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Article

Effects of Substrate Composition on the Growth Traits of Grafting Seedling in Macadamia Nuts

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Abstract: Macadamia nut plantings in China are expanding year by year. In order to further promote the superior varieties, this study analyzed the effects of different varieties of rootstock and scion on the survival rate as well as the effects of 18 different ratios of substrate composition on seedling growth were analyzed. The results showed that: The survival rate of HAES788 varieties as rootstock and Guire No.1 as scion was the highest, reaching 96%. The optimal grafting time in December was better than that in March. Substrate formulations 12, 13, 15 and 16 had agglomerated soil and well-developed root systems compared to the controls. Plant height, stem diameter, leaf length, leaf width, and dry weight of aboveground and underground parts were significantly higher than those of the control. In addition, the substrate formulations 12, 13, 15 and 16 significantly improved the organic matter, total nitrogen, and total potassium content of the substrate soils, but little on total phosphorus content. All in all, macadamia grafting times are best in December, with HAES788 and Guire No.1 being the best rootstock and scion. Substrate formulations 12, 13, 15 and 16 are the optimal formula. This study provides a solid foundation to produce high-quality macadamia seedlings.

Keywords: macadamia nuts; substrate; surviving rate; growth; grafted seedling

1. Introduction

Grafting is a widely used practice for asexual propagation of fruit trees[1]. It can increase production, Physico-Chemical characteristics, nutritional quality as well as yield of fruit [2,3]. Selection of rootstock and scion is the top priority in fruit tree grafting because differential responses between rootstock and scion [4]. This is because rootstock mediates transcriptional regulation and accumulation of secondary metabolites[5,6]. Furthermore, grafting also affect endogenous hormones contents [7] and oxidative defense reaction [8], even enhance resistance to biotic and abiotic stresses[9,10]. For grafting technique, the substrate or matrix composition are important because it affects the hormonal crosstalk, protein and small molecules movement, nutrients uptake, and transport in the grafted trees[1].

Macadamia nut, also known as *Macadamia integrifolia* Maiden & Betche, is an evergreen fruit tree of the family Proteaceae, native to Australia[11]. Macadamia nuts kernel are rich in oil nutrition[12], can use as medicine and food, and have the reputation of "the king of dried fruits"[13]. *Macadamia* species are generally grown in an environment with an average annual temperature of 19~23 °C, an annual rainfall of more than 1, 000 mm, an altitude of less than 1,300 m, a soil depth of more than 1m, and a soil pH value ranged from 4.5 to 6.5. *Macadamia nuts* begin to bear fruit after 3~4 years of planting, and enter the bumper yield period in 8~10 years, and the economic benefits are increasing year by year[14]. *Macadamia* cultivation is a research hotspot to achieve premature and high yield by cultivating dwarf rootstock varieties, combined with pruning, and dwarfing trees. Macadamia

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seedlings mainly include cuttings, grafting and high-altitude strips, but their wood is brittle and hard, and the survival rate of grafting is low. The grafting method is mainly used for macadamia seedlings at home and abroad, and the seedling grafting techniques of *Macadamia nuts* are different under different ecological and geographical conditions. After years of practice, *Macadamia nuts* are now commonly grafted by splitting, tongue, ventral and dorsal grafting. The survival rate of macadamia nut grafting was 83.30. The grafting rate of "Guire No. 1" macadamia nuts was grafted by branch-belly grafting, and the grafting survival rate was the highest (86.40%), followed by cutting grafting, with a grafting survival rate of 70.60%. Besides, grafting period, grafting method, rootstock quality and rootstock combination are the main factors affecting the survival rate of grafting, and the grafting survival rate is different for different rootstock combinations, and its adaptability, stress resistance and high yield performance will be different. Another important factor is the substrate of seedling [15,16]. The proportion of the substrate composition affects the phenotype, survival rate and biomass of grafting seedlings. The purpose of this study is to screen out Macadamia varieties with strong grafting affinity for rootstock and scion, explore the optimal rootstock combination, and investigate the growth of seedlings through 18 different substrate compositions, and to compare their effects on the biomass of seedlings such as plant height, stem diameter, leaf length, leaf width, etc., and to select the most favorable substrate ratio for seedling growth, so as to provide a scientific basis for the promotion and application of seedlings. The results will provide useful guide for macadamia seedling production.

