

GRAFT COMPATIBILITY OF *CAMELLIA OLEIFERA* CHANGLIN 4 CLONE WITH FIVE MAIN ROOTSTOCK

YE HANG^{1,2*}, WANG DONGXUE^{1,2}, WEI WEI¹, WEN RUSI^{1,2},
ZHOU ZHAODI^{1,2} AND JIANG ZEPENG^{1,2}

Guangxi Forestry Research Institute, Nanning city, Guangxi province,
530002, People's Republic of China

Key words: *Camellia oleifera*, Root stock, Graft, Survival, Compatibility

Abstract

Studies were conducted on graft compatibility of *C. oleifera* Changlin 4 superior clone scion and *C. gigantocarpa*, *C. semiserrata*, *C. osmantha*, and *C. vietnamensis* as rootstocks in the seedling stage by using *Camellia oleifera* as control root stock. It shows that the graft survival rate and preservation rate reached 92.67 and 96.79%, respectively when *C. osmantha* was used as the root stock grafting Changlin 4 clone. Compared *C. oleifera* as rootstock, there are no significant differences in sprout and growth of other grafted seedlings. In addition, graft compatibility is the best among four *Camellia* species. The graft survival and preservation rates of *C. vietnamensis*, the second best, they reached 93.33 and 96.80% respectively, but its leaf N, P content was significantly lower than the control after growing one year. *C. semiserrata* shows some incompatibility in the grafting process, of which graft survival rate is just 76.67% and growth is inhibited later. The graft preservation rate of *C. gigantocarpa* is only 11.81% after growing one year and stops growing afterwards, which shows strong incompatibility.

Introduction

With the advancement of *Camellia* new afforestation in recent years, reforestation production brings problems of low survival rate, and early slow growth and so on. However, breeding rootstock is one of the most effective ways to solve such problems, but there are a few reports related to root stock selection of *C. oleifera*. So in this paper, studies were carried on graft compatibility of *C. oleifera* Changlin 4 superior clone scion and *C. gigantocarpa*, *C. semiserrata*, *C. osmantha*, *C. vietnamensis* and *C. oleifera* as rootstocks in the seedling stage to build a foundation for further screening fine *Camellia* rootstocks.

Materials and Methods

Oil-tea *Camellia* that belongs to *Camellia* plants (*Camellia* spp.) contains higher amount of seed oil. It originated from China and was mainly cultivated in China as one of the world's four major woody oil plants with a certain cultivation and management area (Chen 2008). Its main products are tea oil containing more than 90 per cent of unsaturated fatty acid. Tea oil is also known as "Orient olive oil" on which FAO focused the promotion of healthy edible oil (Zhang 2003, Zhang *et al.* 2006).

Test rootstock species including *C. gigantocarpa*, *C. semiserrata*, *C. osmantha* and *C. vietnamensis* and *C. oleifera* was used as the positive control. At the end of October 2011, fruits from those five *Camellia* species mentioned above were collected and dried in natural shade. Then

¹Guangxi Key Laboratory of Special Nonwood Forest Cultivation and Utilization, Nanning city, Guangxi province, 530002, People's Republic of China. ²Improved variety and cultivation engineering Research Center of oil-tea camellia in Guangxi, Nanning city, Guangxi province, 530002, People's Republic of China. *Author for correspondence: Dr. Ye Hang, Yongwulu No. 23, Nanning city of Guangxi province. <ye_hang2010@163.com>.

the seeds were taken out after split and set aside. Changlin 4 scion was collected from *Camellia* cutting orchard in Guangxi Forestry Research Institute.

Test was carried out in Guangxi Forestry Research Institute which located in the northern suburb of Nanning city, latitude 22°56', longitude 108°21', with subtropical monsoon climate, annual average temperature around 20-21°C, January average temperature above 11.8°C, July average temperature at 27.6°C.

Seeds were sterilized by soaking them in 0.1% potassium permanganate solution, then followed by sand germination. Early February 2012, bud seedling rootstocks of which the germ length was at 4 ~ 5 cm and thickness was at 0.2 ~ 0.3 cm cleft grafting Changlin 4 lignification scion were selected. Then they were planted in 8 ~ 12 cm height nutrition bag containing a matrix of pure yellow soil, with the arch cover film, shade nets moisturizing and shading treatment. One hundred plants of each species were grafted with three replicates.

