of Semida et al. [45], who examined *S. melongena* growth under deficit irrigation with nanoparticle foliar application. In reference to the work of Sanwal et al. [46], who evaluated salinity effects on tomato

grafted with eggplant rootstocks, we ascertained that the RWC values were significantly higher when the plants were well irrigated (at T0 moments) but the values were similar at T40 in relation to the ungrafted control that they used. Furthermore, we registered RWC values at T40, showing insignificant variations from the ones obtained in the manuscript of Plazas et al. [47], who tested eggplant relatives under drought stress. Concerning the water potential (WP), our results aligned with those achieved by Oda et al. [48], which evaluated the WP after 8 weeks from the grafting with different combinations using tomato as scion in *S. torvum* and eggplant rootstocks. Particularly, we obtained overlapping WP values at the T0 for the *S. torvum* grafting. However, notably higher values were evident at T20 and T40 conditions due to the extended duration without irrigation in comparison to the previously mentioned study. Notably, in our experiment, the 40 h period without irrigation had a notable effect on the water potential in leaves, resulting in an increase in its value. Consequently, the amount of water within the leaves decreased, as confirmed by the relative water content. The increase in WP values from T0 to T40 can be attributed to the reduced water content in the plant's tissues after 40 h without irrigation. This resulted in a higher pressure requirement for water to seep out from the leaf tissue during the WP analysis. Consequently, this shift led to a notable decrease in the relative water content in the leaf tissue from T0 to T40, as indicated by the physiological analysis. In this study, histological analysis was conducted with the intention of gaining a deeper understanding of the complex relationship between the rootstock and the scion, particularly within the context of grafting incompatibility observed in the Be/Bb combination. In this framework, histological analysis confirmed that the combination of Black Bell cultivar as the scion grafted onto the Beaufort F1 rootstock exhibited a higher vigour and water uptake compared to the grafting combination with *S. torvum* and the non-grafted Black Bell. The results of our study showed that the areas and diameters of the xylem vessels (AXVs and DXVs) in the Black Bell (Bb) and To/Bb grafting combinations were consistent with those reported by Magdaleno-Garcia et al. [49], who measured the area of xylem vessels in response to the application of zinc oxide nanoparticles. However, the Be/Bb genotype showed higher values for AXVs and DXVs, likely due to its higher vigour and the disequilibrium between the two bionts. In particular, the DXV values for Be/Bb were higher than those reported by Wu et al. [50], who analysed histological parameters of wild eggplants in response to *Verticillium* wilt. The notable increase in both the area and diameter of the xylem vessels in Be/Bb is probably associated with the greater water uptake capacity of the Beaufort F1 rootstock compared to *S. torvum* and the non-grafted plant. Increased water absorption applies pressure to the xylem vessels, resulting in their dilation. The increased water adsorption, as previously mentioned, can be attributed to the strong root system of Beaufort F1, as corroborated by the high fresh and dry weights reported by Johnson et al. [40].

## 5. Conclusions

The data acquired enables us to evaluate the impact of the affinity between the rootstock and the scion utilized on plant growth and development, and the water physiology in response to the tested environmental conditions.

Significant variations emerged in our study regarding the impact of greenhouse and soil temperatures on leaf count and fruit weight. Particularly, the Be/Bb combination exhibited a significant vegetative biomass increase under cold greenhouse conditions, while displaying the highest affinity index and resulting in the greatest fruit weight in the heated greenhouse. Our physiological and histological analyses further corroborated these variations. Specifically, the interspecific combination Be/Bb showcased the highest absolute water potential (WP) values, coupled with elevated relative water content, validating its excessive vegetative growth.

The main outcome of the present work is the mitigation of the huge plant vigour of the interspecific combination Be/Bb as a consequence of the reduction in the temperature of both thermal air and substrate. In this context, the grafting technique proved to be an

effective response to the various environmental conditions examined. This work allows us to establish a foundation for the development of innovative, sustainable cultivation protocols within the framework of organic farming, with a forward-looking perspective on the future of agriculture. Additionally, the utilization of suitable rootstock–scion combinations can promote better vegetative growth in plants, leading to improved water resource management.

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