agronomy

MDPI

Article

A Novel Graft between Pac Choi (*Brassica rapa* var. *chinensis*) and Daikon Radish (*Raphanus sativus* var. *longipinnatus*)

Tian Gong, Zachary T. Ray, Kylee E. Butcher, Zachary E. Black, Xin Zhao * and Jeffrey K. Brecht

Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA; tiangong@ufl.edu (T.G.); ray.ztyler@ufl.edu (Z.T.R.); kbutcher@ufl.edu (K.E.B.); zackblack@ufl.edu (Z.E.B.); jkbrecht@ufl.edu (J.K.B.)
* Correspondence: zxin@ufl.edu; Tel.: +1-352-273-4773

Received: 15 August 2020; Accepted: 16 September 2020; Published: 24 September 2020

check for updates

Abstract: Vegetable grafting has primarily been used in the commercial production of high-value crops in the Solanaceae and Cucurbitaceae families. In this study, we explored the feasibility of making a novel graft between pac choi (*Brassica rapa* L. var. *chinensis*) and daikon radish (*Raphanus sativus* L. var. *longipinnatus*) to create a plant with harvestable pac choi leafy vegetable above-ground, and a daikon radish taproot below-ground. 'Mei Qing Choi' pac choi (scion) was grafted onto 'Bora King' daikon radish (rootstock). Grafted pac choi–daikon radish plants did not show a decrease in SPAD value, canopy size, leaf number, leaf area, or above-ground weight compared with self-grafted pac choi plants. However, taproot formation was reduced in grafted pac choi–daikon radish plants, as shown by decreased taproot length, diameter, fresh weight, and dry weight compared with non- and self-grafted daikon radish plants. Surprisingly, grafting with radish increased the photosynthetic rate of the pac choi. This pilot study demonstrated the potential of creating a new pac choi–daikon radish vegetable product to help save growing space and minimize waste at consumption, as pac choi roots are not eaten and radish leaves are usually discarded. The inter-generic grafting between *B. rapa* var. *chinensis* and *R. sativus* var. *longipinnatus* could also provide a unique model system to help elucidate scion-rootstock synergy and above- and below-ground sink competition in horticultural crops.

Keywords: Brassicaceae; growth; mineral content; photosynthesis; rootstock; taproot

1. Introduction

Grafting has become an effective practice in the production of high-value solanaceous and cucurbitaceous vegetables to help overcome biotic and abiotic stresses and improve crop productivity [1–3]. Although grafting also has been used as a tool in plant physiology studies of Arabidopsis (*Arabidopsis thaliana* L.) [4], for accelerating the breeding work of common beans (*Phaseolus vulgaris* L.) [5], and for combating Verticillium wilt of globe artichoke (*Cynara cardunculus* L. subsp. *Scolymus*) [6], grafting in other vegetable species beyond Solanaceae and Cucurbitaceae is generally not practiced commercially. Interestingly, some attempts have been made to explore the feasibility of grafting vegetable plants in *Brassicaceae*. Oda et al. [7] tested inter-varietal, inter-specific, and inter-generic grafting among cabbage (*Brassica oleracea* L. var. *capitata*), kale (*Brassica oleracea* var. *sabellica*), kohlrabi (*Brassica oleracea* var. *gongylodes*), Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*), turnip (*Brassica rapa* subsp. *rapa*), Japanese mustard (Takana) (*Brassica juncea* L. var. *integrifolia*), and Japanese radish (*Raphanus sativus* L. var. *longipinnatus*) and obtained successful grafts. Particularly, an adhesive and hardener system was developed for making grafts between Chinese cabbage (scion) and turnip (rootstock) [8]. Recently, Chen et al. [9] evaluated the survival rate of cabbage grafted onto Chinese kale (*B. oleracea* Alboglabra group) rootstocks and assessed the feasibility of using grafting to improve cabbage head quality.

The effort of cruciferous vegetable grafting has not only broadened the potential use of vegetable grafting as a management tool, but also presents the possibility of creating a novel vegetable product with added value. In the case of grafted Chinese cabbage/turnip plants, the above-ground portion of Chinese cabbage—a leafy vegetable, and the below-ground portion of turnip—a root vegetable, can be harvested from the same plant. This type of rootstock–scion combination holds promise for space saving in small-scale intensive cultivation systems. Moreover, the grafted Chinese cabbage/turnip vegetable may possess added economic value with minimal waste, since many consumers may prefer not to eat turnip leaves and could be drawn to the novelty of this new product. However, the Chinese cabbage and turnip grafting study of Oda and Nakajima [8] only reported a 50% graft survival rate and observed restricted development of the Chinese cabbage head.

In this proof of concept study, we grafted the pac choi (*B. rapa* var. *chinensis*) scion onto the daikon radish (*R. sativus* var. *longipinnatus*) rootstock to generate a vegetable plant that produced a pac choi leafy vegetable above-ground and an edible daikon radish root below-ground. Pac choi and daikon radish are among specialty vegetables increasingly grown for local markets in the U.S. Although edible, daikon radish leaves are often discarded at consumption. Recent genetic studies supported the feasibility of making successful inter-generic grafts between *B. rapa* and *R. sativus*. Yang et al. [10] sequenced the chloroplast noncoding region and found that *R. sativus* was closely related to *B. rapa/oleracea* and proposed that *Raphanus* was derived from hybridization between *B. rapa/oleracea* and *B. nigra,* the two evolutionary lineages in the genus Brassica. Furthermore, the reciprocal hybridization between *R. sativus* and *B. rapa* has been proven viable [11]. Vigorous growth was also observed for most of the successful primary hybrids between *B. rapa* and *R. sativus* [12]. On the other hand, according to Tonosaki et al. [13], when hybridized with *R. sativus*, only one particular breeding line of *B. rapa* ('Shogoin-kabu') successfully produced hybrid seeds, whereas most other lines failed due to embryo breakdown.

By grafting the pac choi scion onto the daikon radish rootstock, the objectives of this pilot experiment were to examine the feasibility of developing successful grafts for harvesting both pac choi leaves and daikon radish taproot from the same plant, and to compare the growth and development of grafted plants with self-grafted and non-grafted pac choi and daikon radish plants.

2. Materials and Methods

Two experiments were carried out in this study. The first experiment was a pilot study to test the feasibility of grafting pac choi onto daikon radish. The second experiment was intended to provide a better understanding of above-ground growth and below-ground development of this unique scion-rootstock system over an extended post-grafting period of plant establishment. In both experiments, 'Bora King' (BK), a daikon radish with purple taproots (Johnny's Selected Seeds, Winslow, ME, USA) was used as the rootstock, while 'Mei Qing Choi' (MQ) pac choi (Johnny's Selected Seeds) was used as the scion. They were selected based on our preliminary study in which these two cultivars were found to be compatible for grafting and have similar hypocotyl diameters.

2.1. Setup of the Pilot Experiment

Pac choi and daikon radish were seeded on 7 and 13 November 2016, respectively. The pac choi was seeded 6 d earlier than the daikon radish in order to match the stem diameter of the seedlings at grafting, as the daikon radish germinated and emerged much quicker than the pac choi based on a preliminary seeding test. All the seeds were sown in 72-cell Speedling trays (Speedling Inc., Ruskin, FL, USA) and filled with Fafard-2 potting mix (Sun Gro Horticulture, Agawam, MA, USA) containing a mixture of peat moss, perlite, vermiculite, and dolomite lime. Plants were grown in a greenhouse at the University of Florida campus (Gainesville, FL, USA). Water-soluble fertilizer 20N-8.7P-16.7K (Jack's Classic; Jr Peters Inc., Allentown, PA, USA) was applied on 17 and 28 November at a nitrogen (N) concentration of 200 mg L^{-1}.

Plants were grafted on 30 November 2016 (0 d after grafting (DAG)) using the splice grafting method [1]. Twenty-four plants were grafted using seedlings with the most consistent growth. With the purpose of ensuring consistent grafting quality, only a small number of plants were grafted in this pilot experiment after earlier attempts at practicing the grafting technique. The daikon radish seedlings were severed using a double edge razor blade at approximately a 45-degree angle below cotyledons to remove the shoots, with pac choi scions cut at the hypocotyl with the same angle just above the soil surface. The cut surfaces of the pac choi scion and the daikon radish seedlings with shoot removal were conjoined using a 1.5 mm silicone grafting clip (Johnny's Selected Seeds). Grafted plants were then placed in a healing chamber constructed by wrapping a metal shelving unit with thin plastic film (Uline Econo-Wrapper (0.02 mm), Uline corporation, Pleasant Prairie, WI, USA) in a temperature-controlled room with air temperature set at 23 °C and relative humidity (RH) at 99%. Light was provided by two, 54-watt T5 fluorescent lights (Philips Lighting Company, Somerset, NJ, USA) at a photosynthetic photon flux density (PPFD) of 56 μmol m^{-2} s^{-1} at seedling canopy level for 12 h each day. An additional plastic tray with a wet sheet of germination paper was placed inside the healing chamber to help maintain humidity.

