Effects of Different Seasons on Cutting Propagation of Five Citrus Rootstocks

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Abstract [**Objectives**] To explore the cutting propagation mode of citrus rootstock, improve the survival rooting rate of citrus rootstock, and provide theoretical guidance and technical reference for the rooting research of difficult-to-root plants such as citrus. [**Methods**] Five citrus rootstocks *Citrus tangerina* Tanaka 'Hongju', *Citrus haniana* Hort 'Suanju', *Citrus limonia* Osbeck 'Hongningmeng', *Citrus sinensis* × *Poncirus trifoliata* 'Zhicheng' and *Poncirus trifoliate* (L) Raf. 'Zhike' were used as experimental materials to select the suitable cutting substrate for citrus rootstocks by measuring the physical properties of the substrate. Cutting was carried out in spring, summer, autumn and winter respectively. After cutting, the morphological changes of cuttings were observed regularly, and the callus rate, germination rate and rooting rate of cuttings were recorded. [**Results**] The best substrate for citrus rootstock cutting was peat soil, vermiculite and fine river sand (2∶1∶1). The callus of citrus rootstock in different cutting seasons began to appear in 10 - 22 d, and the callus rate reached 55% - 100%. In terms of budding, the budding time was the earliest in summer and autumn, and slightly later in spring and winter; the germination rate of *C. limonia* Osbeck 'Hongningmeng' was the highest, and the germination rate of *C. sinensis* × *P. trifoliata* 'Zhicheng' and *P. trifoliate* (L) Raf. 'Zhike' was lower; in terms of rooting, *C. limonia* Osbeck 'Hongningmeng' had the earliest rooting time and the highest rooting rate and could reach 100% in all seasons; the rooting rate of *C. tangerina* Tanaka 'Hongju' was 50% - 80%; the rooting rate of *C. haniana* Hort 'Suanju' was 60% - 80%; *C. sinensis* × *P. trifoliata* 'Zhicheng' and *P. trifoliate* (L) Raf. 'Zhike' showed the earliest rooting time and the highest rooting rate in summer, and the latest rooting time and the lowest rooting rate in winter, which were only 14.5%. Therefore, different citrus rootstock varieties should choose the appropriate cutting time according to their own characteristics. [**Conclusions**] The results of this study can provide a scientific basis for a large number of cutting propagation of different citrus rootstocks, and have practical guiding significance for large-scale planting.

Key words Citrus, Cutting propagation, Rooting rate, Varieties

1 Introduction

Citrus, belonging to the subfamily Aurantioideae of Rutaceae, is the largest fruit in the world, and its cultivation area and yield rank first[1]. Grafting is the most commonly used propagation method in citrus production. Rootstock plays a fundamental role in the grafted seedlings of citrus. It not only fixes and supports the scion, but also forms plants together with the scion after healing, thus affecting the growth, fruiting, yield and quality of citrus[2]. In production, citrus rootstocks are mainly propagated by seeds, but their long growth cycle and low reproductive rate limit the development of citrus industry. Cutting propagation can maintain the characteristics of the female parent, is not affected by the season, is convenient to obtain materials, has fast seedling formation and low cost, and is a technology for realizing large-scale rapid propagation. At present, the research on citrus cutting propagation mainly focuses on citron[3], lemon[4-6], *Citrus sinensis* × *Poncirus trifoliata* 'Zhicheng'[7], and mostly focuses on rooting agent[8-9] and substrate[7]. Wang Xiaoan's research shows that branch maturity and leaf retention can promote the rooting and survival of citron cuttings, and rooting agent + mature branches + two and a half leaves are most conducive to the germination of adventitious roots of citron branches[3]. The application of *C. sinensis* × *P. trifoliata* 'Zhicheng' semi-lignified branches, and the cutting effect was the best when the ratio of peat soil: river sand: vermiculite was 2∶1∶2[7]. Luo Junqin found that the cutting effect of "Beijing" with 1 to 2 leaves was better, and the survival rate was higher than 93%[5]. The screening based on lemon cuttage substrate indicated that 1 part of red soil + 1 part of perlite or vermiculite is the ideal cuttage substrate for perfume lemon[6]. Huang Zongbo *et al.*[8] used different concentrations of ABT series and GGR series for cutting seedlings of Satsuma Mandarin twigs, and found that the rooting rate of ABT1 was the highest when it was soaked in 60 mg/kg for 12 h. Lemon branches were treated with different concentrations of IAA, and the rooting effect of 500 mg/L branches was better, and the rooting rate was 90.8% after 50 d of cutting[9]. There are many studies on the cutting of lemons, such as

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'Beijing' lemon[5], perfume lemon[6] and common lemon[9], but there are few studies on other citrus rootstocks. In this study, five citrus rootstocks *Citrus tangerina* Tanaka 'Hongju', *Citrus haniana* Hort 'Suanju', *Citrus limonia* Osbeck 'Hongningmeng', *C. sinensis* × *P. trifoliata* 'Zhicheng' and *Poncirus trifoliate* (L) Raf. 'Zhike' commonly used in Zhaoqing area were used as used as experimental materials. Cutting in different seasons was carried out to evaluate its effects on tree growth, fruit quality and photosynthetic characteristics, so as to provide a reference for citrus rootstock propagation.

