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Propagation of Citrus by Stem Cuttings and Seasonal Variation in Rooting Capacity

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Abstract: The rooting capacity of spring cuttings from various citrus species and varieties, and the seasonal variation in the rooting potential of stem cuttings of two trifoliate orange strains were determined. Rooting abilities varied among species and varieties (0-100 %). Rough lemon and lemon had 100 % rooting while Tengu and 'Kuno' satsuma mandarin had 0 %. In common trifoliate orange, the maximum rooting (100 %) was found in June cuttings followed by July cuttings (90 %) and the minimum rooting (30 %) was recorded in September. In Flying Dragon cuttings, the maximum rooting (100 %) was in July cuttings followed by June cuttings (96.7 %) and the minimum was in May cuttings (26.7 %). In both strains, cuttings collected from mature trees in all months and from juvenile trees between October to March failed to form callus and root. June and July seem to be the most appropriate months for the collection of stem cuttings for the propagation of juvenile common and Flying Dragon trifoliate oranges.

Key words: Stem cuttings, callus, rooting percentage, IBA, juvenile

Introduction

Stem cutting is important, particularly, in horticulture for mass production of improved materials within a short time, and for perpetuating the characteristics of the parent plant (Hartmann *et al.*, 1997). For species that can be propagated easily by cuttings, this method has numerous advantages. Many new plants can be started in a limited space from a few stock plants. It is inexpensive, rapid, simple and does not require the special techniques necessary in grafting, budding or micro propagation. When seed of desired rootstocks is not available in sufficient quantities, rootstocks can be propagated as stem cuttings. Such rootstocks have greater uniformity because of absence of the variation which sometimes appears as a result of the variable seedling rootstocks of grafted plants (Hartmann *et al.*, 1997). Furthermore, cutting propagation is important in clonal selection and plant improvement because large genetic advances can be made in single selection step.

There is considerable variability in the rooting of stem cuttings of different citrus species. Some root much more readily than others do. With difficult-to-root cuttings, the ontogenetic age of the plant source can be very critical. Generally, cuttings from plants in the juvenile growth phase root more readily than plants in the adult phase of growth (Heuser, 1976). The rooting potential of the softwood cuttings has been found to diminish with the increase in age of the mother shoots (Sing *et al.*, 1957). Seasonal timing, the period of the year which cuttings are taken, can play an important role in rooting. With many species there is an optimal period of the year for rooting (Anand and Heberlein, 1975).

So the objectives of this experiment were to determine the rooting capacity of various citrus species and varieties by spring shoot cuttings and to find the seasonal variation in rooting potential of stem cuttings.

Materials and Methods

The experiment was conducted at the University Farm, Ehime University, Japan during June 1998 to March 2001. This work was carried out in two experiments over two seasons. The 29 citrus species and varieties used in the first experiment are shown in Table 1. Current spring shoot cuttings were collected from two-year-old trifoliate orange seedlings and from mature trees grafted on trifoliate orange for the other species, which were mostly more than ten years old. Rough lemon, Cleopatra mandarin and Shiikuwasha were collected from the Ehime Fruit Tree Experiment Station and the remaining species from the Experimental Farm of Ehime University. Cuttings were prepared in early June, 1998. The cuttings were 8 cm long with 3-4 well matured plump buds. Only the top-most leaf was retained and cut transversely into half. Cuttings were inserted in rows into "Kanuma"-soil media in 50 x 35 cm^2 size plastic trays. There were two treatments for each species. A 4000 ppm indole-3-butyric acid (IBA) solution was prepared in 50 % aqueous ethanol and the basal parts of cuttings were dipped in IBA solution for 5 seconds. The control cuttings were placed directly into the media after the cutting preparation. There were four replications for each treatment with ten cuttings. This experiment was conducted under greenhouse conditions and misting was done for one minute after every hour. Thus the rooting media was constantly moist and the relative humidity (RH) was high.

Each cutting was uprooted after 20 days and callus formation was scored with the help of callus formation index (5 for good callus and 0 for no callus). After 45 days, rooting percentage, number and length of primary roots, and number of secondary roots were recorded for each cutting. Cuttings were immediately returned into the media after data recording. Final rooting percentage was evaluated after three months in early September 1998.