From 5 DAG, the healing chamber was gradually cut open and the ambient RH setting was reduced to 60%. At 8 DAG, the plastic film was completely removed, and grafts remained exposed in the temperature-controlled room until 13 DAG. Water was applied to plants by filling the bottom of the tray for absorption. All the grafted plants were transferred to a greenhouse at 13 DAG, and graft survival rate was determined by counting the number of live and dead plants; only plants with turgid leaves were counted as living. At harvest, the number of surviving grafted plants was counted again for calculation of the final graft survival rate, as some plants severely declined following transplanting into pots.

At 16 DAG, surviving grafted plants were transplanted into 11.36 L black plastic pots (1200C; Hummert International, Earth City, MO, USA) filled with Fafard-2 soilless mix (Sun Gro Horticulture) for continued monitoring of the survival of the grafted plants. In addition, five plants of non-grafted 'Mei Qing Choi' and 'Bora King' were potted as controls. All the plants were placed on the greenhouse bench following a completely randomized design. Organic fertilizer MicroSTART60 3N-0.9P-2.5K (Perdue AgriRecycle, LLC., Seaford, DE, USA) was applied to each pot at the rate of 80 g/pot. Drip irrigation was used by placing one 1.89 L h^{-1} emitter (Woodpecker pressure compensating junior dripper; Netafim USA, Fresno, CA, USA) in each pot; plants were watered once a day for 3 min. Irrigation increased to twice per day for 2 min each time starting at 56 DAG. Insecticidal soap (Safer Brand; Woodstream Corporation, Lancaster, PA, USA) was sprayed at 56 DAG and lacewing larvae (*Chrysoperla rufilabris* (Neuroptera: Chrysopidae); Rincon-Vitova Insectaries, Ventura, CA, USA) were released at 64 DAG for aphid control. The average day and night temperatures of the greenhouse during the plant growth were 22.8 °C and 16.5 °C, respectively.

2.2. Setup of the Follow-Up Experiment

A follow-up experiment was conducted in 2019 to further explore the above-ground growth and below-ground taproot development in grafted pac choi–daikon radish plants. 'Mei Qing Choi' (MQ) pac choi was grafted onto 'Bora King' (BK) daikon radish (MQ/BK), while non-grafted pac choi (MQ) and daikon radish (BK) as well as self-grafted pac choi (MQ/MQ) and daikon radish (BK/BK) were used as controls. A randomized complete block design with four replications (blocks) and ten grafted plants per treatment per replication (block) was used in the grafting experiment. MQ and BK were seeded into 72-cell trays on 8 and 14 February 2019, respectively. Fish & seaweed organic liquid fertilizer 2N-1.3P-0.8K (Neptune's Harvest, Gloucester, MA, USA) and 0N-0P-41.5K potassium sulfate (Big K; JHBiotech, Inc., Ventura, CA, USA) were applied at concentrations of 200 mg L^{-1} N and 200 mg L^{-1} K_2O at the seedling growth stage on 18 and 26 Feb. Plants were grafted on 1 March 2019 using the aforementioned grafting method. Grafted plants were healed in an air-conditioned laboratory room with the same set up of healing chamber as in 2016. Supplemental light was provided for 10 h each day during the healing process. The air temperature and RH of the laboratory room were 23.8 ± 0.5 °C and 47.6 ± 13.1%. The plastic film was completely removed at 8 DAG. Water was gently sprayed onto the soil surface using a wash bottle when needed to avoid wetting foliage. All the grafted plants were moved into a greenhouse at 13 DAG where the average day and night temperatures were 27.1 °C and 20.7 °C, respectively. Graft survival rate was determined for each grafting treatment in each replication by counting the number of live and dead plants at 17 DAG. At 19 DAG, 24 plants from each treatment with healthy and consistent growth were chosen and randomly reassigned to four blocks with six plants in each block for further evaluation of the growth of the grafted plants in a greenhouse pot study, following a randomized complete block design. Plants were transplanted into 11.36 L black plastic pots filled with PRO-MIX premium organic vegetable and herb mix (Premier Tech Ltd., Quakertown, PA, USA) which contained 60–75% peat moss plus peat humus, compost, perlite, gypsum, limestone, organic fertilizer, and mycorrhizae. Drip irrigation was used by placing one 1.89 L h^{-1} emitter in each pot; the plants were watered twice a day for 3 min per cycle between 21 and 39 DAG and irrigation increased to 4 min per cycle thereafter. Adventitious roots developed from the graft union area were monitored and removed once a week as needed after the plants were transplanted into the pots.

2.3. Plant Growth Measurements

In the 2019 follow-up experiment, leaf relative chlorophyll content and canopy size were measured at 33 and 41 DAG. A SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Aurora, IL, USA) was used to measure leaf relative chlorophyll content on three randomly chosen plants per treatment per block by averaging four readings obtained from two distal areas of the leaf blade for each of the two most recent mature leaves per plant. The canopy size was measured on 3 plants of MQ/BK, MQ/MQ, and MQ for each block using digital photographs processed with ImageJ/Fiji (version 2.0.0) [14]. A ruler held in the frame of each photograph set the scale for pixels per linear cm and enabled digital measurement of length and width of the plant canopy. The canopy size was then determined by multiplying the canopy length and width.

2.4. Gas Exchange Measurements

Gas-exchange was measured in the 2019 follow-up experiment at 34 and 46 DAG between 10:00 am and 3:00 pm by using an open gas exchange system (Li-6800; Li-Cor Inc., Lincoln, NE, USA) on three plants per treatment per block. Leaf transpiration rate (E, mmol H_2O m^{-2} s^{-1}), net CO_2 assimilation rate (A, mmol CO_2 m^{-2} s^{-1}), intercellular CO_2 concentration (Ci, μmol CO_2 mol^{-1} air), and stomatal conductance to water (gsw, mmol H_2O m^{-2} s^{-1}) were measured at steady-state on the third (fully expanded) leaf from the top of each plant [15]. The PPFD was set at 800 μmol m^{-2} s^{-1}, with CO_2 concentration at 400 ppm, vapor pressure deficit at 1.2 kPa, and leaf temperature at 27–29 °C. Instantaneous water use efficiency (iWUE) (μmol CO_2 $mmol^{-1}$ H_2O) was calculated as A/E [16] and stomatal conductance (Gs, mol m^{-2} s^{-1}) was calculated as gsw/1.6 [17].

2.5. Yield Components and Biomass Accumulation at Harvest

For the 2016 pilot study, the above-ground part (above soil line) of all the plants of MQ/BK, non-grafted MQ, and non-grafted BK were harvested at 68 DAG. The number of leaves longer than 4 cm were counted for each plant. The MQ/BK and non-grafted BK were then uprooted, and the taproots were separated and rinsed with water to remove excess potting soil from the roots. Taproot length (from the stem base to the end of the radish taproot) of each harvested plant was recorded, and the diameter of the widest part of each radish taproot was measured with a digital caliper. For the 2019 follow-up experiment, harvest and destructive sampling were carried out at 47 DAG. Five out of six plants per treatment per replication were randomly sampled. The above-ground part (above soil line) of each plant was removed from the pot, and leaves longer than 4 cm were counted and scanned with a leaf area meter (LI-3100; Li-Cor Inc., Lincoln, NE, USA). Only taproots from MQ/BK, BK, and BK/BK were harvested and cleaned. Pak choi and daikon radish leaves and taproots from the 2016 and 2019 experiments were first weighed then dried at 65 °C for 7 d (until constant weight) to determine the above-ground and below-ground fresh and dry biomass.

2.6. Mineral Nutrient Contents in Leaf and Root Tissues

In the 2019 experiment, the dried root samples from the BK, BK/BK, and MQ/BK treatments and dried leaf samples from the MQ, MQ/MQ, and MQ/BK treatments were ground using a Thomas Wiley Laboratory mill (Model 4; Arthur H. Thomas Company, Philadelphia, PA, USA) and sent to Waters Agricultural Laboratories (Camilla, GA, USA) to measure the concentrations of the macronutrients N, phosphate (P), sulfate (S), potassium (K), calcium (Ca), and magnesium (Mg) and the micronutrients boron (B), zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu). Nutrient accumulation was calculated by multiplying the nutrient concentration by dry tissue biomass.