2. Results

2.1. Effect of Macadamia Scion and Rootstock on Survival Rate

The main Macadamia varieties H2, HAES344, O.C, HAES788, Guire No.1 and HAES695 were used as rootstocks, and Guire No.1, HAES695, O.C and A16 were used as scion, and bud grafting experiments were carried out in December and March of the following year, respectively. The results showed that the bud grafting survival rates of Guire No.1, HAES695, O.C and A16 scion in December and March were 82.25%, 73.33%, 77.13%, 48.67%, 62.04%, 38.67%, 60.56% and 33.67%, respectively. Survival rate ranking as Guire No. 1> HAES695>OC>A16. The budding survival rate in December was significantly higher than that in March. The best combinations were December HAES788 grafted Guire No.1, the survival rate is 96% and March H2, the survival rate is 92% for Guire No.1 (Figure 1). Compared with the respective controls, the survival rate of different rootstocks significantly improves the grafting survival rate.

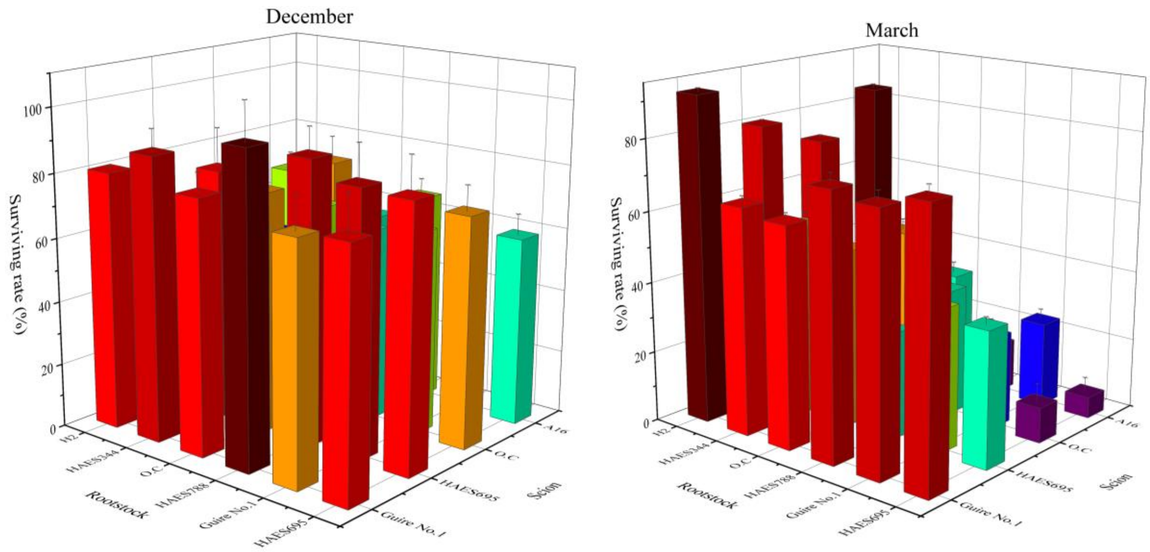

Figure 1. Effects of different scion and rootstock grafting on survival rate.

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2.2. *Effect of Different Substrate Composition on the Growth of Graft Seedlings*

2.2.1. Root Phenotypes of Graft Seedlings Cultivated with Different Substrate Composition

As can be seen from Figure 2, the root growth of the substrate composition T12, T13, T15 and T16 was significantly higher than that of the control (CK). The main change is that the control root system has less soil attachment and less root system of shoots. T12, T13, T15 and T16 treated seedlings showed that the root soil was tightly fixed, the taproot of the shoot-grafted seedlings was thick, and the number of fine roots increased significantly (Figure 2).