2 Materials and methods

2.1 Overview of the experimental site The experiment was carried out in the Citrus Base of Fruit Tree Research Institute of Zhaoqing University, Zhaoqing City, Guangdong Province. Zhaoqing has a south subtropical monsoon climate, located between 22°47′ – 24 °24′ N and 111°21′ – 112°52′ E. The annual average temperature is 21.2 ℃ and the annual average rainfall is about 1 650 mm.

2.2 Experimental materials In spring, summer, autumn, winter, the robust, pest and disease-free branches with full buds were randomly selected from *C. tangerina* Tanaka 'Hongju', *C. haniana* Hort 'Suanju', *C. limonia* Osbeck 'Hongningmeng', *C. sinensis* × *P. trifoliata* 'Zhicheng' and *P. trifoliate* (L) Raf. 'Zhike' plants.

2.3 Experimental methods

2.3.1 Determination of physical properties of substrates. There were 11 treatments in the experiment. They are fine river sand (T1), vermiculite (T2), perlite (T3), peat soil (T4), loess (T5), loess : fine river sand (1 : 1) (T6), loess : perlite (1 : 1) (T7), loess : vermiculite (1 : 1) (T8), loess : perlite : vermiculite (2 : 1 : 1) (T9), peat soil : fine river sand : loess (2 : 1 : 1) (T10); peat soil : vermiculite : fine river sand (2 : 1 : 1) (T11). The physical properties of the substrate were determined with reference to the method of Pu Shenghai[10].

2.3.2 Cuttage treatment method. Five citrus rootstock materials were cut into cuttings with mature branches of about 20 cm, with 5 – 8 plump buds, the upper end of the cuttings was cut flat, and the base was cut obliquely at 45 degrees. The lower cut of the cutting was soaked in rooting agent to the middle of the cutting for 2 h, and the upper cut was coated with Guoguang Hutu wound healing agent with a brush. The rooting powder was GGR produced by Beijing ABT Company with a concentration of 200 mg/L, and Hutu wound healing agent product was produced by Guoguang Company.

A planting pond with a length of 2 m and a width of 1 m was used for cutting, and the row spacing of cuttings was 5 cm × 10 cm. An arch shed with a height of 50 – 60 cm was built with bamboo chips on the slotting bed, covered with plastic film (to prevent rainwater infiltration), and then covered with a black sunshade net (shading). Automatic intermittent mist spray devices were installed in the pool and on the roof of the shed. The water spraying time was set to be 5 min each time, and the water spraying frequency was adjusted according to the weather, and the frequency was controlled to be 3 – 6 times. Each treatment of 30 branches was repeated 3 times.

2.4 Measurement indicators

2.4.1 Determination of growth indicators of cuttings. After 10 d of cuttage, the callus formation rate, budding time and rooting time were observed and recorded. After 90 d of cutting, the germination rate, callus rate and rooting rate of each treatment were counted; 5 plants were randomly selected from each treatment, and the number of new buds, new leaves and total number of roots were counted. The growth indicators of bud length and root length were measured with vernier caliper. The longest root length was the longest root measured from 5 randomly selected plants.

2.4.2 Calculation formula.

Germination rate (%) = Number of germinated cuttings/Total number of cuttings × 100%;

Callus formation rate (%) = Number of cuttings formed by callus/Total number of cuttings × 100%;

Rooting rate (%) = Rooting cuttings/Total cuttings × 100%.

2.5 Morphological observation Cuttings cut from citrus trees were used as materials, and the morphology of cuttings was observed regularly with a microscope and photographed with a camera.

3 Results and analysis

3.1 Physical property analysis of different substrate ratios The substrate plays a very important role in cuttage propagation. The tree species that are easy to root do not have high requirements for the substrate. On the contrary, the tree species that are difficult to root, such as citrus, have strict requirements for the substrate. The substrate needed by the cuttage can fix the citrus cuttage seedlings and enable the root system to have a good growth environment. Generally, roots with strong heat preservation, air permeability and water holding capacity are conducive to root growth. The results show that among the physical properties of fine river sand, the bulk density is large, the grain is small, the water retention and air permeability are poor, and the nutrient is insufficient, which is not conducive to root germination. Perlite has light texture and good air permeability, but its bulk density is small, which is not conducive to the fixation of roots; loess has large bulk density and good water retention, but poor air permeability. Combined with some substrates such as perlite with light texture or fine river sand with high air permeability, the effect is more advantageous than that of single substrate[11-12].

From Fig. 1, it can be seen that in this experiment, the maximum bulk density was T1, and the minimum bulk density was T3. Too large bulk density or too small bulk density is not conducive to cutting. Generally, the reasonable range of bulk density is 0.2 – 0.8 g/cm^3; the total porosity is 60% – 70%, and the suitable substrates are T2, T10 and T11; the water retention capacity is 55% – 75%, and the suitable substrate is T11. According to the

physical properties of various substrates and the successful application of soilless culture, the substrate of peat soil : vermiculite : fine river sand (2 : 1 : 1) was selected as the cutting substrate for the follow-up experiment.