In the second experiment, common trifoliate orange (*Poncirus trifoliata* [L.] Raf.) and Flying Dragon trifoliate orange (*Poncirus trifoliata* [L.] Raf.) were propagated from both juvenile and mature shoot cuttings once every month from early April 2000 to early March 2001. The juvenile cuttings were obtained from two-years-old trees and the mature ones were from more than 15-years-old trees. The minimum and maximum monthly temperature for one year, inside the experimental mist house is shown in Table 2. Cuttings were inserted into the media without any treatment, with other procedures for cutting preparation and insertion in media being the same as in the first experiment. Final rooting percentage was recorded three months after cuttings were inserted into the media.

Results and Discussion

In the first experiment, rooting percentage and callus formation were evaluated 45 days after insertion of the cuttings, and the final rooting percentage three months later, in early September, 1998. Cuttings from all species varieties

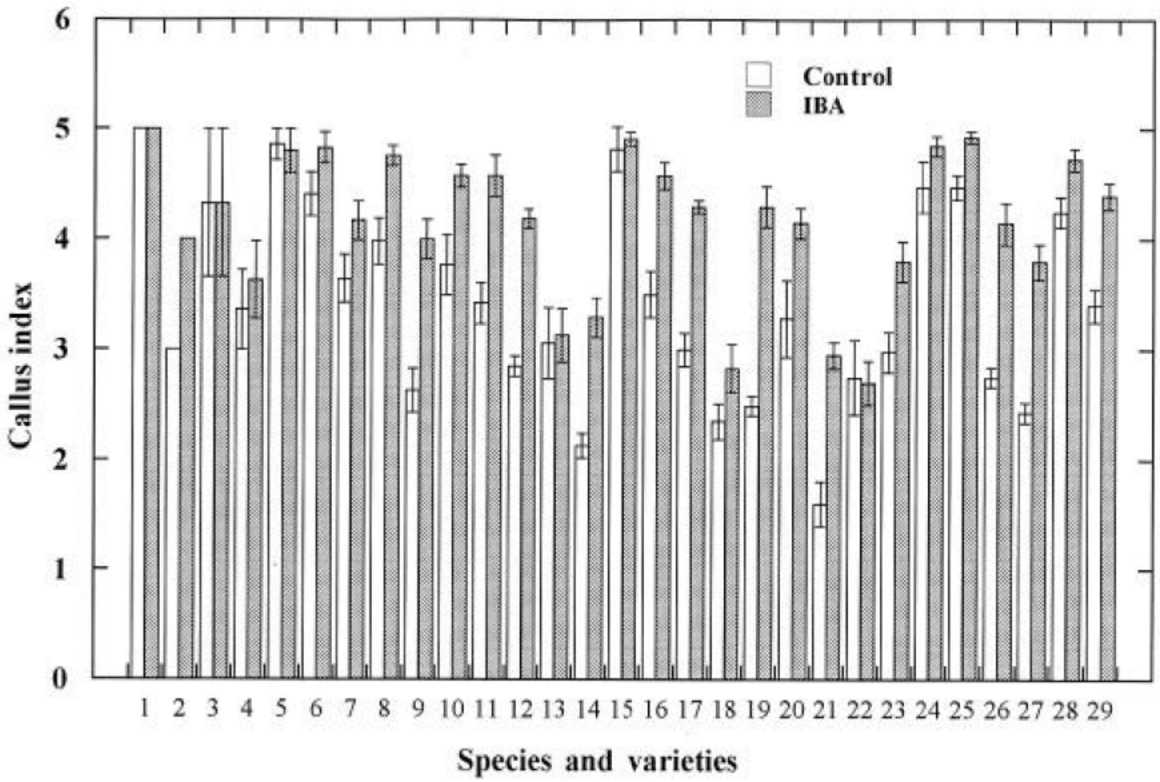

Fig. 1: Callus formation of citrus cuttings

Table 1: Citrus species and varieties used in the first experiment.