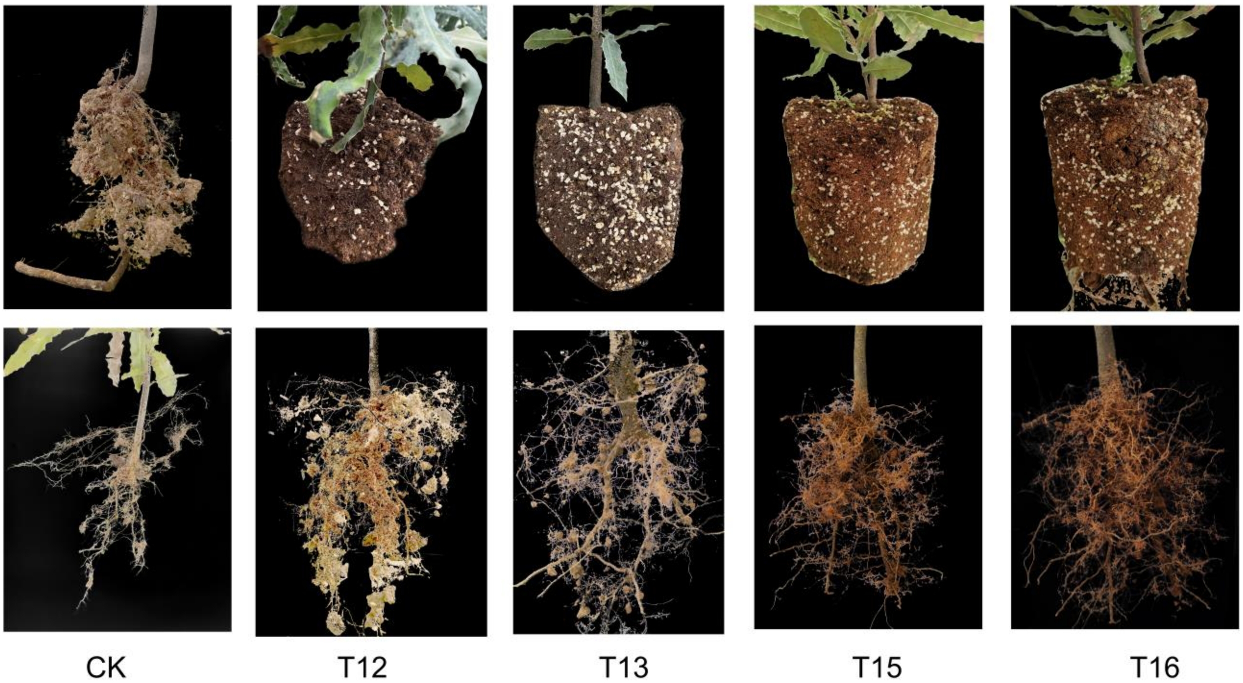

Figure 2. Root phenotypes under T12, T13, T15 and T16 substrate composition.

2.2.2. Effect of Substrate Composition on the Growth of Graft Seedlings

In order to reveal the effect of 18 substrate compositions (Table 1) on the growth and development of grated seedlings, the growth changes of grafted seedlings were continuously observed once every three months. As can be seen from Figure 3, the plant height continued to increase with the extension of cultivation time, and the control plant length increased from 10.4 cm to 61.41 cm. The bud seedlings cultivated in T12, T13, T15 and T16 medium increased from 14.8, 11.2, 13.3 and 12.7 cm to 112.56, 115.85, 128.48 and 126.58 cm, respectively. The stem diameter control grew from 2.82 cm to 9.45 cm. The sprout seedlings cultivated in T12, T13, T15 and T16 media increased from 2.98, 2.93, 2.48 and 2.82 cm to 11.56, 12.58, 12.41 and 13.57 cm, respectively. The leaf length control ranged from 9.8 cm to 24.13 cm. The sprouts cultivated in T12, T13, T15 and T16 media increased from 13.8, 11.5, 9.3 and 14.5 cm to 22.4, 25.63, 25.43 and 28.14 cm, respectively. The leaf width control grew from 1.9 cm to 3.8 cm. The growth of bud seedlings cultivated in T12, T13, T15 and T16 medium increased significantly from 2.6, 2.3, 2.7 and 2.00 cm to 4.5, 4.4, 4.6 and 4.2 cm, respectively (p <0.05).

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Table 1. experimental design of substrates.