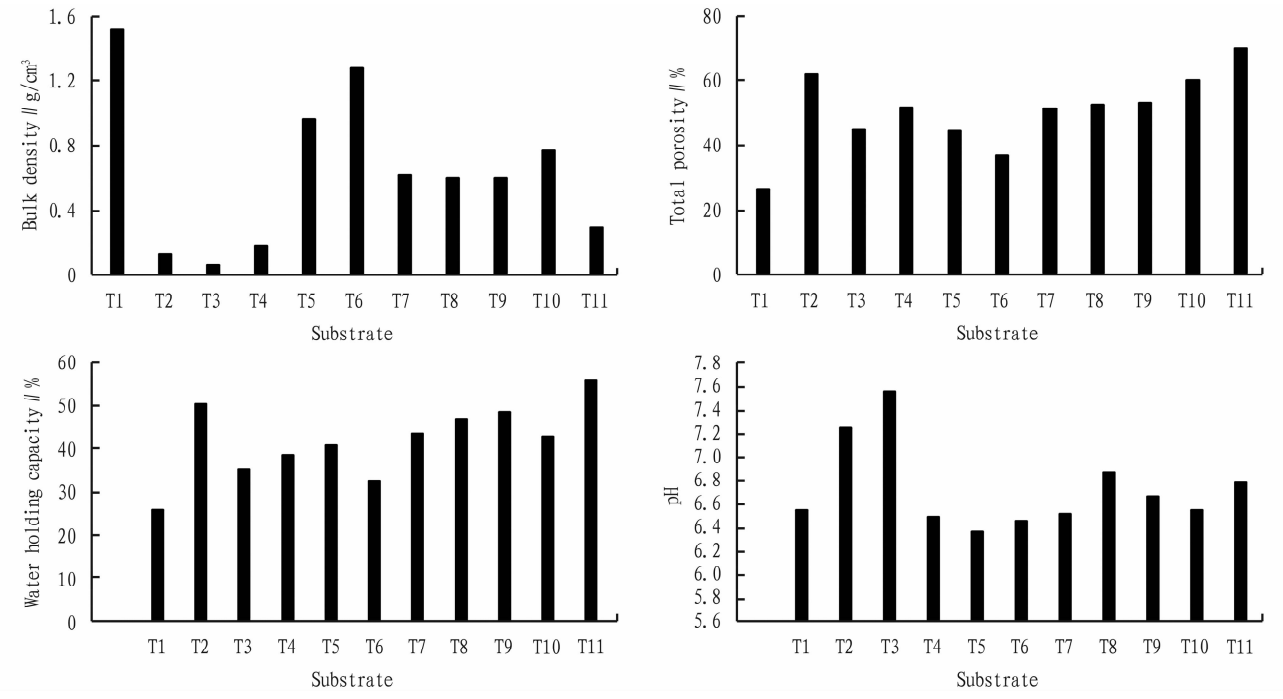


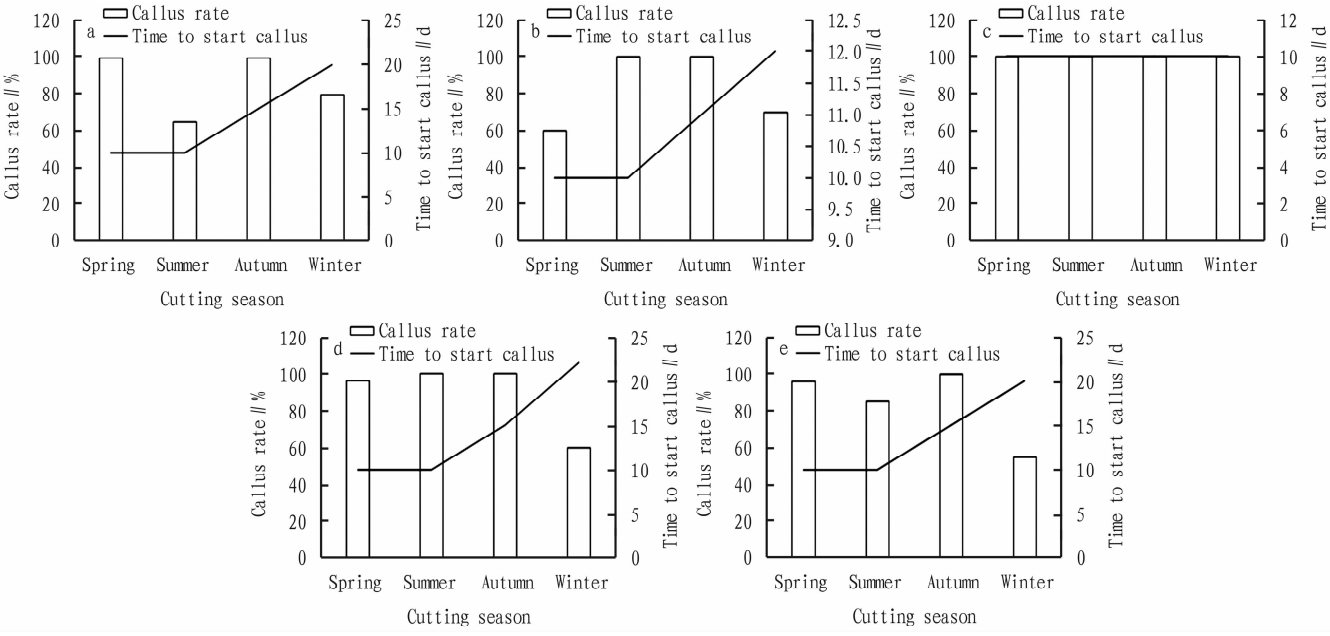
Fig. 1 Comparison of physical properties of different substrate ratios