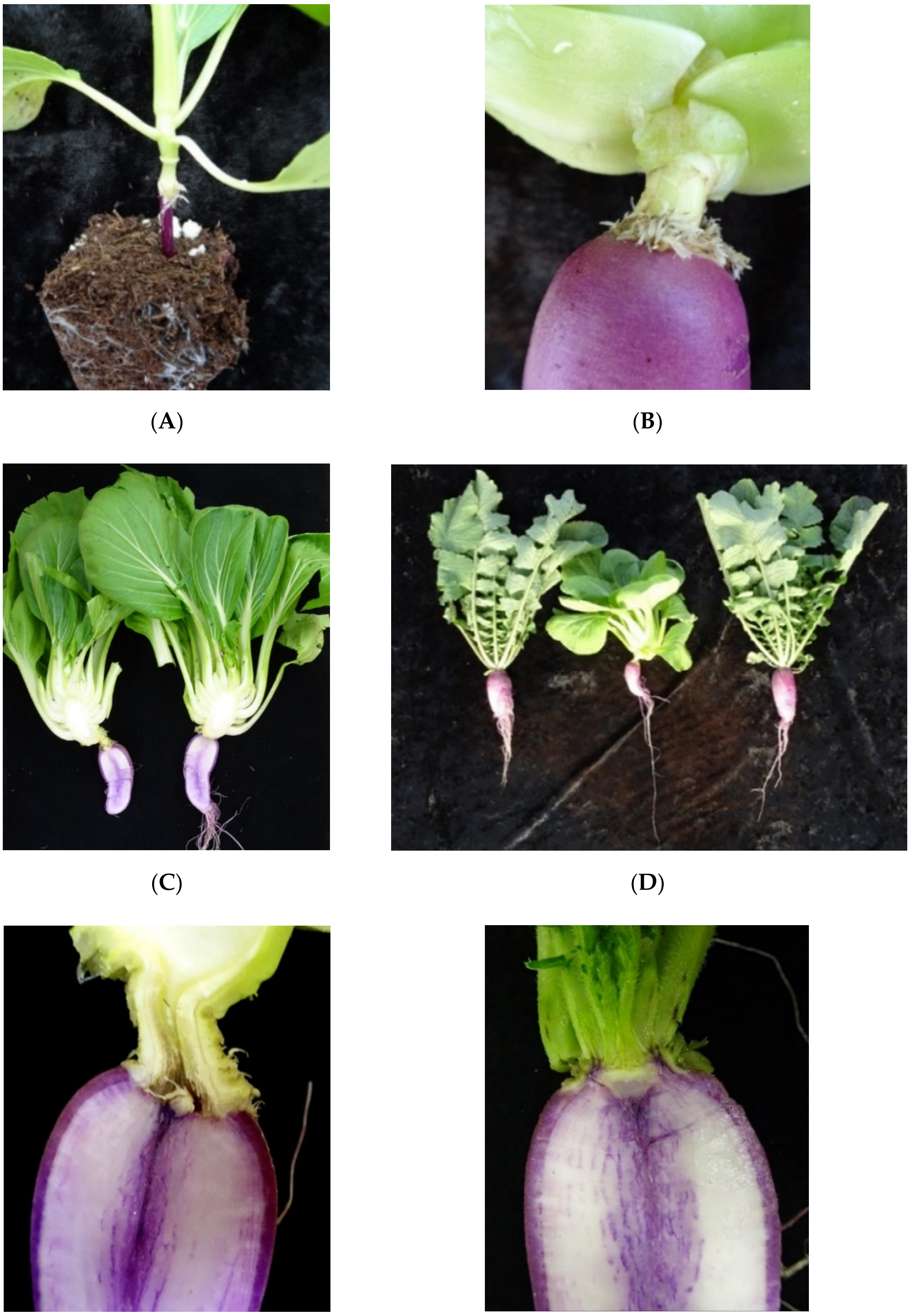
2.7. Statistical Analyses

The pilot study followed a completely randomized design with five replications and one plant per replication. In the follow-up experiment, a randomized complete block design with four replications (blocks) and ten plants per experimental unit was used before plants were transplanted to larger pots, when the number was reduced to six plants per experimental unit. Data were analyzed using a linear mixed model in the GLIMMIX procedure of SAS (SAS Version 9.4 for Windows; SAS Institute, Cary, NC, USA). Some data were transformed by taking the square root to meet the assumptions of the model (normality, homogeneity, linearity) as needed, while results were presented using the original data following statistical analysis. Fisher's least significant difference (LSD) test ($\alpha = 0.05$) was conducted for multiple comparisons of different measurements among treatments.

3. Results and Discussion

3.1. Graft Survival Rate

In the 2016 pilot study, the survival rate of MQ/BK was 95.8% at 13 DAG but decreased to 87.5% at 68 DAG (data not shown). In the 2019 experiment, there was no difference in survival rate ($p = 0.483$) between BK/BK (79%), MQ/MQ (92%), and MQ/BK (77%) at 17 DAG (data not shown). The relatively high survival rates of MQ/BK indicated good graft compatibility between 'Mei Qing Choi' pac choi and 'Bora King' daikon radish. Both the edible pac choi leafy green part of the plant and the radish taproot developed in MQ/BK (Figure 1A–C). According to Oda and Nakajima [8], 'Taibyoh 60-nichi' Chinese cabbage grafted onto 'Taibyoh hikari' turnip had a survival rate of only approximately 50%; however, it was attributed to the small size of the seedlings at grafting rather than graft incompatibility. In our studies, we also used 1.5 mm or even smaller grafting clips to graft younger and tender seedlings as the hypocotyl tissue of radish and pac choi plants tend to become more lignified as they grow. The lower survival rate observed in the 2019 experiment may have been due to an issue with properly matching the plant stem diameters at grafting since the hypocotyl of the daikon radish plant had grown thicker than expected at the time of grafting. As stem diameter and alignment of cambial tissues affect the success of grafting [18–20], matching plant stem diameters between these two species, which have thin hypocotyls at the optimum stage for grafting, is a challenge for achieving successful grafts. In this study, daikon radish was seeded 6 d earlier than pac choi to help match their stem diameters, but with less than desirable results, especially in the 2019 experiment owing to the seasonal variability of greenhouse conditions that led to unpredictable growth rate of plants. As shown by Hayashida et al. [21] and Kwack et al. [22], the hypocotyl growth of pac choi and radish seedlings can be manipulated by light quality and intensity. Employing a more controlled environment for growing seedlings until ready for grafting seems to be advisable for future work.



(A) (B) (C) (D) (E) (F)

Figure 1. *Cont.*

(**G**)

Figure 1. Grafted plants with 'Mei Qing Choi' pac choi as scion and 'Bora King' daikon radish as rootstock. (**A**) A well-healed grafted pac choi–daikon radish seedling. (**B**) Formation of the taproot of daikon radish grafted with pac choi. (**C**) Longitudinal section of the grafted pac choi–daikon radish plant at harvest. (**D**) Comparison between self-grafted 'Bora King' (left), 'Mei Qing Choi' pac choi grafted onto 'Bora King' daikon radish (middle), and non-grafted 'Bora King' (right) at harvest. (**E**) Longitudinal section of the graft union area of grafted pac choi–daikon radish plant at harvest. (**F**) Longitudinal section of the graft union area of self-grafted 'Bora King' daikon radish at harvest. (**G**) Longitudinal section of the graft union area of self-grafted 'Mei Qing Choi' pac choi plant at harvest.

3.2. Plant Growth Parameters

SPAD and canopy size were measured at 33 and 41 DAG in the 2019 experiment (Table 1). There was a significant difference in SPAD among treatments at 33 DAG, but no difference was found at 41 DAG. At 33 DAG, non-grafted pac choi showed lower levels of leaf SPAD values than non- and self-grafted daikon radish. The lack of difference in SPAD between MQ/BK, MQ, and MQ/MQ indicated that BK as a rootstock did not impair accumulation of chlorophyll by MQ. The similar canopy size between MQ/BK, MQ, and MQ/MQ at both 33 and 41 DAG (Table 1) suggested that grafting with daikon radish did not reduce the leaf growth and expansion of pac choi.

Table 1. Relative chlorophyll content and canopy size of grafted, self-grafted, and non-grafted pac choi and daikon radish plants at 33 d after grafting (DAG) and 41 DAG in the 2019 experiment.