S. No	Botanical names	Common names
1	*Citrus jambhiri* Lush.	Rough lemon
2	*Poncirus trifoliata* [L.] Raf.	Trifoliate orange
3	*Citrus limon* [L.] Burm. f.	Lemon
4	*Citrus reshni* hort. ex Tanaka	Cleopatra mandarin
5	*Citrus junos* hort. ex Tanaka	Yuzu
6	*Citrus grandis* [L.] Osbeck	Ban-okan
7	*Citrus natsudaidai* Hayata	'Kawano' natsudaidai
8	*Citrus sinensis* Osbeck	Kosuito
9	*Citrus depressa* Hayata	Shiikuwasha
10	*Citrus glaberrima* Tanaka	Kinukawa-mikan
11	*Citrus medioglobosa* hort. ex Tanaka	Kikudaidai
12	*Citrus medioglobosa* hort. ex Tanaka	Naruto-mikan
13	*Citrus aurantium* L.	Hana-daidai
14	*Citrus murcot* Smith	Murcot
15	*Citrus sudachi* hort. ex Shirai	Sudachi
16	*Citrus unshiu* Marc.	'Okitsu wase' satsuma mandarin
17	*Citrus sinensis* Osbeck	'Tanaka' navel orange
18	*Citrus iyo* hort. ex Tanaka	'Miyauchi' iyo
19	*Citrus* iyo hort. ex Tanaka	'Katsuyama' iyo
20	*Citrus natsudaidai* Hayata	Natsudaidai
21	*Citrus tengu* hort. ex Tanaka	Tengu
22	Citrus aurantium L.	Shuto
23	*Citrus grandis* Osbeck	'Hirado' buntan
24	*Citrus yamabuki* hort. ex Y. Tanaka	Yamabuki
25	*Citrus unshiu* Marc.	'Kuno' satsuma mandarin
26	*Citrus unshiu* Marc.	'Miyagawa wase' satsuma mandarin
27	*Citrus reticulata* Blanco	Ponkan
28	(*C. unshiu* Marc. x *C. sinensis*	'Shiranui'
29	Osbeck) x *C. reticulata* Blanco	
	Citrus unshiu Marc.	'Aoshima' satsuma mandarin

formed callus to varying degrees (Fig. 1), but some did not root even after three months (Tengu and 'Kuno' satsuma mandarin). Some species with a low rooting percentage in the initial recording time (Fig. 2) showed a significant increase in rooting at the final recording. Cuttings treated with IBA increased both root number and length relative to non-treated cuttings, but did not differ significantly among IBA treatments and control in the final rooting percentage. Species and varieties used in this experiment had big variations in rooting ability, with rooting percentages ranging from 0-100 percent. According to final rooting percentages (Fig. 3), Rough lemon, trifoliate orange and lemon had 100 % rooting ability , while Tengu and 'Kuno' satsuma mandarin had 0%. Cleopatra mandarin, Yuzu, Ban-okan, 'Kawano' natsudaidai, Kosuito, Shiikuwasha, Kinukawa-mikan, Hana-daidai, Murcot, Sudachi, Natsudaidai and Shuto had good rooting ability; Kikudaidai, 'Tanaka' navel orange, 'Miyauchi' iyo, 'Katsuyama' iyo, Yamabuki and Shiranui had medium ability; 'Okitsu wase' satsuma mandarin, 'Hirado' buntan, 'Miyagawa wase' satsuma mandarin, Ponkan and 'Aoshima' satsuma mandarin had poor ability. The rooting ability of the 29 different citrus species and varieties when treated with IBA (Fig. 3) are listed below in descending order. Rough lemon = Trifoliate orange = Lemon > Natsudaidai > Cleopatra mandarin > 'Kawano' natsudaidai > Bano-kan = Kosuito > Yuzu > Murcot > Kinukawa-mikan > Shiikuwasha = Hana-daidai > Sudachi > Naruto-mikan > Shuto > 'Katsuyama' iyo > 'Tanaka' navel orange > Shiranui > Yamabuki > Kikudaidai > 'Miyauchi' iyo > Ponkan > 'Hirado' buntan > 'Miyagawa wase' satsuma mandarin >