3.2 Morphological observation on rooting process of citrus rootstock cuttings In the process of adventitious root formation, a key stage is the formation of callus[13]. This tissue not only plays a dual role of absorption and defense, but also plays a vital role before the cuttings take root[14]. Specifically, the callus can accelerate the healing process of the wound at the base of the cutting, effectively prevent the wound from rotting and mildewing due to exposure, and reduce the loss of effective substances inside[14]. In addition, it also undertakes the important task of transporting nutrients and water, providing necessary support for the subsequent growth of cuttings and the formation of adventitious roots[14]. Therefore, the emergence of callus plays an important role in the success of plant cutting propagation. In this study, the changes of the base of cuttings were observed regularly from the 0 d of cutting. Callus began to appear in 10 – 22 d in the five rootstocks in different seasons, and the callus rate reached 55% – 100% (Fig. 2). In the early stage of cutting, the process of cell proliferation and differentiation was initiated at the incision at the base of the cutting, which showed that the cells at the edge of the incision swelled, and then the initial white translucent callus was formed (Fig. 3A). In the middle stage of cutting, the callus entered the rapid growth period, and the volume and quantity of the callus increased significantly. Some cuttings induced adventitious roots under the callus or epidermis (Fig. 3B). With the extension of cutting time, the adventitious roots underwent further morphological development, including elongation, thickening and significant increase in number (Fig. 3C). In the late stage of cutting, the secondary lateral roots gradually appeared, the root system became darker, and the rooting process was completed (Fig. 3D).

The adventitious roots of cuttings can be divided into latent root primordium and induced root primordium according to their sources[15]. Latent root primordium is a potential adventitious root origin formed before the cutting is intercepted, which is originally in a dormant state. When encountering a suitable cutting environment, the dormant root primordium is activated to continue its growth and development process, and eventually differentiate into adventitious roots[15]. Tree species with latent root primordia often show stronger rooting ability in cutting propagation, which makes the whole cutting process relatively easy to succeed[16]. Induced root primordium is induced by external environment or hormone after cutting[17]. In this study, the morphological observation showed that 'Hongningmeng' was easier to root, which may belong to the latent rooting type. The rootstocks of the other four varieties were all induced rooting type. In addition, it was observed that there were four stages in cutting propagation of citrus rootstock, which were callus induction stage, adventitious root formation stage, root primordium transformation into adventitious root and adventitious root further growth and development into plants.

3.3 Effects of different cutting seasons on the germination of five citrus rootstocks The experimental results showed that the germination time of citrus was different in different cutting periods, except 'Zhicheng' the germination time of other varieties was summer, the germination time of autumn was the earliest, which was 3 – 5 d, and that of spring and winter was slightly later. The germination time of 'Zhicheng' was later than that of the other four rootstocks, from 18 to 43 d. 'Zhike' had a very late germination time of 52 d. The average bud number was between 0 and 4. The germination rate of 'Hongningmeng' was the highest, which

was 100%, followed by 'Hongju' and 'Suanju', which were between 50% and 100%; Both 'Zhicheng' and 'Zhike' had lower germination rates of 0 – 17.1% (except for the germination rate of 'Zhicheng' in autumn), as shown in Fig. 4.



NOTE a. 'Hongju', b. 'Suanju', c. 'Hongningmeng', d. 'Zhicheng', e. 'Zhike'