Proportion / Substrate formulations	Peat soil	perlite	Wood bran	loess
CK	0	0	0	10
T1	6	1	1	2
T2	6	1	2	1
T3	6	2	1	1
T4	5	1	1	3
T5	5	1	3	1
T6	5	3	1	1
T7	5	1	2	2
T8	5	2	1	2
T9	5	2	2	1
T10	4	2	2	2
T11	4	1	2	3
T12	4	2	1	3
T13	4	2	3	1
T14	4	3	2	1
T15	4	3	1	2
T16	4	1	3	2

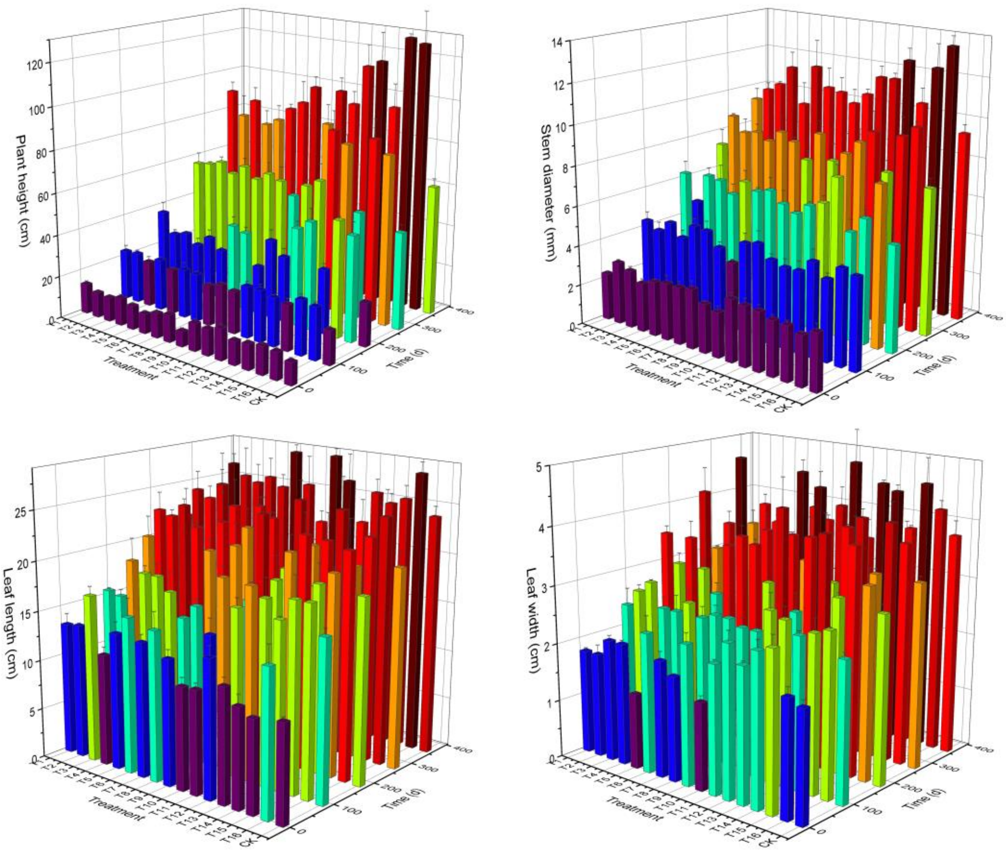

Figure 3. Effects of substrate composition on the plant height, Stem diameter, leaf length and leaf width of grated seedling.

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As can be seen from Figure 4, with the extension of cultivation time, the fresh weight and dry weight of the aboveground and underground parts of the plant continue to increase. The fresh weight of the above-ground parts increased from 4.13 g to 142.36 g. The bud seedlings cultivated in T12, T13, T15 and T16 medium increased from 5.37, 6.83, 5.01 and 6.68 g to 186.36, 210.32, 217.53 and 180.36 g, respectively, with a significant difference (p<0.01). The dry weight of the aerial parts increased from 2.67 g to 48.62 g. The bud seedlings cultivated in T12, T13, T15 and T16 medium increased from 2.21, 2.71, 1.87 and 2.73 g to 85.63, 98.47, 102.36 and 89.47 g, respectively, with a significant difference (p<0.01). The dry weight moisture content of the aboveground parts increased from 35.35% to 65.85%. The bud seedlings cultivated in T12, T13, T15 and T16 medium decreased from 58.85, 60.32, 61.87 and 59.13% to 54.05, 53.18, 52.94 and 50.39%, respectively, with a significant difference (p <0.01).