Treatment [z]	Relative Chlorophyll Content (SPAD)		Canopy (cm^2 $Plant^{-1}$)	
	33 DAG	41 DAG	33 DAG	41 DAG
BK	37.2 ± 0.8 a [y]	40.6 ± 0.7 a	-	-
BK/BK	36.7 ± 0.8 ab	40.3 ± 0.7 a	-	-
MQ	34.1 ± 0.8 c	40.7 ± 0.7 a	54.26 ± 2.71 a	78.32 ± 3.35 a
MQ/MQ	34.6 ± 0.8 bc	42.1 ± 0.7 a	58.90 ± 2.71 a	76.90 ± 3.35 a
MQ/BK	33.3 ± 0.8 c	40.0 ± 0.7 a	52.32 ± 2.71 a	75.42 ± 3.35 a
p value	0.006	0.194	0.150	0.813

[z] BK = Non-grafted 'Bora King' daikon radish; BK/BK = Self-grafted 'Bora King' daikon radish; MQ = Non-grafted 'Mei Qing Choi' pac choi; MQ/MQ = Self-grafted 'Mei Qing Choi' pac choi; MQ/BK = 'Mei Qing Choi' pac choi grafted onto 'Bora King' daikon radish. [y] Mean ± SE (standard error); means followed by the same letter are not significantly different at $p \le 0.05$ according to Fisher's LSD test.

As shown in Figure 1B, above-ground pac choi and below-ground daikon radish taproot developed normally in MQ/BK plants. We observed cavities in the vascular bundle connections at the graft union area in grafted pac choi plants with the daikon radish rootstock but not in self-grafted daikon radish plants (Figure 1E,F), similar to what was reported in grafted Chinese cabbage/turnip plants [8]. In our experiments, grafting was carried out at 16 d after sowing (DAS) for daikon radish and 23 DAS for pac choi. According to Liu et al. [23], as early as 16 DAS, tuberization began in turnip (*B. rapa* subsp. *Rapa*) and the center part of the upper hypocotyl which accounted for about 50% of the cross section area was occupied by pith cells, with an actively dividing cambium circle and a thin xylem ring. When pac choi was grafted at 23 DAS, more than 50% of the center part of the upper hypocotyl of pac choi consisted of secondary xylem cells with highly lignified cell walls. This discrepancy in hypocotyl structure between scion and rootstock seedlings likely resulted in the formation of the cavity inside the graft union during healing as observed in our study (Figure 1E), especially considering that the cavity did not exist in self-grafted daikon radish (Figure 1F) or pac choi (Figure 1G).

Leaf number and taproot length and diameter were measured at harvest in both experiments (Table 2). In the 2016 pilot study, MQ/BK had more leaves than non-grafted BK, but did not differ significantly from MQ. In the 2019 experiment, MQ/BK had 35% and 26% more leaves than self- and non-grafted BK, respectively, but no difference was found between self- and non-grafted BK. Similar leaf numbers were observed for MQ/BK, MQ/MQ, and MQ. Total leaf area was also measured in the 2019 experiment and MQ/BK had smaller leaf area than all other treatments. In both 2016 and 2019, MQ/BK produced significantly shorter and smaller taproots than non-grafted BK and in 2019, MQ/BK was also smaller in taproot diameter than BK/BK (Table 2 and Figure 1D). Our results were consistent with Zheng et al. [24], who reported that the diameter of turnip was significantly smaller when grafted with rapeseed (*B. rapa* subsp. *oleifera*) than self-grafted turnip. BK/BK did not differ significantly from BK in taproot length but was 7% smaller in taproot diameter.

Table 2. Total leaf number and area and taproot length and diameter of grafted, self-grafted, and non-grafted pac choi and daikon radish plants at harvest in the 2016 pilot study and the 2019 experiment.

Treatment [z]	Leaf Number (no. Plant^{-1})	Leaf Area (mm^2 Plant^{-1})	Taproot Length (cm)	Taproot Diameter (mm)
		2016		
BK	18.9 ± 2.3 b [y]	-	10.9 ± 0.5 a	68.55 ± 3.98 a
MQ	26.6 ± 2.4 ab	-	-	-
MQ/BK	28.1 ± 2.4 a	-	8.2 ± 0.5 b	51.92 ± 3.56 b
p value	0.020		0.005	0.017
		2019		
BK	15.7 ± 0.6 b	2521.73 ± 60.62 a	9.2 ± 0.5 a	45.24 ± 0.71 a
BK/BK	14.7 ± 0.5 b	2401.06 ± 60.62 ab	8.4 ± 0.5 a	42.20 ± 0.71 b
MQ	20.4 ± 0.6 a	2423.26 ± 60.62 ab	-	-
MQ/MQ	19.5 ± 0.6 a	2311.67 ± 60.62 b	-	-
MQ/BK	19.8 ± 0.6 a	2080.12 ± 60.62 c	6.8 ± 0.5 b	34.94 ± 0.71 c
p value	<0.001	0.002	0.005	<0.001

[z] BK = Non-grafted 'Bora King' daikon radish; BK/BK = Self-grafted 'Bora King' daikon radish; MQ = Non-grafted 'Mei Qing Choi' pac choi; MQ/MQ = Self-grafted 'Mei Qing Choi' pac choi; MQ/BK = 'Mei Qing Choi' pac choi grafted onto 'Bora King' daikon radish. [y] Mean ± SE (standard error); means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's LSD test.

The primary root axis of radish consists of two anatomically distinct parts. The upper part originates from the hypocotyl whereas the lower part is true root tissue. Both lower and upper regions of the radish root thicken to form succulent tissue by increases in both cell number and cell size [25,26]. In this grafting experiment, the cut made on the daikon radish plant was in the thickening region of the hypocotyl as demonstrated by the longitudinal section of the graft union area of self-grafted daikon radish plant (Figure 1F). Very likely, grafting pac choi with radish shortens the hypocotyl part that could contribute to the formation of the taproot, leading to reduced taproot length compared with non-grafted radish, while self-grafting radish does not involve any loss of hypocotyl tissue. Furthermore, it has been found that in turnip the hypocotyl tissue is the main contributor to underground tuber development, and hypocotyl excision led to a lower expression level of genes controlling tuberization, leading to a substantial inhibition of tuber formation [24].

3.3. Gas-Exchange Parameters

Leaf transpiration rate, net CO_2 assimilation rate, intercellular CO_2 concentration, stomatal conductance, and instantaneous water use efficiency (iWUE) were compared for MQ/BK, MQ, MQ/MQ, BK, and BK/BK at 34 and 46 DAG in the 2019 experiment (Table 3). No difference in leaf transpiration rate was observed at 34 DAG, while at 46 DAG, MQ/BK had a similar transpiration rate as MQ and MQ/MQ, and all three treatments showed a 95% increase of transpiration rate on average than BK and BK/BK. Grafting significantly increased the net CO_2 assimilation rate of MQ/BK compared with MQ and MQ/MQ by 15% and 28%, respectively, at 34 DAG, while no difference was observed between MQ/BK and BK/BK. At 46 DAG, MQ/BK showed a net CO_2 assimilation rate that was 48% and 45% higher than MQ and MQ/MQ, respectively, but it did not differ significantly from BK/BK. MQ/BK also had a 21% higher net CO_2 assimilation rate than BK at 46 DAG. Very likely, MQ/BK had a stronger sink strength than MQ/MQ and MQ, which contributed to the higher photosynthetic rate observed [27]. Interestingly, at 34 DAG, MQ and MQ/MQ had similar intercellular CO_2 concentrations, which were significantly higher than that of other treatments. At 46 DAG, MQ/BK, MQ, and MQ/MQ had higher intercellular CO_2 concentration than BK and BK/BK and the same trend was observed for stomatal conductance at 46 DAG although no difference in stomatal conductance was detected at 34 DAG. At 34 DAG, MQ/BK had 36% and 53% higher iWUE than MQ and MQ/MQ, but did not differ from BK and BK/BK. However, at 46 DAG, MQ/BK, MQ, and MQ/MQ exhibited a similar level of iWUE which was significantly lower than that of BK and BK/BK.

Table 3. Leaf transpiration rate (E), net CO_2 assimilation rate (A), intercellular CO_2 concentration (Ci), stomatal conductance (gs), and instantaneous water use efficiency (iWUE) of grafted, self-grafted, and non-grafted pac choi and daikon radish plants at 34 d after grafting (DAG) and 46 DAG in the 2019 experiment.