Fig. 2 Callus of citrus rootstock cuttings

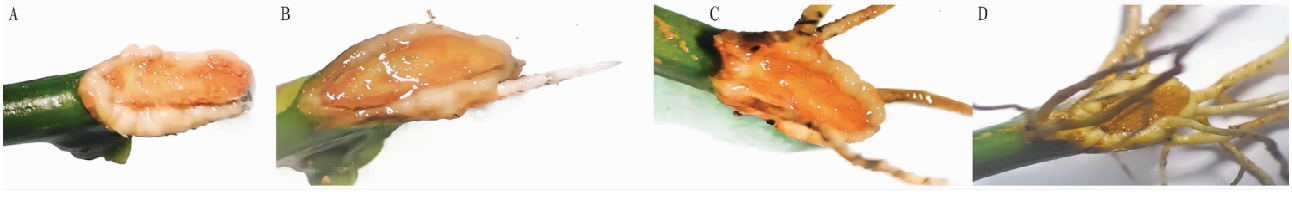
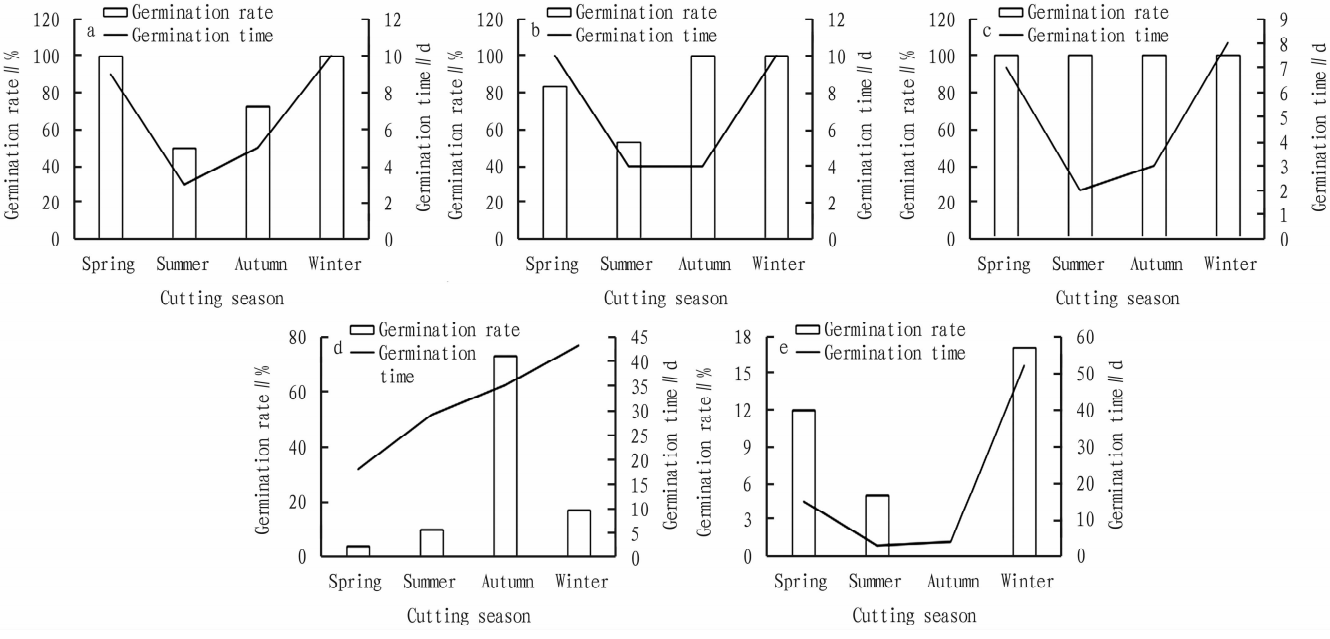
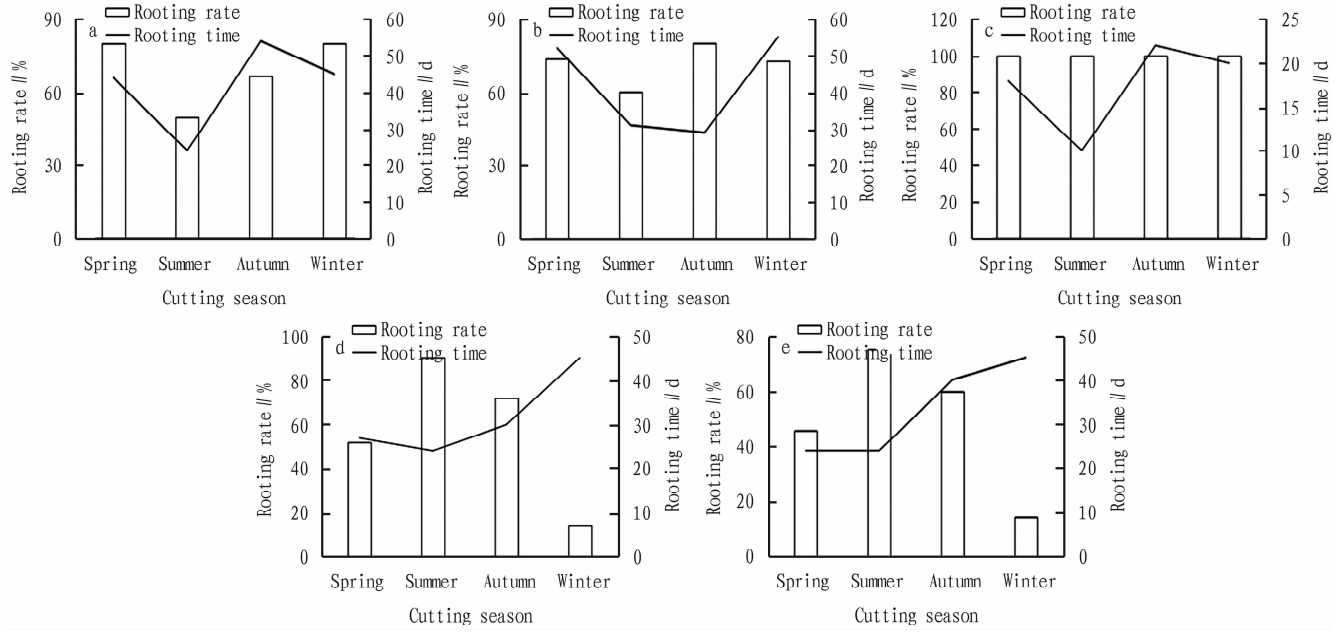