The fresh weight of the underground part grew from 1.43 g to 58.64 g. The bud seedlings cultivated in T12, T13, T15 and T16 medium increased from 1.96, 3.19, 2.2 and 2.82 g to 81.62, 89.64, 96.35 and 92.45 g, respectively, with a significant difference (p<0.01). The dry weight of the underground part grew from 1.18g to 36.74g. The bud seedlings cultivated in T12, T13, T15 and T16 medium increased from 0.71, 1.11, 0.80 and 1.09 g to 45.63, 52.36, 53.24 and 56.32 g, respectively, with a significant difference (p<0.01). The dry weight moisture content of the underground part increased from 31.79% to 37.64%. The bud seedlings cultivated in T12, T13, T15 and T16 medium decreased from 63.78, 65.20, 63.64 and 61.35% to 44.09, 41.58, 44.74 and 39.08%, respectively, with a significant difference (p<0.01).

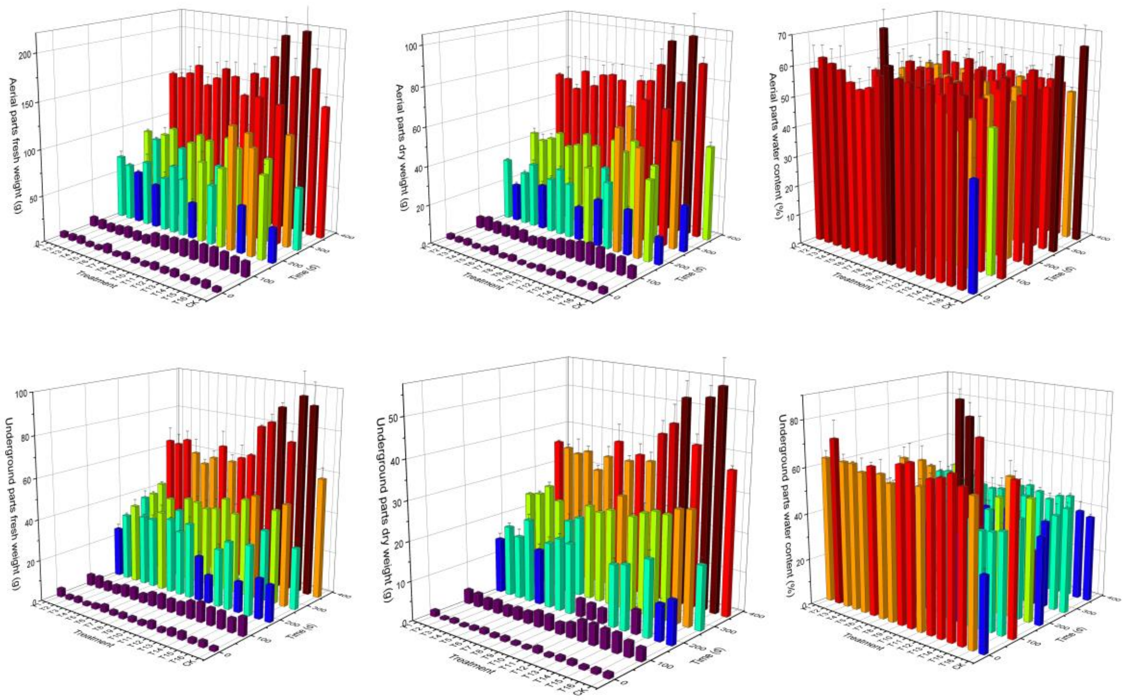

Figure 4. Effects of substrate composition on the Aerial parts fresh weight, Aerial parts dry weight, Aerial parts water content, Underground parts fresh weight, Underground parts dry weight, and Underground parts water content of grated seedling.