Treatment [y]	E (mmol H_2O m^{-2} s^{-1})		A (μmol CO_2 m^{-2} s^{-1})		Ci (μmol CO_2 mol^{-1} air)		Gs (mol m^{-2} s^{-1})		iWUE (μmol CO_2 $mmol^{-1}$ H_2O) [z]	
	34 DAG	46 DAG	34 DAG	46 DAG	34 DAG	46 DAG	34 DAG	46 DAG	34 DAG	46 DAG
BK	4.81 ± 0.71 a [x]	2.80 ± 0.79 b	21.41 ± 0.75 a	17.22 ± 0.89 b	298.14 ± 11.10 b	251.33 ± 10.59 b	0.28 ± 0.05 a	0.15 ± 0.05 b	4.92 ± 0.56 a	7.26 ± 0.54 a
BK/BK	4.68 ± 0.71 a	4.18 ± 0.79 b	19.66 ± 0.75 ab	18.93 ± 0.89 ab	305.43 ± 11.10 b	273.09 ± 10.59 b	0.27 ± 0.05 a	0.25 ± 0.05 b	4.55 ± 0.56 ab	6.27 ± 0.54 a
MQ	4.95 ± 0.71 a	6.61 ± 0.79 a	16.10 ± 0.75 c	14.06 ± 0.89 c	330.36 ± 11.10 a	345.22 ± 10.59 a	0.32 ± 0.05 a	0.41 ± 0.05 a	3.59 ± 0.56 bc	2.49 ± 0.54 b
MQ/MQ	5.51 ± 0.71 a	6.33 ± 0.79 a	14.41 ± 0.75 c	14.31 ± 0.89 c	331.63 ± 11.10 a	346.80 ± 10.59 a	0.33 ± 0.05 a	0.40 ± 0.05 a	3.20 ± 0.56 c	2.51 ± 0.54 b
MQ/BK	4.35 ± 0.71 a	7.47 ± 0.79 a	18.44 ± 0.75 b	20.76 ± 0.89 a	299.39 ± 11.10 b	336.66 ± 10.59 a	0.25 ± 0.05 a	0.45 ± 0.05 a	4.88 ± 0.56 a	2.94 ± 0.54 b
p value	0.564	<0.001	<0.001	<0.001	0.011	<0.001	0.362	0.001	0.024	<0.001

[z] Instantaneous water use efficiency (iWUE) = net CO_2 assimilation rate (A)/transpiration rate (E). [y] BK = Non-grafted 'Bora King' daikon radish; BK/BK = Self-grafted 'Bora King' daikon radish; MQ = Non-grafted 'Mei Qing Choi' pac choi; MQ/MQ = Self-grafted 'Mei Qing Choi' pac choi; MQ/BK = 'Mei Qing Choi' pac choi grafted onto 'Bora King' daikon radish. [x] Mean ± SE (standard error); means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's LSD test.

Lower leaf transpiration rate and intercellular CO_2 concentration of radish compared with pac choi observed in the later growth stage could be owing to different leaf structures. Pac choi leaves are fleshy and glossy, while radish has trichomes on both upper and lower leaf surfaces [28]. It has been suggested that trichome density is negatively related to transpiration rate and CO_2 diffusion rate as trichomes can increase boundary layer resistance [29–31]. The trichomes on daikon radish leaves might have affected the leaf transpiration rates and intercellular CO_2 concentrations measured in this study. Further examination is needed to directly compare the intrinsic leaf structures of pac choi and daikon radish plants for their effects on gas exchange and gas exchange measurements.

Most water loss from leaves of intact plants is generally through open stomatal apertures [32], thus the higher stomatal conductance of MQ/BK was likely the driving factor for its lower iWUE compared with BK and BK/BK despite its higher net CO_2 assimilation rate. It has been found in tobacco (*Nicotiana tabacum* L.) that stomatal conductance did not always parallel photosynthetic capacity changes [33,34], which could partially explain the relatively high stomata conductance, but low net CO_2 assimilation rate observed in MQ and MQ/MQ.

3.4. Leaf and Taproot Harvest and Biomass Partition

In the 2016 pilot study, MQ and MQ/BK had 151% and 104% higher above-ground fresh weight (FW) than BK, and the former two did not differ significantly (Table 4). No difference was detected in above-ground dry weight (DW) between these three treatments. MQ/BK produced significantly lower below-ground FW and DW compared with BK, while similar levels of total FW and DW were observed between MQ/BK and BK.

Table 4. Above-ground, below-ground, and total fresh weight (FW) and dry weight (DW) of grafted, self-grafted, and non-grafted pac choi and daikon radish plants at harvest in the 2016 pilot study and the 2019 experiment.

Treatment [z]	Above-Ground FW (g Plant^{-1})	Below-Ground FW (g Plant^{-1})	Above-Ground DW (g Plant^{-1})	Below-Ground DW (g Plant^{-1})	Total FW (g Plant^{-1})	Total DW (g Plant^{-1})
			2016			
BK	240.07 ± 54.58 b [y]	332.15 ± 33.23 a	20.41 ± 2.52 a	19.84 ± 1.56 a	572.21 ± 67.48 a	41.08 ± 4.00 a
MQ	602.76 ± 48.82 a	-	25.50 ± 2.25 a	-	-	-
MQ/BK	490.63 ± 48.82 a	110.44 ± 29.72 b	24.42 ± 2.25 a	7.60 ± 1.20 b	601.07 ± 60.36 a	32.02 ± 3.10 a
p value	0.001	<0.001	0.331	<0.001	0.792	0.142
			2019			
BK	171.64 ± 6.62 d	103.47 ± 5.25 a	12.85 ± 0.25 a	6.58 ± 0.33 a	275.17 ± 4.44 b	19.45 ± 0.41 a
BK/BK	162.27 ± 6.62 d	77.55 ± 4.57 b	12.34 ± 0.25 a	5.33 ± 0.30 b	239.91 ± 4.44 c	17.67 ± 0.41 b
MQ	329.83 ± 6.62 a	-	10.09 ± 0.25 b	-	-	-
MQ/MQ	293.51 ± 6.62 b	-	8.98 ± 0.25 c	-	-	-
MQ/BK	269.08 ± 6.62 c	43.59 ± 3.45 c	9.43 ± 0.25 bc	2.24 ± 0.20 c	312.93 ± 4.44 a	11.68 ± 0.41 c
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z] BK = Non-grafted 'Bora King' daikon radish; BK/BK = Self-grafted 'Bora King' daikon radish; MQ = Non-grafted 'Mei Qing Choi' pac choi; MQ/MQ = Self-grafted 'Mei Qing Choi' pac choi; MQ/BK = 'Mei Qing Choi' pac choi grafted onto 'Bora King' daikon radish. [y] Mean ± SE (standard error); means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's LSD test.

In the 2019 study, MQ/BK showed a significant reduction in above-ground FW compared with MQ and MQ/MQ by 18% and 8%, respectively (Table 4). MQ, MQ/MQ, and MQ/BK on average had 78% higher above-ground FW than the average of BK and BK/BK. However, BK and BK/BK had significantly higher above-ground DW than other treatments and MQ/BK did not differ significantly in above-ground DW from MQ and MQ/MQ. Grafting with pac choi significantly decreased the below-ground FW and DW of daikon radish compared with non- and self-grafted daikon radish. BK had higher below-ground FW and DW than BK/BK by 33% and 23%, respectively. MQ/BK exhibited a significantly higher total FW but a reduction in total DW compared with BK and BK/BK. The water content of both radish taproots and leaves was about 93%, whereas it reached 97% in the pac choi leaves, indicating that the pac choi leafy part had a disproportional contribution to the FW of MQ/BK. The differences in total FW and DW between 2016 and 2019 studies may be due to the different growing seasons and the time between grafting and harvest as the period from grafting to harvesting was 45% longer in 2016 compared with the 2019 study. Overall, the two experiments suggested that grafting between 'Mei Qing Choi' pac choi and 'Bora King' daikon radish had a greater impact on radish taproot development than the influence on pac choi leaf growth as the taproot DW of MQ/BK was significantly lower than BK, but the leaf DW was similar to that of MQ.

The grafting procedure per se negatively affected biomass accumulation in edible part in both self-grafted BK/BK and MQ/MQ compared with their non-grafted counterparts. Grafting can be viewed as mechanical wounding that triggers redistribution of the local resources or mobilization of resources from neighboring tissues to the injured part [35], which may divert the resources that could be used for plant growth. Moreover, wounding could induce jasmonic acid production, leading to suppression of mitosis [36], and thus reduce cell number and lead to reduced plant growth.

Gibberellins (GAs) have long been known to inhibit potato tuberization possibly by their involvement in photoperiodic control of tuber formation [37,38]. Grafting potato (*Solanum tuberosum* L.) with tomato (*Solanum lycopersicum* L.) decreased stolon (underground shoot) number and length as well as tuber number, but increased the gibberellic acid (GA_3) content of stolon and tuber compared with self-grafted potato [39]. The level of GAs has also been reported to play a vital role in carrot (*Daucus carota* L. var. *sativus*) elongation and expansion [40]. Leaf application of GA_3 inhibited tuberous root growth but improved shoot growth in radish, while leaf application of paclobutrazol, an inhibitor of gibberellin biosynthesis, improved taproot growth [41]. Auxin may also affect hypocotyl-tuber growth in turnip as show in in-vitro studies [23]. Peres et al. [38] grafted tomato mutants, which were incapable of certain hormone biosynthesis or photomorphogenesis, onto potato plants and suggested that failure to produce certain chemicals by the tomato mutant scion may have impeded formation of the potato tuber. Hence, modifications of signal molecules produced in and transported from pac choi may have led to reduced growth of the daikon radish taproot.