Fig. 3 Morphological changes at the base of cuttings during rooting of citrus rootstock



NOTE a. 'Hongju', b. 'Suanju', c. 'Hongningmeng', d. 'Zhicheng', e. 'Zhike'.

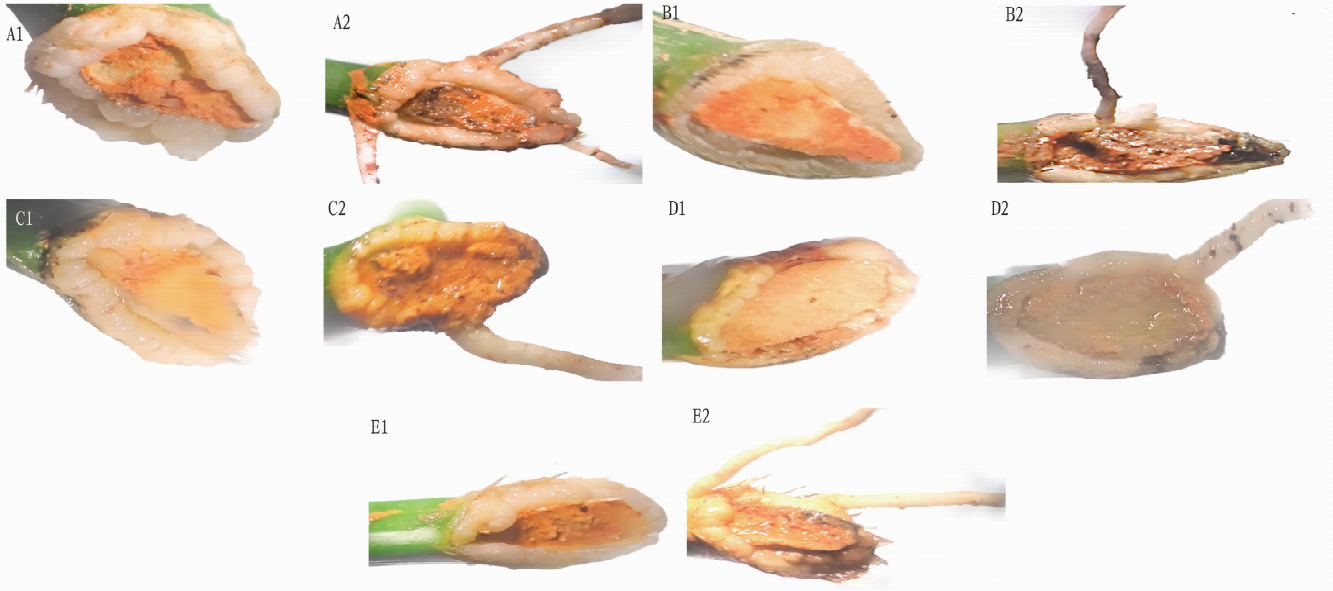
Fig. 4 Germination of five citrus rootstocks in different cutting seasons



NOTE a. 'Hongju', b. 'Suanju', c. 'Hongningmeng', d. 'Zhicheng', e. 'Zhike'.

