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A Study of Rooting Characteristics and Anatomical Structure of Feijoa Cuttings

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Abstract: Softwood and 1-year-old hardwood from 4-year-old mother trees of feijoa cultivar 'Unique' were used to study the rooting capacity of cuttings during their development. The result showed that the rooting of softwood cuttings was better than that of hardwood cuttings. The secondary structure of stems and the development process of adventitious root were observed by means of paraffin section and microscopy on feijoa cuttings. The result of the observation showed that the adventitious root primordium of feijoa cuttings is a type of induced root primordium. This adventitious root primordium originated from the junction of the vascular cambium and pith rays. There were cyclical and sequential sclerenchyma cells in the phloem and no root primordium in the stems. The adventitious roots of cuttings generated from the edge of the cut section to 0.1-2 cm above the cut. These structural features may be one of the main reasons that our feijoa cuttings showed a low rooting rate.

Key words: *Feijoa sellowiana* berg., stem, cutting, adventitious roots, anatomy

INTRODUCTION

Feijoa sellowiana is a subtropical sp., belonging to the Myrtaceae family. It is native to South Brazil with a secondary dispersion in Uruguay (Thorp and Bieleski, 2002). The fresh fruit is enjoyed for its characteristic flavour and aroma, which are similar to pineapple. For this reason, it is also called 'pineapple guava'. Owing to its easy adaptability in subtropical regions, nowadays it is extensively cultivated in America, France, Spain, Russia, Australia, Japan and especially in New Zealand, where the fruits are popular. In China, this species was introduced at the end of the 20th century, initially as an ornamental plant. Recently, the species has assumed some economic relevance, with orchards planted in the Southern regions of the country, such as Jiangsu, Shanghai, Sichuan and Zhejiang. As a newly rising species for its edible fruits, ornamental and medicinal properties, feijoa shows great potential in foods, drugs and cosmetics (Hideki, 2001; Hardy and Micbapl, 1970; Kolesnik *et al*., 1991). Supported by the overseas intellectual resource introduction program of Sichuan Bureau of Foreign experts administration, professor Wang *et al*. (2007) in Southwest University of Science and Technology (SWUST) introduced a small number of fruit seedlings from New Zealand in 2004. She considers feijoa to have great potential for development of ornamental trees and edible fruits because it retains its fine qualities in Sichuan climatic conditions (Wang *et al*., 2007).

Feijoa can be propagated either from seed or by cuttings. They are relatively easy to grow from seed, but the quality of fruit from seed-propagated trees varies considerably from one seedling tree to the next. Cutting propagation can maintain the original properties and is therefore, widely used in propagation. However, there is a low rooting rate in the process of cutting propagation (Figueiredo *et al*., 1995). It is urgent to speed up the rate of reproduction in order to meet production demand. To date, there has been no anatomical study of the reason for this low rooting rate. In this study, the anatomy of occurrence and development of adventitious roots was studied to explain the mechanisms of root system formation, in order to provide a theoretical and technical basis for more extensive planting.

MATERIALS AND METHODS

On May 20th, June 20th and October 20th, 2007, 1-year-old hardwood and softwood cuttings were taken from *Feijoa sellowiana* Berg. 'Unique' 4-years-old mother

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plants. Cuttings were collected from each tree top, keeping 2-3 nodes and 2 pairs leaves (half-leaf pruned) and all cuttings were 8-12 cm long.

The experiment was carried out in the Agriculture Laboratory of SWUST. Cuttings were dipped in 1000 mg L^{-1} IBA for 10 sec, then inserted into cell trays containing a perlite medium. The experiment was a completely randomized design with 3 replications and 30 subsamples (cuttings) per replication. All cuttings were evaluated for root length, root number and rooting percentage after 40 days.

In order to discover, the source of adventitious roots, the secondary structure of stems and the development process of adventitious root were observed by means of paraffin section and microscopy on feijoa cuttings taken on June 20. Five cuttings were observed every 5 days. Material for light microscopy was fixed in FAA, dehydrated in an ethanol series (30-100%) and imbedded in paraffin (Li, 1987). Sections 10 μm thick were made on a microtome and stained with safranine-brilliant green. Sections were observed using a Olympus-DMB5 transmission electron microscope.

RESULTS

The rooting capacity of feijoa cuttings taken at different times: The rooting capacity of feijoa cuttings was closely related to physiological maturity and cutting time. As Table 1, cutting date had a greater impact on root length, root number and rooting rate and the rooting of softwood cuttings was better than that of the hardwood cuttings. The rooting capacity of softwood cuttings taken on June 20th was best, obviously much better than softwood cuttings on May 20th and hardwood cuttings on October 20th. Therefore, in order to obtain a higher rooting rate, softwood should be chosen for cuttings, which should optimally be taken around mid-June.

External morphological observation of rooting process of feijoa cuttings: The external morphology of cutting rooting process on feijoa was observed by collecting softwood cuttings on June 20th. It took 30 days from cutting to rooting. The lower incision organization started to become loose after 10 days and after 15 days the cortex of the lower incision of some cuttings showed minor cracking. In a small minority of cuttings, the incision began to show a small quantity of crystal-shaped callus neurites along the vascular cambium and cortex, which gradually formed a zonal pattern, but there was no callus seen in the incision of most cuttings after 20 days. The basal position of cuttings began to enlarge after 25 days.