In most cases, plants partition photosynthates preferentially to vegetative organs in the early to middle growth stage and to reproductive or storage organs in late growth stage [42]. However, many root vegetable plants grow the vegetative biomass and develop the storage root at the same time, leading to a balance between them [42,43]. Grafting may disturb the source–sink balance between scion and rootstock [44]. Both the leaf apical meristem of pac choi and the taproot part of daikon radish are strong sinks [45] and possibly competed for photosynthates in the pac choi–daikon radish grafts. The source-sink relationship was likely altered to support the growth of pac choi leaves at a cost of reduction in radish taproot development in the grafted plants. The dry mass of the leafy part accounted for 81% of the total DW of MQ/BK, but for non-grafted daikon radish, the leafy part only accounted for 66% of the total DW.

For Chinese cabbage grafted onto turnip, the heading of the Chinese cabbage was restricted by the thickening of the turnip taproot, resulting in a small Chinese cabbage head when the turnip taproot was harvested [8]. This imbalance was attributed to the discrepancy in crop maturity and growth cycle requirement as the heading of the Chinese cabbage requires more time than the development of the turnip taproot. In our study, 'Mei Qing Choi' pac choi and 'Bora King' daikon radish were both fast-growing cultivars with 45 and 49 d to maturity, respectively (Johnny's Selected Seeds). However, we seeded the faster maturing pac choi 6 d earlier than the slower maturing daikon radish in order to better match their stem diameters for grafting. Further examinations are needed to elucidate the scion–rootstock interactions for grafting scenarios in which accumulated biomass of both scion (shoots) and rootstock (enlarged taproot) are harvested together for economic yield. In this special scenario, competition for water and nutrients, photosynthetic capacity, and photosynthate partitioning between above- and below-ground sinks are of particular importance. Interestingly, the reciprocal grafting experiment by Sugiura et al. [42] using *Raphanus sativus* genotypes with differential hypocotyl sink activities demonstrated the genotype-dependent autonomous regulation of the hypocotyl sink activity.

3.5. Mineral Nutrient Contents in Pac Choi Leaves and Daikon Radish Roots of Grafted Plants

Dried leaves of MQ, MQ/MQ, and MQ/BK, and dried taproots of BK, BK/BK, and MQ/BK from the 2019 experiment harvest were used to examine the macronutrient and micronutrient concentrations (Table 5) and accumulation (Table 6). Leaf N concentration did not differ significantly between MQ/BK and MQ. Interestingly, MQ/MQ had a significantly higher N concentration in the leaf tissue compared with MQ/BK. However, this seems contradictory to the finding that MQ/BK had a higher leaf photosynthetic rate (Table 3) than MQ/MQ. It needs to be pointed out that the entire above-ground leaf tissue was sampled for leaf nutrient analysis, whereas the most recently mature leaves were used for photosynthesis measurements. While MQ/BK had a lower level of leaf N concentration in the above-ground biomass, its higher leaf photosynthetic rate could be due to remobilization of N compounds from the older leaves to the most recently mature leaves that were used for photosynthesis measurement [46]. Compared with non-grafted pac choi, grafting with the daikon radish rootstock significantly decreased K and S concentrations in the leaf tissue of pac choi (by 21% and 45%, respectively), but it increased Zn concentration in the leaf tissue by 37%. MQ/BK had significantly higher concentrations of N (by 14%), K (by 30%), Mg (by 47%), Ca (by 38%), B (by 36%), and Zn (by 63%) in the taproot compared with the average of BK and BK/BK, while there were no differences between BK and BK/BK. By contrast, the concentration of S in the taproot of MQ/BK was reduced by 44% compared with BK and BK/BK. Overall, there was not a clear relationship between plant nutritional status and reduction of taproot in MQ/BK. Nutrient uptake of grafted pac choi–daikon radish in relation to scion-rootstock interactions is an intriguing area to explore.

Table 5. Mineral nutrient concentrations in leaves of grafted, self-grafted, and non-grafted pac choi and in taproots of grafted, self-grafted, and non-grafted daikon radish plants at harvest in the 2019 experiment.

Treatment [z]	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Mg (mg g^{-1})	Ca (mg g^{-1})	S (mg g^{-1})	B (μg g^{-1})	Zn (μg g^{-1})	Mn (μg g^{-1})	Fe (μg g^{-1})	Cu (μg g^{-1})
						Leaves					
MQ	51.5 ± 0.9 ab [y]	10.3 ± 0.3 a	92.1 ± 1.7 b	6.4 ± 0.2 a	30.2 ± 1.0 a	15.4 ± 0.4 a	44.6 ± 1.6 a	77.5 ± 3.5 b	180.4 ± 7.1 a	103.5 ± 6.8 a	0.7 ± 0.1 a
MQ/MQ	53.7 ± 0.9 a	10.5 ± 0.3 a	96.1 ± 1.7 a	6.7 ± 0.2 a	31.3 ± 1.0 a	15.4 ± 0.4 a	45.9 ± 1.6 a	76.7 ± 3.5 b	195.3 ± 7.1 a	101.7 ± 6.8 a	0.7 ± 0.1 a
MQ/BK	48.4 ± 0.9 b	9.7 ± 0.3 a	73.1 ± 1.7 c	6.4 ± 0.2 a	32.0 ± 1.0 a	8.5 ± 0.4 b	45.6 ± 1.6 a	106.5 ± 3.5 a	180.8 ± 7.1 a	120.1 ± 6.8 a	1.1 ± 0.1 a
p value	0.008	0.147	<0.001	0.352	0.437	<0.001	0.835	<0.001	0.175	0.070	0.057
						Taproot					
BK	28.2 ± 0.7 b	7.6 ± 0.3 a	51.1 ± 2.4 b	1.5 ± 0.1 b	3.3 ± 0.2 b	10.3 ± 0.3 a	20.7 ± 0.9 b	63.2 ± 3.8 b	27.0 ± 1.5 a	105.2 ± 17.9 a	0.9 ± 0.2 a
BK/BK	28.2 ± 0.7 b	7.4 ± 0.3 a	49.7 ± 2.4 b	1.4 ± 0.1 b	3.3 ± 0.2 b	10.6 ± 0.3 a	20.2 ± 0.9 b	65.0 ± 3.8 b	23.4 ± 1.5 a	92.2 ± 16.8 a	1.0 ± 0.2 a
MQ/BK	32.2 ± 0.7 a	8.1 ± 0.3 a	65.7 ± 2.4 a	2.2 ± 0.1 a	4.6 ± 0.2 a	5.9 ± 0.3 b	27.8 ± 0.9 a	104.3 ± 3.8 a	25.5 ± 1.5 a	90.9 ± 16.7 a	1.1 ± 0.2 a
p value	0.008	0.128	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.201	0.664	0.677

[z] BK = Non-grafted 'Bora King' daikon radish; BK/BK = Self-grafted 'Bora King' daikon radish; MQ = Non-grafted 'Mei Qing Choi' pac choi; MQ/MQ = Self-grafted 'Mei Qing Choi' pac choi; MQ/BK = 'Mei Qing Choi' pac choi grafted onto 'Bora King' daikon radish. [y] Mean ± SE (standard error); means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's LSD test.

Table 6. Mineral nutrient contents accumulated in leaves of grafted, self-grafted, and non-grafted pac choi and in taproots of grafted, self-grafted, and non-grafted daikon radish plants at harvest in the 2019 experiment.