Grafting survival rate and sprout situation were investigated and quantified after 100 days. The survived grafted seedlings were transplanted into 14 ~ 16cm nutrition bowl, with regular water and fertilizer. One year after transplanting, preservation rate and growth of grafted seedlings were analyzed. Also the diameter in upper and lower of graft union each 1cm was measured. One hundred mature leaves were randomly collected for each treatment. Yaxin-1241 leaf area instrument was used to measure leaf area, and Weighting method and Drying method were applied to determine fresh weight and dry weight respectively. Acetone extraction - spectrophotometer measurements was used to detect chlorophyll content (Yuan *et al.* 2009); Kjeldahl constant nitrogen determination method, molybdenum blue colorimetric method and flame photometric method were used for measuring N, P, K content respectively (Zhang *et al.* 2010).

DPS data processing system - Duncan new multiple range method (Clyde. 1956) was applied to variance analysis after using Excel 2003 to calculate the average value.

Results and Discussion

Field survey is the most intuitive method to identify graft compatibility of rootstocks and scion. Early incompatibility of rootstocks and scion affects the formation of graft symbiont and finally leads to death, so graft compatibility can be evaluated through graft survival rate (Yang 2007). Rootstock and grafting healing form symbiotic generally takes 1 to 2 months (Moore and Walker 1981, Jeffree and Yeoman 1983), graft survival rate often was carried in 60 to 100 days later. As shown in table 1, 100 days after grafting, the graft survival rate of *C. gigantocarpa*, *C. vietnamensis* and *C. osmantha* were all over 90%, without any significant differences compared with *C. oleifera* 91.33%; while the graft survival rate in *C. semiserrata* was only 76.67%, which was significantly lower than that of *C. oleifera*. Graft sprout rate of four *Camellia* species was more than 95% among grafting survival plants, having no significant differences with *C. oleifera*, the same as grafted seedling of height, sprout length and coarseness. But the number of newborn leaves of *C. semiserrata* was only 5.33, which was significantly lower than *C. oleifera* 7.03 pieces. Remaining three *Camellia* species, namely, *C. gigantocarpa*, *C. vietnamensis* and *C. osmantha* showed no incompatibility phenomenon from the survival and sprout situation point of view 100 days after grafting.

One year after transplanting, preservation rate and growth of grafted seedlings were examined (Table 2). The preservation rate of grafted seedlings that *C. oleifera* grafted Changlin 4 clone was 96.72%, while *C. semiserrata*, *C. vietnamensis* and *C. osmantha* were 95.65, 96.80 and 96.79% respectively, with no obvious difference compared to *C. oleifera*. However, *C. gigantocarpa* was just 11.81%, which was significantly lower than other four *Camellia* species. Among all the survived plants, the height of *C. gigantocarpa* and *C. semiserrata* grafted seedlings were 16.34

and 14.76 cm respectively, which was significantly lower than *C. oleifera* 23.44 cm, while the *C. vietnamensis* and *C. osmantha* grafted seedlings were 22.26 and 18.86 cm tall respectively, with no significant difference between *C. oleifera*.

Table 1. Survival and sprout situation of different *Camellia* species grafting with Changlin 4.

<i>Camellia</i> species	Survival rate (%)	Sprout rate (%)	Seedling height (cm)	Sprout length (cm)	Sprout coarseness (mm)	Leaf number (piece)
<i>C. oleifera</i>	91.33a	97.45ab	11.34ab	6.99ab	1.50a	7.03ab
<i>C. gigantocarpa</i>	90.67a	96.31ab	12.67a	7.04ab	1.58a	7.23a
<i>C. semiserrata</i>	76.67b	95.69b	9.82b	4.86b	1.52a	5.33c
<i>C. vietnamensis</i>	93.33a	95.71b	11.80a	7.77a	1.60a	6.63abc
<i>C. osmantha</i>	92.67a	98.57a	10.75ab	7.33ab	1.48a	5.50bc

In a vertical column different letters indicate a significant difference in the level of 0.05, respectively. The same as the following tables.

Grafting symbiont formation disrupts, transpiration of water, nutrients and assimilates, which will cause late incompatibility and a "big feet" symptoms. Eventually, it will lead to scion of weak growth or even death, so morphological observation of graft union position and plant growth measurement are used to make identification (Hartmann *et al.* 1997). The results showed that *C. gigantocarpa* rootstock and scion got thicker than other rootstocks. The diameter of upper and lower each 1 cm at the place of graft union were both less than 3 mm (Table 2), but graft union position obviously expanded. Moreover, the maximum diameter was up to 6.26 mm, which is significantly higher than *C. oleifera* grafted seedlings. These lead to the diameter ratio of graft union up and down place each 1 cm and the biggest enlargement place of graft union are 0.46 and 0.37, respectively, which is significantly smaller than *C. oleifera* 0.70 and 0.77, respectively,

Table 2. Seedling preservation, growth and graft union healing situation of different *Camellia* species grafting with Changlin 4.