2.2.3. Nutrient Composition Changes in Different Substrate Compositions

In order to analyze the effective nutrient composition of the substrate, we compared and analyzed the main nutrients of the substrate after 18 months. As can be seen from Figure 5, the contents of organic matter, total nitrogen, total phosphorus and total potassium in the control changed from 14.6 g kg-1, 0.32%, 0.09% and 0.29% to 39.506 g kg-1, 1.77%, 0.08% and 0.56%, respectively. substrate composition 1-16 had significantly higher levels of organic matter and total nitrogen content than controls. The organic matter content was 2.03~7.21, the total nitrogen was 1.29~3.07 times that of the control, the total phosphorus was 0.88~1.26 times that of the control, and the total potassium was 0.85~2.23 times that of the control. substrate composition 9 had the highest organic matter and total nitrogen contents, which were 284.59 g kg-1 and 5.45 g kg-1, respectively. The total phosphorus of substrate composition 5 was the highest, which was 0.097 g/100g, and the total potassium of substrate composition 13 was the highest, which was 0.73 g/100g.

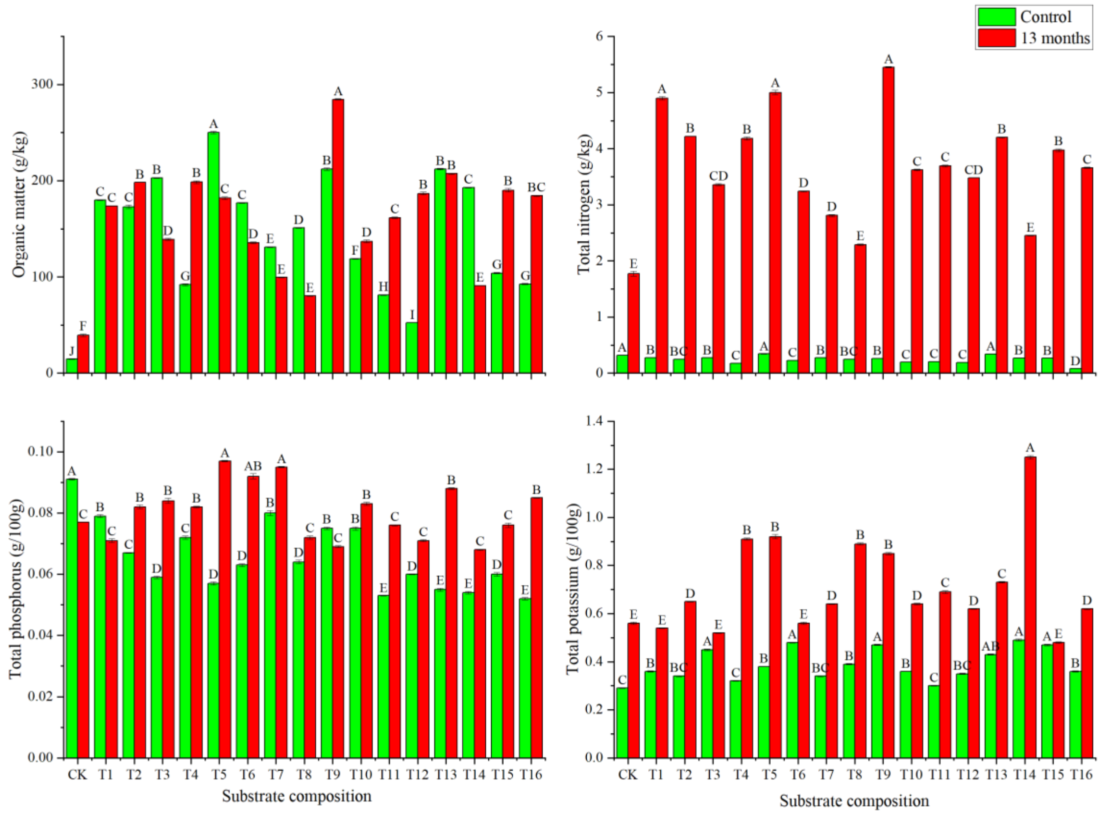

Figure 5. Effect of substrate composition on nutrient composition after 13 months.