Treatment [z]	N (mg $Plant^{-1}$)	P (mg $Plant^{-1}$)	K (mg $Plant^{-1}$)	Mg (mg $Plant^{-1}$)	Ca (mg $Plant^{-1}$)	S (mg $Plant^{-1}$)	B (μg $Plant^{-1}$)	Zn (μg $Plant^{-1}$)	Mn (μg $Plant^{-1}$)	Fe (μg $Plant^{-1}$)	Cu (μg $Plant^{-1}$)
						Leaves					
MQ	519.6 ± 13.3 a [y]	104.2 ± 3.8 a	929.3 ± 25.1 a	64.6 ± 1.9 a	304.6 ± 10.8 a	155.0 ± 5.2 a	450.0 ± 21.1 a	781.9 ± 37.5 b	1819.5 ± 84.7 a	1043.8 ± 69.4 a	6.9 ± 1.2 a
MQ/MQ	481.6 ± 13.3 ab	93.8 ± 3.8 a	862.5 ± 25.1 a	59.9 ± 1.9 a	280.7 ± 10.8 a	138.1 ± 5.2 b	412.9 ± 21.1 a	688.1 ± 37.5 c	1753.8 ± 84.7 a	911.5 ± 69.4 a	6.4 ± 1.2 a
MQ/BK	456.2 ± 13.3 b	91.3 ± 3.8 a	690.4 ± 25.1 b	60.1 ± 1.9 a	300.7 ± 10.8 a	79.7 ± 5.2 c	429.8 ± 21.1 a	1003.4 ± 37.5 a	1707.4 ± 84.7 a	1133.1 ± 69.4 a	10.5 ± 1.2 a
p value	0.025	0.103	<0.001	0.123	0.172	<0.001	0.487	<0.001	0.636	0.081	0.081
						Taproot					
BK	185.3 ± 5.7 a	49.9 ± 1.5 a	333.8 ± 11.5 a	9.9 ± 0.3 a	21.8 ± 0.5 a	67.4 ± 2.4 a	135.3 ± 4.1 a	414.8 ± 15.9 a	178.4 ± 10.0 a	723.3 ± 106.9 a	6.0 ± 1.2 a
BK/BK	150.2 ± 5.7 b	39.3 ± 1.5 b	264.5 ± 11.5 b	7.7 ± 0.3 b	17.3 ± 0.5 b	56.4 ± 2.4 b	107.5 ± 4.1 b	346.6 ± 15.9 b	124.4 ± 10.0 b	503.8 ± 106.9 ab	5.5 ± 1.2 a
MQ/BK	72.1 ± 5.7 c	18.3 ± 1.5 c	148.2 ± 11.5 c	4.8 ± 0.3 c	10.2 ± 0.5 c	13.2 ± 2.4 c	62.9 ± 4.1 c	235.2 ± 15.9 c	57.8 ± 10.0 c	207.3 ± 106.9 b	2.5 ± 1.2 a
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.027	0.120

[z] BK = Non-grafted 'Bora King' daikon radish; BK/BK = Self-grafted 'Bora King' daikon radish; MQ = Non-grafted 'Mei Qing Choi' pac choi; MQ/MQ = Self-grafted 'Mei Qing Choi' pac choi; MQ/BK = 'Mei Qing Choi' pac choi grafted onto 'Bora King' daikon radish. [y] Mean ± SE (standard error); means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's LSD test.

With respect to leaf nutrient accumulation, MQ/BK had 37% greater Zn accumulation compared with the average of MQ/MQ and MQ (Table 6). However, MQ/BK decreased N, K, and S content by 12%, 26%, and 49%, respectively, compared with MQ. In terms of nutrient accumulation in the taproot, MQ/BK showed significantly lower accumulation of all measured minerals except Fe and Cu relative to BK and BK/BK, which was likely associated with the small size of the taproot in MQ/BK. The lower accumulation of nutrients in the taproot demonstrated that pac choi–daikon radish favored growth of the pac choi leaves at the expense of the radish taproot.

4. Conclusions

Successful grafts were produced between *B. rapa* var. *chinensis* and *R. sativus* var. *longipinnatus* in this study, resulting in a novel 'pac choi–daikon radish' product that may help save growing space and have added-value as perceived by farmers and consumers. More research is needed to optimize the seeding time and management of seedling production to help further improve the graft survival rate. Grafting pac choi with daikon radish did not severely impair the growth of the above-ground parts as grafted pac choi had similar SPAD value, canopy size, leaf number, and above-ground DW compared with non-grafted pac choi. Interestingly, grafting with radish increased the photosynthetic ability of the pac choi. However, grafting the daikon radish with pac choi decreased the taproot formation as reflected by the reduced length, diameter, FW and DW of the taproot. Future studies could explore different approaches such as cultivar selection and nutrient management to better balance the sizes of the above- and below-ground parts of this new pac choi–daikon radish product. Given the wide range of *B. rapa* var. *chinensis* and *R. sativus* var. *longipinnatus* cultivars, it would be interesting to explore different grafting combinations to characterize the range of graft performance. We only tested the graft performance under greenhouse conditions, and the grafted plants need to be further evaluated in field growing systems where biotic and abiotic stressors can be intensified. Generally, grafting between pac choi and daikon radish showed more negative impacts on mineral nutrient levels in radish taproots than in pac choi leaves. Sensory properties of the 'pac choi–daikon radish' product are unknown, and this aspect deserves further assessment. The inter-generic grafting between *B. rapa* var. *chinensis* and *R. sativus* var. *longipinnatus* could also provide a unique model system to further our understanding of scion-rootstock synergy and above- and below-ground sink competition in horticultural crops toward improving the use of grafting technology in sustainable vegetable production.

Author Contributions: Conceptualization, X.Z.; Data curation, T.G., Z.T.R. and K.E.B.; Formal analysis, T.G.; Funding acquisition, X.Z.; Investigation, T.G., Z.T.R. and K.E.B.; Methodology, T.G., Z.T.R., Z.E.B. and X.Z.; Project administration, X.Z.; Resources, X.Z. and J.K.B.; Supervision, X.Z. and J.K.B.; Writing—original draft, T.G. and X.Z.; Writing—review & editing, T.G., Z.T.R., K.E.B., Z.E.B., X.Z. and J.K.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work is partly supported by the Specialty Crop Research Initiative grant No. 2016-51181-25404 from the USDA National Institute of Food and Agriculture.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lee, J.M.; Kubota, C.; Tsao, S.J.; Bie, Z.; Echevarria, P.H.; Morra, L.; Oda, M. Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Sci. Hortic.* **2010**, *127*, 93–105. [CrossRef]
2. Louws, F.J.; Rivard, C.L.; Kubota, C. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Sci. Hortic.* **2010**, *127*, 127–146. [CrossRef]
3. Guan, W.; Zhao, X.; Hassell, R.; Thies, J. Defense mechanisms involved in disease resistance of grafted vegetables. *HortScience* **2012**, *47*, 164–170. [CrossRef]
4. Chen, A.; Komives, E.A.; Schroeder, J.I. An improved grafting technique for mature Arabidopsis plants demonstrates long-distance shoot-to-root transport of phytochelatins in Arabidopsis. *Plant Physiol.* **2006**, *141*, 108–120. [CrossRef]
5. Gurusamy, V.; Bett, K.E.; Vandenberg, A. Grafting as a tool in common bean breeding. *Can. J. Plant Sci.* **2010**, *90*, 299–304. [CrossRef]
6. Temperini, O.; Calabrese, N.; Temperini, A.; Rouphael, Y.; Tesi, R.; Lenzi, A.; Carito, A.; Colla, G. Grafting artichoke onto cardoon rootstocks: Graft compatibility, yield and Verticillium wilt incidence. *Sci. Hortic.* **2013**, *149*, 22–27. [CrossRef]
7. Oda, M.; Tsuji, K.; Nagaoka, M. Inter-generic, inter-specific and inter-varietal grafting in Cruciferae. *Acta Hortic.* **1990**, *319*, 425–430. [CrossRef]
8. Oda, M.; Nakajima, T. Adhesive grafting of Chinese cabbage on turnip. *HortScience* **1992**, *27*, 1136. [CrossRef]