Fig. 5 Rooting of five citrus rootstocks in different cutting seasons

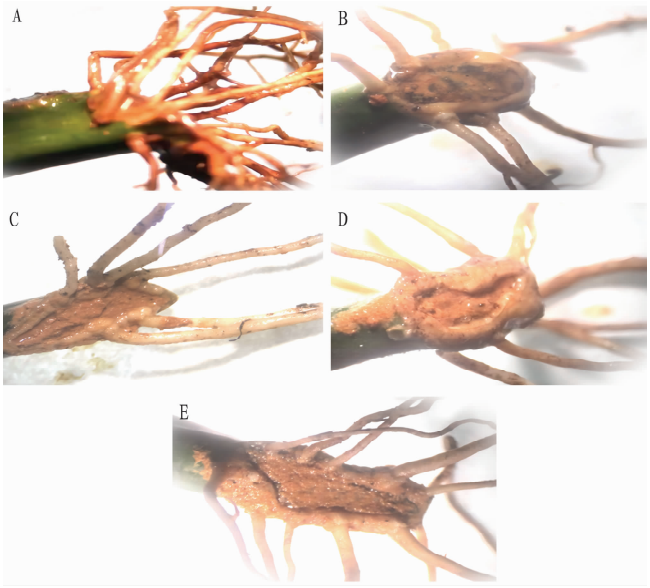
3.4 Effects of different cutting seasons on rooting of 5 citrus rootstocks There is a close correlation between the rooting rate of cuttings and the cutting season, and different cutting seasons have a significant impact on the survival rate of seedlings[18]. Studies have shown that whether cuttings can take root is the key to their survival[19]. It can be seen from Fig. 5 that the rooting time and rooting rate of different citrus rootstocks were different in different seasons. The rooting time of 'Hongningmeng' was the earliest and the rooting rate was the highest, which could reach 100% in each period. The rooting rate of the 'Suanju' was between 60% and 80%. The rooting rate of 'Suanju' ranged from 50% to 80%, and the rooting rate was the lowest in summer. Both 'Zhicheng' and 'Zhike' showed the earliest rooting time and the highest rooting rate in summer, and the latest rooting time and the lowest rooting rate in winter, which were only 14.5%. Taking spring cuttage as an example, 'Hongningmeng', 'hongju', 'Suanju', 'Zhike', the callus formation time of 'Zhicheng' was 10 d, and 'Hongningmeng' had formed adventitious roots 18 d after cutting, followed by 'Zhike' (24d), 'Zhicheng' (27 d); 'hongju' and 'Suanju', formed adventitious roots later, 44 d and 52 d, respectively (Fig. 6).



NOTE A1: 'Hongningmeng' 10 d; A2: 'Hongningmeng' 18 d; B1: 'Hongju' 10 d; B2: : 'Hongju' 44 d; C1: 'Zhicheng' 10 d; C2: 'Zhicheng' 27 d; D1: C. 'Suanju' 10 d; D2: C. 'Suanju' 52 d; E1: 'Zhike' 10 d; E2: 'Zhike' 24 d.

Fig. 6 Rooting of citrus rootstock cuttings in spring

From Fig. 7 and Table 1, it can be seen that the average number of roots of 'Hongningmeng' is the most, which is significantly better than that of other varieties; as far as 'Hongningmeng' variety was concerned, the average number of roots in summer was the most, which was 12.75%, and there was no significant difference among other seasons. From the point of view of the maximum rooting number, there were differences among different rootstocks in different cutting periods. The 'Hongningmeng' of cutting in summer had the largest number of hairy roots, reaching 20 roots per plant; the rooting number of 'hongju' and 'Suanju' were the most in autumn and winter, which were 7 and 6 per plant, respectively; the rooting number of 'Zhicheng' was the most in summer (12 roots per plant) and the least in winter, and that of 'Zhike' was the most in spring (10 roots per plant) and the least in winter.



NOTE A: 'Hongningmeng'; B: 'hongju'; C: 'Suanju'; D: 'Zhicheng'; E: 'Zhike'.

Fig. 7 Rooting of different citrus rootstocks

4 Discussion and conclusions

4.1 Discussion

4.1.1 Selection of citrus rootstocks for cuttage. The substrate has an important impact on the rooting of plant cuttings, and it determines the root growth environment. The substrate should have the characteristics of air permeability and water retention, which is the key factor to determine the survival rate and growth of seedlings[4]. Different types of substrates have different water and fertilizer retention capacity, air permeability, nutrient types and contents and other physical and chemical properties and microbial environment, which will affect the rooting effect of cuttings[20]. At present, a single substrate or a mixed substrate such as peat soil, perlite, vermiculite and that like is usually use for the cuttage substrate, and the cuttage substrate is adjusted accord to the characteristics of the cuttage plant. In this experiment, peat soil, vermiculite and fine river sand (2 : 1 : 1) were used to determine the physical properties of substrate, combined with the characteristics of citrus seedling. The results showed that the substrate was excellent in all aspects of citrus cutting, and it was suitable for citrus cutting.

Table 1 Effect of cutting season on rooting of citrus rootstock cuttings

Rootstock	Cutting season	Average number of roots pcs/plant	Average root length // cm	Max rooting number pcs/plant
'Hongju'	Spring	2.80 ±0.28 a	2.76 ±0.17 b	5
	Summer	2.10 ±0.41 a	2.54 ±0.66 b	4
	Autumn	2.00 ±0.16 a	8.81 ±0.58 a	3
	Winter	2.17 ±0.17 a	7.95 ± 1.36 a	7
'Suanju'	Spring	1.00 ±0.06 a	3.30 ± 0.22 c	3
	Summer	2.00 ±0.60 a	6.30 ±0.22 b	5
	Autumn	1.13 ±0.24 a	6.22 ±1.19 b	6
	Winter	1.33 ±0.26 a	11.34 ±2.63 a	3
'Hongningmeng'	Spring	7.60 ±0.67 b	6.99 ±0.41 c	12
	Summer	12.75 ±0.86 a	14.00 ±0.00 a	20
	Autumn	5.00 ±0.53 b	8.40 ±1.57 b	7
	Winter	5.20 ±0.66 b	6.05 ±0.50 c	7
'Zhicheng'	Spring	1.00 ±0.25 a	5.45 ±0.41 b	5
	Summer	2.25 ±0.57 a	6.26 ±0.85 b	12
	Autumn	2.45 ±0.47 a	9.92 ±1.14 a	2
	Winter	1.03 ±0.14 a	11.97 ±1.01 a	2
'Zhike'	Spring	1.96 ±0.55 a	5.32 ±0.38 b	10
	Summer	1.30 ±0.26 a	4.77 ±0.54 b	4
	Autumn	1.10 ±0.41 a	5.00 ±0.18 b	1
	Winter	1.05 ±0.12 a	11.97 ±3.39 a	1