Table 1: The rooting capacity of cuttings

Cutting time	Branch type	Root length (cm)	Root no.	Root rate (%)
May 20	Softwood	10.5	3.2	51.1
June 20	Softwood	13.5	4.7	67.8
October 20	Hardwood	6.8	1.7	20.0

After 30 days the outside of the periderm near the incision thickened slightly, swelling and forming many small white protrusions (Fig. 1a), then the tips of these protrusions burst and transparent white young adventitious roots erupted successively in all cuttings (Fig. 1b).

It was observed that cuttings in which callus was undeveloped or absent had better rooting and cuttings with developed callus had fewer roots, or even took no root. The adventitious roots of cuttings generated from the verge of the cut section to 0.1-2 cm above the cut (Fig. 1c).

Anatomical investigation of cuttings rooting process in feijoa: The anatomical investigation of the softwood cuttings of feijoa collected on June 20th was observed and recorded. The stem is composed of periderm (including skin debris), cortex and secondary vascular tissues from the outside to the inside (Fig. 1d). The cortical round cells were arranged more loosely, with a greater gap among cells, containing a large number of visible crystals (Fig. 1e). The cortex shows a vascular tissue system, which includes the phloem, vascular cambium, xylem, vascular rays and pith. The phloem lateral side shows several layers of selerenchyma cells (Fig. 1f), arranged in a more continuous loop which can strengthen the support capacity of the stem.

From stem transection viewing, latent root primordia did not exist (Fig. 1d), indicating that there was no root primordium produced during the plant's normal growth process.

The cuttings first developed root primordium, then generated adventitious roots. Twenty days after softwood cuttings were taken on June 20th, parenchyma cells located in the junction of cambium and pith ray cells regained meristematic capacity. As a result, the cytoplasm changed concentration and the vacuoles narrowed, following which the parenchyma cells split and formed a parenchyma cell mass which showed larger nuclei, closely arranged, more clearly distinct from the surrounding cells. The cell in the mass continued to split from the cortex end of the mass and in the development direction of the root germ cells, some of the dyed red root primordium cells showed larger nuclei and thick cytoplasm. Root primordium cells near cambium cells stained darker and showed stronger divisibility, gradually forming a group of smaller and darker staining meristematic cells, constituting meristematic tissue mass (Fig. 1g).

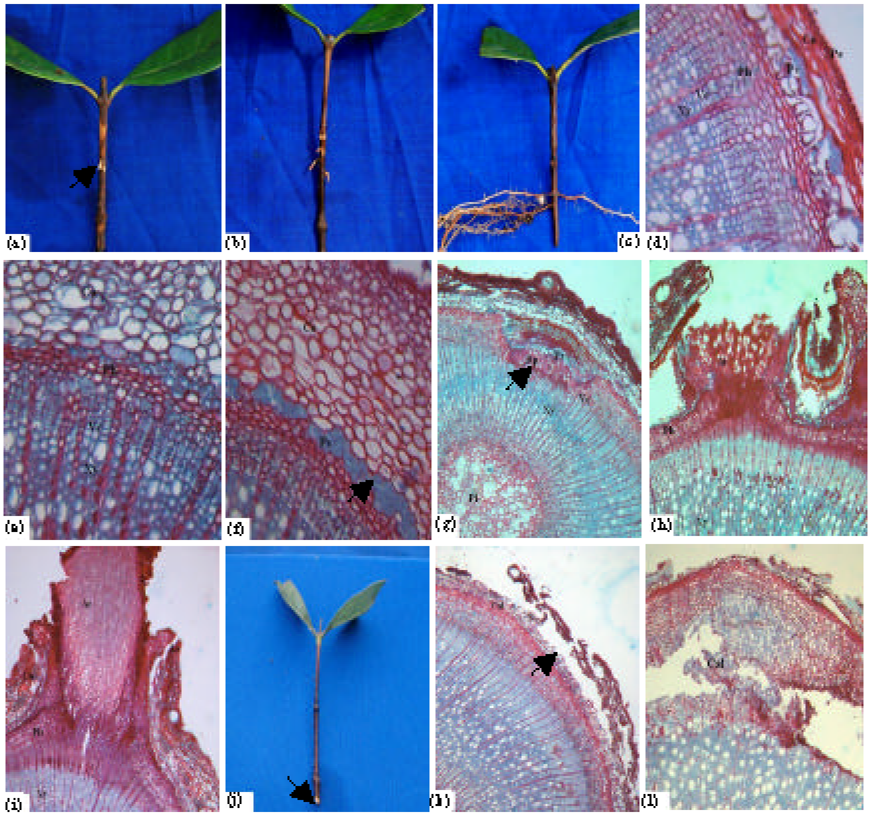

Fig. 1: Morphological and anatomical observation on rooting of feijoa cuttings. Ar: Adventitious root; Cal: Callus; Co: Cortex; Le: Lenticel; Pe: Periderm; Ph: Phloem; Pi: Pith; Ps: Phloem selerenchyma; Rp: Root primordium; Vc: Vascular cambium, Xy: Xylem. (a): White dot in cutting, b): Adventitious root emerging from stem, c): Adventitious root, d): Stem transection (40×), e): Crystal structure in stem cortex (40×), f): Selerenchyma in stem phloem (40×), g): Development of root primordium (10×), h) Delaminating phenomena of adventitious root emerging from lenticel (10×), i): Adventitious root emerging from lenticel (10×), j): Callus, k): Parenchyma cell of callus (10×) and l): Callus of stem (10×)