<i>Camellia</i> species	Preservation rate/%	Plant height/cm	Graft union diameter					
			Upper* /cm	Middle /cm	Lower /cm	Upper/middle	Lower/middle	Upper/lower
<i>C. oleifera</i>	96.72a	23.44a	3.54ab	5.05b	3.92a	0.70a	0.77a	0.93a
<i>C. gigantocarpa</i>	11.81b	16.34bc	2.82b	6.26a	2.32b	0.46b	0.37b	1.26a
<i>C. semiserrata</i>	95.65a	14.76c	3.22b	4.78b	3.42ab	0.68a	0.71a	0.95a
<i>C. vietnamensis</i>	96.80a	22.26ab	4.16a	5.25b	4.36a	0.79a	0.83a	0.95a
<i>C. osmantha</i>	96.79a	18.86abc	3.6ab	4.60b	3.3ab	0.79a	0.72a	1.09a

*' refers to the place of upper, enlargement and lower each 1 cm of graft union, respectively.

clearly showing a late grafting incompatibility. While the rest of *C. semiserrata*, *C. vietnamensis* and *C. osmantha* had no obvious enlargement on the graft union diameter, the ratio between graft union up and down place each 1 cm and enlargement place of graft union all showed no significant difference compared to *C. oleifera* grafted seedling. These data indicated that incompatibility phenomenon was not observed in the morphology determination of graft union.

One year after transplanting, the number of leaves from *C. vietnamensis* and *C. osmantha* grafted seedlings were 28.37 and 28.18 pieces, respectively without any significant difference compared with *C. oleifera* grafted seedlings 36.90 pieces, whereas *C. gigantocarpa*, *C. semiserrata* grafted seedlings had 11.87 pieces and 14.49 pieces respectively, which is significantly less than that of *C. oleifera*. The Leaf area, the fresh weight and the dry weight of *C. semiserrata*, *C. vietnamensis* and *C. osmantha* were not significantly different from *C. oleifera*. N, P, K content of leaves from *C. osmantha* grafted seedlings were 12.61 g/kg, 706.52 mg/kg, 8683.90 mg/kg, respectively with no significant difference between *C. oleifera*, while leaf N, P content of *C. vietnamensis* are significantly lower than *C. oleifera* 11.81 g/kg and 648.27 mg/kg, the same as *C. semiserrata*, of which K content reaching 1,003,0.87 mg/kg, is significantly higher than *C. oleifera*. Because mature function leaf number of *C. gigantocarpa* failed to meet the test requirements, it was not included in variance analysis.

Table 3. Differences of leaf area, quality and nutrient element content in different *Camellia* species grafting with Changlin 4.

<i>Camellia</i> species	Leaf number /piece	Leaf area/ mm ²	Fresh weight /g	Dry weight /g	N (g/kg)	P (mg/kg)	K (mg/kg)
<i>C. oleifera</i>	36.90a	903.30a	0.37a	0.14a	12.68a	698.87a	8613.94b
<i>C. gigantocarpa</i>	11.87b	-	-	-	-	-	-
<i>C. semiserrata</i>	14.49b	770.38a	0.29a	0.12a	11.28b	617.4c	10030.87a
<i>C. vietnamensis</i>	28.37a	748.30a	0.28a	0.11a	11.81b	648.27b	8690.07b
<i>C. osmantha</i>	28.18a	967.86a	0.34a	0.13a	12.61a	706.52a	8683.90b

'-' indicates *C. gigantocarpa* was not involved in significant difference analysis because its mature functional leaves number failed to meet the test requirements.

Table 4. Differences of leaf pigment content in different *Camellia* species grafting with Changlin 4 (Unit: mg/g FW).