9. Chen, Y.C.; Chang, W.C.; Wang, S.T.; Lin, S.I. Development of a grafting method and healing conditions to improve cabbage head quality. *HortTechnology* **2019**, *29*, 57–64. [CrossRef]
10. Yang, Y.W.; Tai, P.Y.; Chen, Y.; Li, W.H. A study of the phylogeny of *Brassica rapa*, *B. nigra*, *Raphanus sativus*, and their related genera using noncoding regions of chloroplast DNA. *Mol. Phylogenet. Evol.* **2002**, *23*, 268–275. [CrossRef]
11. Matsuzawa, Y.; Funayama, T.; Kamibayashi, M.; Konnai, M.; Bang, S.W.; Kaneko, Y. Synthetic *Brassica rapa-Raphanus sativus* amphidiploid lines developed by reciprocal hybridization. *Plant Breed.* **2000**, *119*, 357–359.
12. Lange, W.; Toxopeus, H.; Lubberts, J.H.; Dolstra, O.; Harrewijn, J.L. The development of Raparadish (x *Brassicoraphanus*, 2n = 38), a new crop in agriculture. *Euphytica* **1989**, *40*, 1–14.
13. Tonosaki, K.; Michiba, K.; Bang, S.W.; Kitashiba, H.; Kaneko, Y.; Nishio, T. Genetic analysis of hybrid seed formation ability of *Brassica rapa* in intergeneric crossings with *Raphanus sativus*. *Theor. Appl. Genet.* **2013**, *126*, 837–846.
14. Abràmoff, M.D.; Magalhães, P.J.; Ram, S.J. Image processing with ImageJ. *Biophotonics Intern.* **2004**, *11*, 36–42.
15. Chen, X.; Wang, J.; Shi, Y.; Zhao, M.Q.; Chi, G.Y. Effects of cadmium on growth and photosynthetic activities in pak choi and mustard. *Bot. Stud.* **2011**, *52*, 41–46.
16. He, Y.; Zhu, Z.; Yang, J.; Ni, X.; Zhu, B. Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. *Environ. Exp. Bot.* **2009**, *66*, 270–278.
17. Gago, J.; de Menezes Daloso, D.; Figueroa, C.M.; Flexas, J.; Fernie, A.R.; Nikoloski, Z. Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: A multispecies meta-analysis approach. *Plant Physiol.* **2016**, *171*, 265–279.
18. Bumgarner, N.R.; Kleinhenz, M.D. *Grafting Guide: A Pictorial Guide to the Cleft and Splice Graft Methods as Applied to Tomato and Pepper*; The Ohio State University: Columbus, OH, USA, 2013; Available online: https://web.extension.illinois.edu/smallfarm/downloads/50570.pdf (accessed on 19 September 2020).
19. Garner, R.J.; Bradley, S. *The Grafter's Handbook*; Mitchell Beazley: London, UK, 2013.
20. Melnyk, C.W. Plant grafting: Insights into tissue regeneration. *Regeneration* **2017**, *4*, 3–14.
21. Hayashida, T.; Shibato, Y.; Hamachi, Y.; Yamato, Y.; Yamazaki, H.; Miura, H. Effect of light quality with different red/far-red photon flux density ratio on elongation of pak-choi (*Brassica chinensis*) seedlings grown under high temperatures. *J. Jpn. Soc. Hortic. Sci.* **2001**, *70*, 774–776.
22. Kwack, Y.; Kim, K.K.; Hwang, H.; Chun, C. Growth and quality of sprouts of six vegetables cultivated under different light intensity and quality. *Hortic. Environ. Biotechnol.* **2015**, *56*, 437–443.
23. Liu, M.; Bassetti, N.; Petrasch, S.; Zhang, N.; Bucher, J.; Shen, S.; Zhao, J.; Bonnema, G. What makes turnips: Anatomy, physiology and transcriptome during early stages of its hypocotyl-tuber development. *Hortic. Res.* **2019**, *6*, 38. [CrossRef]
24. Zheng, Y.; Luo, L.; Gao, Z.; Liu, Y.; Chen, Q.; Kong, X.; Yang, Y. Grafting induces flowering time and tuber formation changes in Brassica species involving FT signaling. *Plant. Biol.* **2019**, *21*, 1031–1038. [PubMed]
25. Tsuro, M.; Suwabe, K.; Kubo, N.; Matsumoto, S.; Hirai, M. Mapping of QTLs controlling root shape and red pigmentation in radish, *Raphanus sativus* L. *Breed. Sci.* **2008**, *58*, 55–61. [CrossRef]
26. Zaki, H.E.M.; Takahata, Y.; Yokoi, S. Analysis of the morphological and anatomical characteristics of roots in three radish (*Raphanus sativus*) cultivars that differ in root shape. *J. Hortic. Sci. Biotechnol.* **2012**, *87*, 172–178.
27. Bihmidine, S.; Hunter Iii, C.T.; Johns, C.E.; Koch, K.E.; Braun, D.M. Regulation of assimilate import into sink organs: Update on molecular drivers of sink strength. *Front. Plant Sci.* **2013**, *4*, 177. [CrossRef] [PubMed]
28. Abdel, C.G. Stomata and trichrome performances of four radish *Raphanus sativus* L. var. *sativus* cultivars grown in controlled cabinet under varying temperatures and irrigation levels. *Intl. J. Farm. Alli. Sci.* **2016**, *5*, 46–65.
29. Ehleringer, J.; Björkman, O.; Mooney, H.A. Leaf pubescence: Effects on absorptance and photosynthesis in a desert shrub. *Science* **1976**, *191*, 376–377.
30. Choinski, J.S., Jr.; Wise, R.R. Leaf growth development in relation to gas exchange in *Quercus marilandica* Muenchh. *J. Plant Physiol.* **1999**, *154*, 302–309. [CrossRef]
31. Hauser, M.T. Molecular basis of natural variation and environmental control of trichome patterning. *Front. Plant Sci.* **2014**, *5*, 1–7.
32. Thomson, G.E. Role of harvest technique and injuries in water loss from stored pak choi (*Brassica rapa* subsp. chinensis) heads. *N. Z. J. Crop Hortic.* **2005**, *33*, 111–115. [CrossRef]

33. Von Caemmerer, S.; Lawson, T.; Oxborough, K.; Baker, N.R.; Andrews, T.J.; Raines, C.A. Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco. *J. Exp. Bot.* **2004**, *55*, 1157–1166. [CrossRef]
34. Xu, Z.; Zhou, G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* **2008**, *59*, 3317–3325. [CrossRef] [PubMed]
35. Rosenkranz, H.; Vogel, R.; Greiner, S.; Rausch, T. In wounded sugar beet (*Beta vulgaris* L.) taproot, hexose accumulation correlates with the induction of a vacuolar invertase isoform. *J. Exp. Bot.* **2001**, *52*, 2381–2385. [CrossRef] [PubMed]
36. Zhang, Y.I.; Turner, J.G. Wound-induced endogenous jasmonates stunt plant growth by inhibiting mitosis. *PLoS ONE* **2008**, *3*, e3699. [CrossRef] [PubMed]
37. Xu, X.; van Lammeren, A.A.M.; Vermeer, E.; Vreugdenhil, D. The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation in vitro. *Plant Physiol.* **1998**, *117*, 575–584. [CrossRef] [PubMed]
38. Peres, L.E.P.; Carvalho, R.F.; Zsögön, A.; Bermúdez-Zambrano, O.D.; Robles, W.G.R.; Tavares, S. Grafting of tomato mutants onto potato rootstocks: An approach to study leaf-derived signaling on tuberization. *Plant Sci.* **2005**, *169*, 680–688. [CrossRef]
39. Zhang, G.; Mao, Z.; Wang, Q.; Song, J.; Nie, X.; Wang, T.; Zhang, H.; Guo, H. Comprehensive transcriptome profiling and phenotyping of rootstock and scion in a tomato/potato heterografting system. *Physiol. Plant* **2019**, *166*, 833–847. [CrossRef]
40. Wang, G.L.; Xiong, F.; Que, F.; Xu, Z.S.; Wang, F.; Xiong, A.S. Morphological characteristics, anatomical structure, and gene expression: Novel insights into gibberellin biosynthesis and perception during carrot growth and development. *Hortic. Res.* **2015**, *2*, 15028. [CrossRef]
41. Jabir, O.; Mohammed, B.; Benard Kinuthia, K.; Almahadi Faroug, M.; Nureldin Awad, F.; Muleke, E.M.; Ahmadzai, Z.; Liu, L. Effects of gibberellin and gibberellin biosynthesis inhibitor (paclobutrazol) applications on radish (*Raphanus sativus*) taproot expansion and the presence of authentic hormones. *Int. J. Agric. Biol.* **2017**, *19*, 779–786. [CrossRef]
42. Sugiura, D.; Betsuyaku, E.; Terashima, I. Manipulation of the hypocotyl sink activity by reciprocal grafting of two *Raphanus sativus* varieties: Its effects on morphological and physiological traits of source leaves and whole-plant growth. *Plant Cell Environ.* **2015**, *38*, 2629–2640. [CrossRef]
43. Chapin Iii, F.S.; Schulze, E.D.; Mooney, H.A. The ecology and economics of storage in plants. *Annu. Rev. Ecol. Syst.* **1990**, *21*, 423–447. [CrossRef]
44. Kyriacou, M.C.; Rouphael, Y.; Colla, G.; Zrenner, R.; Schwarz, D. Vegetable grafting: The implications of a growing agronomic imperative for vegetable fruit quality and nutritive value. *Front. Plant Sci.* **2017**, *8*, 741. [PubMed]
45. Yu, R.; Xu, L.; Zhang, W.; Wang, Y.; Luo, X.; Wang, R.; Zhu, X.; Xie, Y.; Karanja, B.; Liu, L. De novo taproot transcriptome sequencing and analysis of major genes involved in sucrose metabolism in radish (*Raphanus sativus* L.). *Front. Plant Sci.* **2016**, *7*, 585. [PubMed]
46. Sugiura, D.; Watanabe, C.K.A.; Betsuyaku, E.; Terashima, I. Sink-source balance and down-regulation of photosynthesis in *Raphanus sativus*: Effects of grafting, N and CO_2. *Plant Cell Physiol.* **2017**, *58*, 2043–2056. [PubMed]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).