3. Discussion

There were significant differences in the growth and development of different varieties of macadamia nut [17,18]. For instance, compared with Guire No.1, the plant height of H2 was higher, the diameter of the underground part of HAES695 was thicker, the taproot of A16, O.C and OV was longer, and the fresh weight of H2 was larger [19]. The HAES741[20] genome analysis provides a good basis for yield component traits in macadamia[21]. Our previous studies showed that 12 months after grafting, the survey found that the survival rate of grafting with different rootstock combinations was between 75.33%~84.67%. A comprehensive comparison of scion growth, taproot length, fresh plant weight and seedling strength index of different rootstock combinations showed that H2, A16 and HAES695 were good growth and development when they were rootstocks. There are general differences in the seedling rate and growth and development of different macadamia nut grafted varieties, and the survival rate of conventional grafting is about only 50.00%. In this study, we found that HAES788 and Guire No.1 being the best rootstock and scion (Figure 1). The survival rate of Grafted Guire No.1 scion with H2 rootstock could reach more than 85.00%. There were also differences in the survival rate of grafting by different grafting methods, and the survival rate of grafting was the highest in the short branch cutting method, with an average of 80.0%. The average survival rates were 61.00% and 48.50%, respectively. The number of leaves left in the grafted rootstock was positively correlated with the survival rate and physiological growth of the scion, and the more leaves of the rootstock, the higher the survival rate of the grafting. A small reduction of rootstock leaves does not affect the survival rate of grafting. This technology can improve the survival rate of macadamia seedlings grafting, shorten the seedling rearing time, and reduce the seedling cost. Grafting time, grafting site, and grafting technique all affect the survival rate of macadamia grafting[22]. The grafting time of macadamia nuts is generally from late October to early February of the following year, and some studies have found that the survival of different varieties of rootstocks

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varies greatly when grafted at different times. The grafting time of this study is 12 months, which may influence the survival rate, and further research is needed about physiology of grafted macadamia nut[21].

On the other hand, substrate composition is very important for the growth of grafted seedlings. For example, along with the cultivation, participate soil organic-matter changes accordingly[23]. Scion and rootstock transportation modulates nitrate uptake capacity[24]. Biomass partitioning of Macadamia with high manganese and low phosphorus concentrations showed that dry weight of roots, stems, branches and leaves accounted for 14-20%, 19-30%, 36-52%, and 12-18% of total plant weight, respectively [17]. In this study, we found that different substrate composition enhanced the plant height, aerial parts fresh weight and other characterizations of grafted seedlings (Figures 3 and 4). Among them, substrate composition T12, T13, T15 and T16 were the best composition (Figures 2-4). The reasons are due to the substrate composition T12, T13, T15 and T16 significantly increases the soil structure and nutrient composition, such as, organic matter, total nitrogen and total potassium (Figure 5). It also found that grafting also affected rootstock rhizosphere microbiome assembly, the scion genotype highly influences the rootstock microbiome in *Rosa* sp[25]. These results were also found in other tropical crops, such as rubber tree [26] and watermelon [5].

In summary, the difference between rootstock and scion had significant influence on the survival rate of Macadamia grafted seedlings. HAES788 and Guire No.1 being the best rootstock and scion. Substrate formulations 12, 13, 15 and 16 are the best soil for grafted seedling growth.

4. Materials and Methods

4.1. Materials

The test site was located in the macadamia resource garden of the experimental station of the Guangxi Subtropical Crops Research Institute, and the 7 varieties were A16, HAES788, H2, Guire No.1 (selected and bred at this station)[27], HAES344, O.C and HAES900. The seeds were picked on September 28, 2021, and the rootstock seedlings were cultivated in non-woven bags with a size of 10.5 cm × 15 cm.

4.2. Seedling Cultivation

On the day of picking, the outer pericarp of the ripe fruit was removed, dried naturally for 2 days, the seeds were soaked in carbendazim 500~1000 times for 24 hours, and after some of the husks were cracked, was sown on the sand bed with a thickness of about 40cm and a width of about 1m at October 1, 2021. Before sowing, the sand bed is sprayed with formalin for insecticide and sterilization, and the film is covered for about 10 days, when the seedlings grow up to 8cm, the seedlings are selected to be bagged with the same height, and the nutrient soil is yellow mud and grass peat.

4.3. Grafting Test

On December 1, 2022, the seedlings of H2, HAES344, O.C, HAES695, HAES788, and Guire No.1 were used as rootstocks, and Guire No.1, O.C, A16, and HAES695 were used as scions, and the grafted rootstock of the same variety was used as the control, and each rootstock combination was repeated 3 times, with 100 plants per repeat. After one year of growth, the surviving rate were calculated to screen best rootstock and scion group.