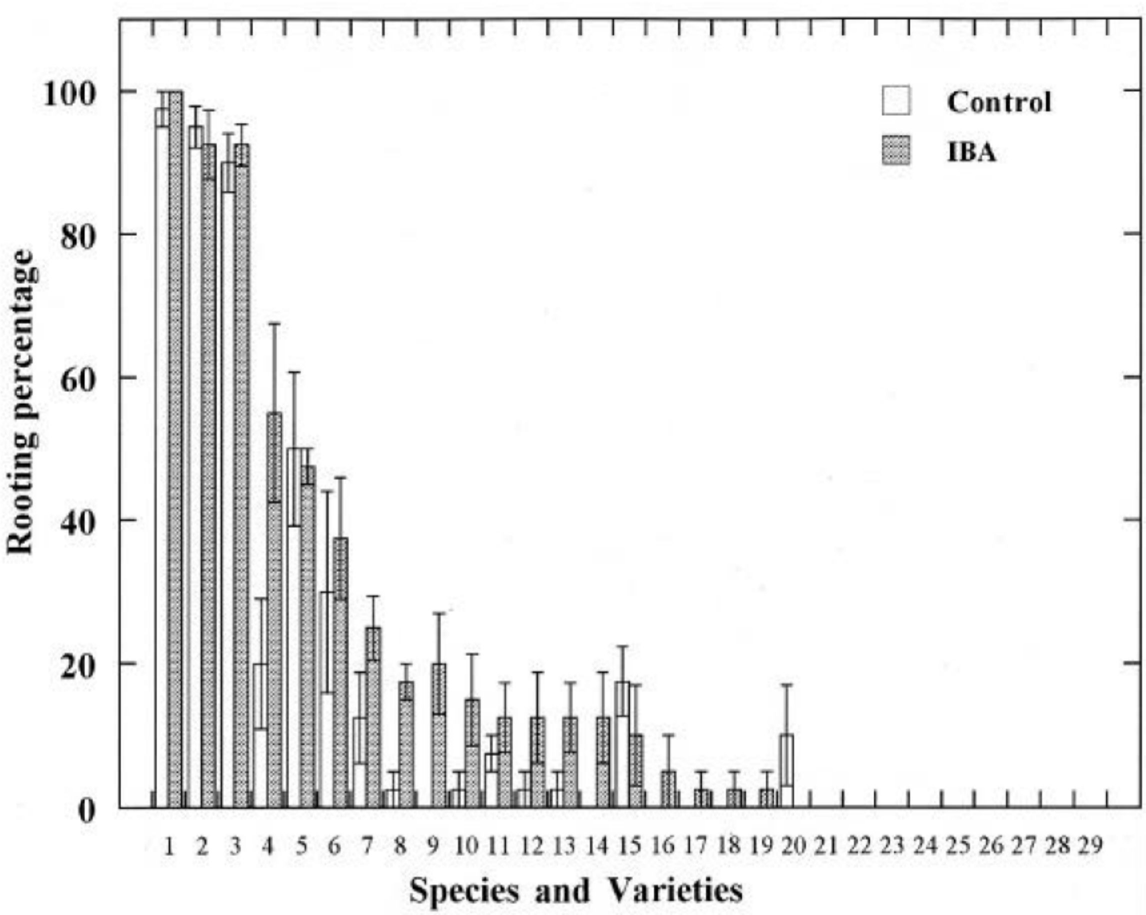

Fig. 2: Rooting percentage of citrus cuttings (after 45 days)

Table 2: Mean minimum and maximum temperature and temperature differences inside the misting greenhouse during April 2000 to March 2001.