Meristematic tissue cells continued periclinal and anticlinal division. The cells connected with the root primordium and around the cambium layer differentiated faster, forming wedge-shaped adventitious root primordia. These extended along the phloem-ray direction, through the cortex and epidermis, ultimately extending outside the stems (Fig. 1h). By the time they reached the periderm, the differentiation of adventitious root vascular systems was completed and connected with the vascular tissues in the stem, by which time distinct layers had emerged, forming a root cap, meristematic zone and an elongation zone (Fig. 1i).

Twenty days after feijoa cuttings were taken, the basal portion had formed callus (Fig. 1j). By anatomical observation, the early callus was composed of parenchyma cells, which were round and more loosely arranged, had no obvious nucleus and initially originated from cambium cells near the clip section of cut surface (Fig. 1k). With callus development, phloem parenchyma cells may also be involved in the formation of callus (Fig. 1l). Through, a lot of slicing observation, no callus differentiated into root primordia.

DISCUSSION

The relationship between the cutting period and rooting status: Different cutting times affected the rooting capacities of our feijoa cuttings, possibly related to anatomical structures, changes in internal hormone and nutrient levels and the growth cycle. Optimum root formation was based on physiological states, rather than fixed dates. Different cutting periods corresponded to different rooting status.

The relationship between stem anatomical structures and adventitious root formation: Stem anatomical structures showed that the secondary layer had 3-4 layers of phloem fiber cells, more cell layers and were arranged in continuous rings in their secondary structure. Being absence of ring sclerenchyma or discontinuous sclerenchyma, the occurrence of adventitious roots is more frequent, whereas ring or multi-layer sclerenchyma does not easily produce roots (Wang, 1989). The poor root formation in *E. maidenii* cuttings may be related to the existence of ring sclerenchyma in its cortex and the lack of potential root primordium (Liu *et al.*, 1997). Poor rooting in dove trees may be related to the presence of continuous ring sclerenchyma cells in stem phloem and no original root primordium in cuttings (Yi *et al.*, 2000). Grape branches have no root primordium, but they root easily, because the cortex of grape cuttings is thin and the pith is large (Wang *et al.*, 1986). Besides, the role of root primordium and callus differentiation in forming new roots, successful cuttings also depend on their anatomical structures. Thus, the multiple layers of sclerenchyma cells of the cortex near the stem phloem is one of the main reasons for poor root formation in feijoa, affecting not only the metabolic activity of the root primordium, but also mechanically hindering growth of root primordium. Therefore, the longer rooting time of feijoa cuttings (about 30 days) may be associated with this.

The production of cuttings adventitious roots and callus formation: The course of adventitious root formation is often associated with the emergence of callus, whose major function is absorption and defence. Studies of softwood cutting in *Larix olgensis* have shown that the induction of adventitious roots was on the premise of callus formation (Liu *et al.*, 1992). But for most species, callus formation and the production of adventitious roots are independent of each other. The formation and development of cutting callus played a stronger inhibitory effect on the generation of adventitious roots, because the volume of callus was too large, consuming too much rooting material followed by aging, resulting in no root formation (Liu *et al.*, 1996). Our study showed callus formation of feijoa cuttings and the production of adventitious roots are independent of each other.

Rooting types of feijoa: Root primordium formation time of adventitious roots generally is divided into latent and induced root primordia (Liu *et al.*, 1997). There are no latent original root bodies and adventitious roots are generated from induced root primordia in one-year-old feijoa cuttings. Therefore, rooting type of feijoa is the induced type. In practical production, the application of rooting accelerator is very important. By means of anatomical observation, root primordium in softwood cuttings occurrs mainly in the parenchyma cells at the junction of the vascular cambium and pith rays. During the process of cutting, the parenchyma cells of feijoa cuttings regain the ability to split and directly form root primordia and then root primordium continues growth and development, extending outward to break through the selerenchyma organizations, cortex and periderm and grow adventitious roots.

CONCLUSION

Anatomical investigation initially clarified that in feijoa the production of adventitious roots is the induced type. The rooting rate of softwood cuttings is higher than that of hardwood cuttings. There were cyclical and sequential sclerenchyma cells in the phloem and no root primordium in the stems and these structural features may be one of the main reasons that our feijoa cuttings showed a low rooting rate. The adventitious roots of cuttings generated from the edge of the cut section to 0.1-2 cm above the cut. Callus formation and the production of adventitious roots are independent of each other.

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