4.4. Substrate Composition Analysis

To investigate the growth of seedlings through 18 different substrate compositions (Table 1), and to compare their effects on the biomass of seedlings, including plant height, stem diameter, leaf length, leaf width, aerial parts fresh weight, Aerial parts fresh weight, Aerial parts water content, Aerial parts water content, Underground parts dry weight, and Underground parts dry weight to select the best substrate compositions for seedling growth. Each substrate composition randomly selects 5 seedlings with consistent growth and labels, measures the plant height, stem diameter, leaf length and leaf width of the seedlings every three months, and takes the average value, in which the length from the stem base to the growth point is measured with a ruler as the plant height (cm), the stem diameter (mm) at the cotyledons is measured with vernier calipers, and the leaf length (cm) and the leaf width (cm) of the leaf are measured with a ruler.

Two seedlings were selected from 16 substrate composition treatments, and the dry and fresh quality of the aerial parts and underground parts were measured. When measuring the dry and fresh quality of the aerial parts, the stems and leaves were first cut and packed into a file bag, the fresh quality was weighed with an electronic balance, and then put into the oven for 30 min at 105 °C, and then dried in a 75 °C constant temperature drying oven for 24~32 h, and then the dry weight was weighed; the roots were dug out and rinsed with water, and then put into the oven for 30 min at 105 °C, and then dried in a 75 °C constant temperature drying oven for 24~32 h, and then the dry weight was measured.

4.5. Measurement of Nutrient Composition Substrate Composition

Nutrient composition, organic matter, total nitrogen, total phosphorus and total potassium contents of different substrate composition were measured. For organic matter measurements, 10 g subsamples of soil were dispersed in 30 mL of 5 g L^{-1} sodium hexametaphosphate by shaking for 15 h on a reciprocal shaker. The dispersed soil samples were passed through a 53 μm sieve and, after rinsing several times with water, the material that was retained on the sieve was dried at 50 °C overnight. The soil slurry passing through the sieve contained the mineral-associated and water-soluble C and N. Water in the slurry was evaporated in a forced-air oven at 50 °C and the dried sample was ground with a mortar and pestle and analyzed for total organic C [23]. For total nitrogen measurement, put 20 ml distilled water and 20 g soil in a 1000 ml distillation flask. Add 100 ml of potassium permanganate (0.32%) and 100 ml of sodium hydroxide solution (2.5%) to the flask. Stopper the flask immediately and start distillation. The tip of the condenser should dip in the 20 ml of boric acid solution in the beaker. On heating, ammonia will be liberated which will be absorbed in the boric acid. The original wine red/pink red color turns to green with the absorption of ammonia. Collect nearly 100 l of the distillate in about 30 min and add to 1 l of 0.02 N H_2SO4 to get the original pink red wine color and record the burette reading [28]. For total phosphorus measurement, 70 μL of the 250 g L^{-1} $Na_2S_2O_8$ solution was added to a brown glass bottle containing 25 mL of the soil sample and mixed. Then these bottles were placed in the water boiler at 95°C for 3 h. After digestion, 0.5 mL of the AA and MR solutions were added sequentially to the cooled digested solution, and mixed thoroughly. The characteristic blue color fully developed within 5 min at room temperature. The absorbance of the formed PMB compound was measured at 700 nm with a spectrophotometer using a 5-cm cuvette [29]. For total potassium content measurement, add 25 ml ammonium acetate solution extracting solution to 5 g soil and shake it for 5 min. Filter the contents and collect the filtrate. Atomize the above extract on flame photometer and record the readings [28].

Author Contributions: Conceptualization W.W.L. and W.L.F..; methodology, T.Q.J.; software, T.Q.J.; formal analysis, T.Q.J.; Z.C.H. X.P. H.X.Y. P.Z.Z. and W.Y.R. investigation, T.Q.J.; Z.C.H. X.P. H.X.Y. P.Z.Z. and W.Y.R.; resources W.W.L.; writing—original draft preparation, T.Q.J.; writing—review and editing, W.W.L. W.L.F.); funding acquisition, W.W.L. and T.Q.J. All authors have read and agreed to the published version of the manuscript.

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

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