Months	Average minimum temperature (° C)	Average maximum temperature (° C)	Temperature range (° C)
April	11.5± 0.4	25.9± 0.3	8-31
May	16.5± 0.4	27.1± 0.4	12-31
June	19.6± 0.4	29.5± 0.6	15-36
July	23.6± 0.2	35.7± 0.3	21-26
August	24.5± 0.2	36.9± 0.4	22-40
September	21.0± 0.5	32.4± 0.7	16-37
October	17.4± 0.4	28.6± 0.4	13-33
November	12.4± 0.6	25.0± 0.4	8-27
December	7.3± 0.4	24.5± 0.6	4-26
January	4.5± 0.5	21.1± 1	0.1-26
February	5.4± 0.4	24.0± 0.7	1-27
March	7.4± 0.6	25.2± 0.6	2-27

'Okitsu wase' satsuma mandarin >'Aoshima' satsuma mandarin > Tengu = 'Kuno' satsuma mandarin.

However, it should be noticed that in this experiment only trifoliate orange was two-year-old trees while the others were mature trees. As will be mentioned below, mature trifoliate oranges failed to root. Therefore it seems that the order of rooting potential of mature trifoliate orange should be ranked very low.

Results from the second experiment show that the month of cutting collection significantly affects rooting in both strains. In common trifoliate orange, the maximum rooting (100 %) was achieved in the June cuttings followed by July cuttings (90 %) and the minimum rooting (30 %) was recorded in September cuttings. In the case of Flying Dragon cuttings, the maximum rooting (100 %) was recorded in July cuttings followed by June cuttings (96.7 %) while the minimum rooting was in May cuttings (26.7 %). In both strains, root number and root length were also found to be satisfactory in June and July cuttings (data not presented). In common trifoliate orange the maximum rooting was in June but in Flying Dragon it was in July. Due to late shoot sprouting in Flying Dragon, the shoots were still immature in May, resulting in low rooting percentages. This may explain the one-month difference in the maximum rooting between Flying Dragon and common trifoliate orange. Complete rooting was achieved after a much longer time in the April and May cuttings as compared to the June and July cuttings whose rooting was much faster. Within the one-year evaluated, June and July seem to be the most appropriate months for the collection of stem cuttings for the propagation of common and Flying Dragon trifoliate oranges.