4.1.2 Development of adventitious roots of citrus rootstock cuttings. The key to the survival of tree cuttings is the formation of adventitious roots, which is not only related to the climate conditions and the type of rooting agent selected, but also related to the physiological state of the cuttings themselves[19]. In the process of plant adventitious root formation, the production of callus has the function of absorption and defense, which can promote wound healing, prevent wound decay, mildew and loss of effective substances[14]. In this study, the rooting process of citrus cuttings was dominated by callus rooting. The color of the callus of the cuttings was white at first, and gradually changed to brown with the passage of time, showing an aging state. There were differences in callus morphology and formation time among different citrus cultivars. Some cuttings produced calli, but did not take root or die. The reason for this may be that there were too many calli, which inhibited the rooting process of cuttings.

4.1.3 Effects of different citrus rootstocks on rooting rate of cuttings. Citrus is a fruit tree that is difficult to root by cutting, and the rooting rate of different varieties is different under the same cutting environment[18]. The rooting rate of fruit tree cuttings is affected by genetic factors, cutting position and water content, and the formation of adventitious roots is a relatively complex physiological process[18]. In this experiment, the rooting rates of five cit-

rus rootstocks were different in different cutting seasons. The 'Hongningmeng' rootstock showed no difference in each cutting season and showed good rooting rate, which indicated that the variety was easy to survive by cutting. The rooting rates of 'Suanju' and 'hongju' were slightly different in different seasons, but both were above 50%, indicating that the two varieties were easy to survive. The rooting rates of 'Zhike' and 'Zhicheng' were higher in summer and lowest in winter, indicating that the cutting of these two varieties was greatly affected by time.

4.1.4 Effects of cutting time on rooting of citrus rootstock. The difficulty of cutting survival is not only closely related to the characteristics of the plant itself, but also affected by multiple factors such as temperature, air humidity and cutting season selection[21]. Cutting in spring, especially around the rainy season in late spring, is suitable for rooting and germination of cuttings. For cutting in summer, the plant is in the period of vigorous growth, the nutrient in the cutting is sufficient, and the cell division is active, which is conducive to rooting and germination, but it may also lead to excessive transpiration and water loss of the cutting, thus affecting rooting and survival. Cutting in autumn has the advantages of suitable climate, high rooting rate and less pests and diseases, but it also has the disadvantages of gradually decreasing temperature, increasing difficulty in management, longer rooting time and vulnerability to frost damage. Winter cutting has the advantages of saving labor and rooting before germination, but winter cutting also faces some problems, such as too low temperature, slow metabolism of cuttings in dormancy period, resulting in slow growth of cuttings.

In this study, we found that there were significant differences in the rooting rate of different citrus varieties in different seasons. Five citrus rootstocks 'hongju', 'Suanju', 'Hongningmeng', 'Zhicheng', and 'Zhike' showed significant differences in the rooting rate of citrus varieties in different seasons. In spring, the cutting effect of 'hongju' and 'Hongningmeng' was good, and the rooting rate was high; in summer, the rooting rate of 'Zhicheng' and 'Zhike' was high; in autumn, the rooting rate of 'Suanju' and 'Hongningmeng' was high; in winter, the rooting rate of 'Zhike', 'Zhicheng' cuttings was low. These suggest that each cultivar has its own specific optimum period for rooting cuttings.

4.2 Conclusions In conclusion, the cutting propagation process of citrus is a complex and changeable process, and its success rate and rooting effect are significantly affected by multiple environmental and management factors[22]. Different citrus rootstock varieties should choose the appropriate cutting time according to their own characteristics. In this study, the physical properties, cutting time and cutting varieties were compared and analyzed. The results showed that the substrate of citrus rootstock cutting propagation was peat soil, vermiculite and fine river sand (2∶1∶1). The rooting rate of 'Hongningmeng' was the highest among the five rootstocks, and the rooting rate was 100% in every season. The best cutting time of 'hongju' and 'Suanju' was in spring and winter. The best cutting time of 'Zhicheng' and 'Zhike' was in summer. Therefore, different citrus rootstock varieties should choose the appropriate cutting time according to their own growth characteristics. This study is expected to provide a theoretical basis and technical support for the establishment of citrus rapid propagation system and the promotion of citrus cultivation.

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