<i>Camellia</i> species	Ca	Cb	Car
<i>C. oleifera</i>	1.29b	0.61b	0.42b
<i>C. gigantocarpa</i>	0.60c	0.29c	0.21c
<i>C. semiserrata</i>	1.53a	0.75a	0.51a
<i>C. vietnamensis</i>	1.60a	0.80a	0.51a
<i>C. osmantha</i>	1.57a	0.77a	0.52a

The leaf pigment amount was measured and the results are shown in Table 4. The amount of chlorophyll *a*, chlorophyll *b* and carotenoid in content of *C. oleifera* grafted seedling leaves were 1.29, 0.61 and 0.42 mg/gFW, respectively which was less than in *C. semiserrata*, *C. vietnamensis* and *C. osmantha* grafted seedling leaves. However, the amount of chlorophyll *a*, chlorophyll *b* and carotenoid in *C. gigantocarpa* grafted seedling leaves were 0.60, 0.29 and 0.21 mg/gFW, respectively which are even less than *C. oleifera*.

The growth of the above ground plant parts require root absorption to provide water and inorganic salt. Therefore, the absorption and transport of water and inorganic salt in rootstocks will affect scion of mineral nutrition and water metabolism and other metabolic activities to

change the process of growth and development and eventually form the difference in the tree-shape, fruit quality and yield (Chen and Zou 1993, Agusti *et al.* 2003). Most of the studies showed that rootstock vigor was obviously related to scion growth. The more exuberant rootstock grows, the better scion develops (Cao and Lin 2004). *C. oleifera* that belongs to shrub is difficult to form a crown during early growth. In order to achieve the purpose of early yield, strong growth potential of species should be taken into consideration in the process of screening rootstock. *C. gigantocarpa*, *C. semiserrata*, *C. vietnamensis* and *C. osmantha* are members of faster growing species in *Camellia* plants. Nevertheless, studies have shown this four species as rootstock do not obviously promote the growth of scion Changlin 4 in the stage of seedling. Indeed, whether rootstocks will promote seedling growth after planting still needs to be further investigated.

Acknowledgements

The supports extended by Guangxi Scientific Research and Technology Development (No. GKN1347013-1 & No. GKG1123004-2A), Chinese Forestry Science Data Platform (No. 2005DKA32200-11) , and Extinguished Expert Special Fund--The Flowering Physiology of Guangxi Characteristic Non-wood Forest are gratefully acknowledged.

References

- Agusti M, Almela V, Juan M, Mesejo C and Martinez FA 2003. Rootstock influence on the incidence of rind breakdown in 'Navelat' Sweet-Orange. *J. Hort. Sci. Biotech.* **78**(3): 554-558.
- Cao Jianhua and Lin Weifu 2004. Mutual influence of Brazilian rubber budding trees between rootstock and scion each other. *Chinese J. Tropical Agr.* **24**(5): 55-60.
- Chen Yongzhong 2008. Oil tea *Camellia* superior germplasm resources. China Forestry Publish Press, 15.
- Chen Yongzhong and Zou Junyu 1993. Different rootstocks of orange saplings growth and leaf mineral element content. *J. South China Agr. Univ.* **14**(4): 84-88.
- Clyde Young Kramer 1956. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics.* **12**(3): 307-310
- Hartmann HT, Kester DE, Davies JR FT and Geneve R 1997. Plant propagation: Principles and practices. 6th edition, Upper Saddle River, NJ: Prentice-Hall.
- Jeffree CE and Yeoman MM 1983. Development of intercellular connections between opposing cells in a graft union. *New Phytol.* **93**: 491-509.
- Moore R and Walker DB 1981. Studies of vegetative compatibility incompatibility in higher plants. I. A structural study of compatible auto graft in *Sedum telephiodes* (Crassulaceae). *Amer. J. Bot.* **68**(6): 820-830.
- Yang Rui 2007. Grapes grafted scion combination of screening and early graft compatibility. Lanzhou: Gansu Agricultural University, 34.
- Yuan Fang, Li Xin, Yu Junping, Wang Xuekui, Xu Jiwei and Zhang Lixin 2009. Discussion of spectrophotometric method determinate chlorophyll content and its ratio question. *Plant Physiol. J.* **1**: 63-66.
- Zhang Lijun, Feng Dianqi and Wang Aixi 2010. Apricot leaf and soil nutrient element content analysis. *Chinese Agr. Sci. Bull.* **10**: 192-196.
- Zhang Naiyan 2003. Guangxi *Camellia* improved variety system of current status and development strategy. *Guangxi Forest. Sci.* **3**(4): 211-213.
- Zhang Riqing, Ding Zhilei, Zhang Xu and Wen Li 2006. Literature Review of Genetic Improvement in Oil tea. *Econ. Forest Res.* **24**(4): 1-8.