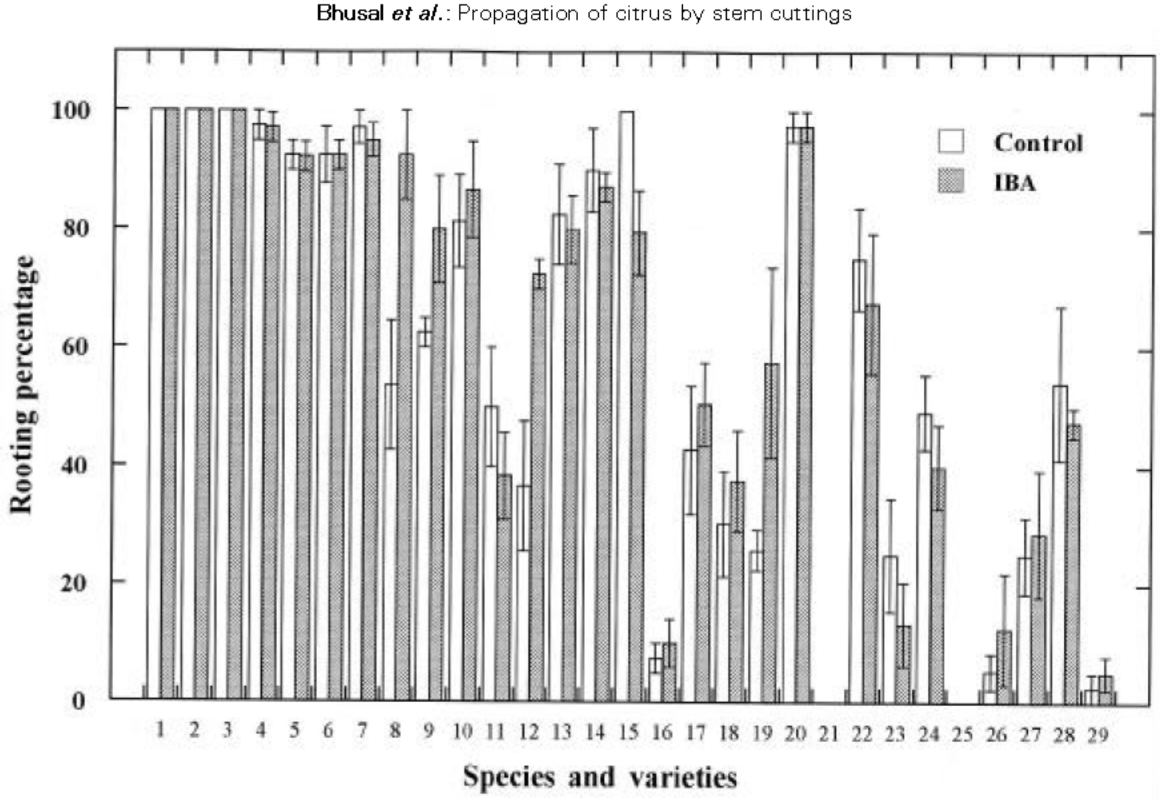

Fig. 3: Final rooting percentage of citrus cuttings (after 90 days)

However, cuttings collected from mature trees failed to form callus and to initiate rooting for both strains. Cuttings collected from juvenile trees of common and Flying Dragon trifoliate oranges between October to March also failed to form callus and to root.

There are many reports on citrus propagation using stem cuttings. Stem cuttings of Sweet lemon had higher rooting ability than those of Cleopatra mandarin (El-Tomi and Galal, 1980). Lemons, lime and citron root most readily; mandarins are the lowest and most difficult to root; sweet orange, sour orange and trifoliate orange are intermediate in their ease of rooting (Platt and Opitz, 1973). Our results also showed that root formation by cuttings is not uniform for all citrus species. In the iyo cultivars, 'Katsuyama' had higher rooting percentage than 'Miyauchi'; in satsuma mandarins 'Okitsu wase,' 'Miyagawa wase' and 'Aoshima' had little rooting ability and 'Kuno' failed to root completely.

When Eureka lemon stem cuttings were treated with different kinds of growth regulators, it was found that IBA at 4000 ppm gave the highest rooting percentage at 95.5-98.0% (El-Shazly *et al.*, 1994). In the initial results of our experiment (after 45 days evaluation) IBA cuttings had higher rooting percentage in Kosuito, Shikuwasha, Kinukawa-mikan, 'Okitsu wase' satsuma mandarin, 'Tanaka' navel orange, 'Miyauchi' iyo and 'Katsuyama' iyo (Fig. 2). But treatment with IBA (4000 ppm) did not have a significant effect on final rooting percentage (Fig. 3). However, root number and length was considerably greater in IBA treated cuttings. Generally there was a positive correlation between rooting percentage and root number.

Physiological variation cannot be avoided in cuttings collected at different times of the year. Rooting is correlated quite strongly with active stock plant growth at the time of cutting collection, indicating that the condition of the growing shoot determines rooting (Howard, 1996). Ozcan (1990) also reported that in the field rooting of the semi-hard wood cuttings of some citrus rootstocks, the highest rooting was achieved from July cuttings followed by May cuttings, and the lowest from October cuttings in all the rootstocks. Cuttings of broad leaved evergreen species usually root most readily, if the cuttings are taken during the late spring or summer from new shoots just after a flush of growth has taken place and the wood is partially matured (Hartmann *et al.*, 1997). Abou-Rawash *et al.* (1998) also did field experiments using some citrus rootstocks and reported that the suitable date for cutting collection and IBA treatments seemed to differ from one rootstock to another.

In the first experiment, trifoliate orange cuttings were taken from juvenile trees, so that they had very good rooting percentage but mature trifoliate cuttings completely failed to root in the second experiment. Thus one important factor that affects the rooting is tree age. Research with many species has shown that the ability to form adventitious roots on stem cuttings decreases with increasing age of seed. Enhanced rooting and increased vegetative vigor are typical in the juvenile phase (Howard, 1996). Cuttings taken from young clones of any species root more readily than those taken from old clones. Ferguson *et al.* (1985) suggested that stem cuttings from juvenile trees are generally recommended over stem cuttings from matured trees for propagation of own-rooted citrus. Summer propagation of citrus cuttings generally gave higher percentage of rooting and root scores than did autumn, winter and spring. Our results showed that the

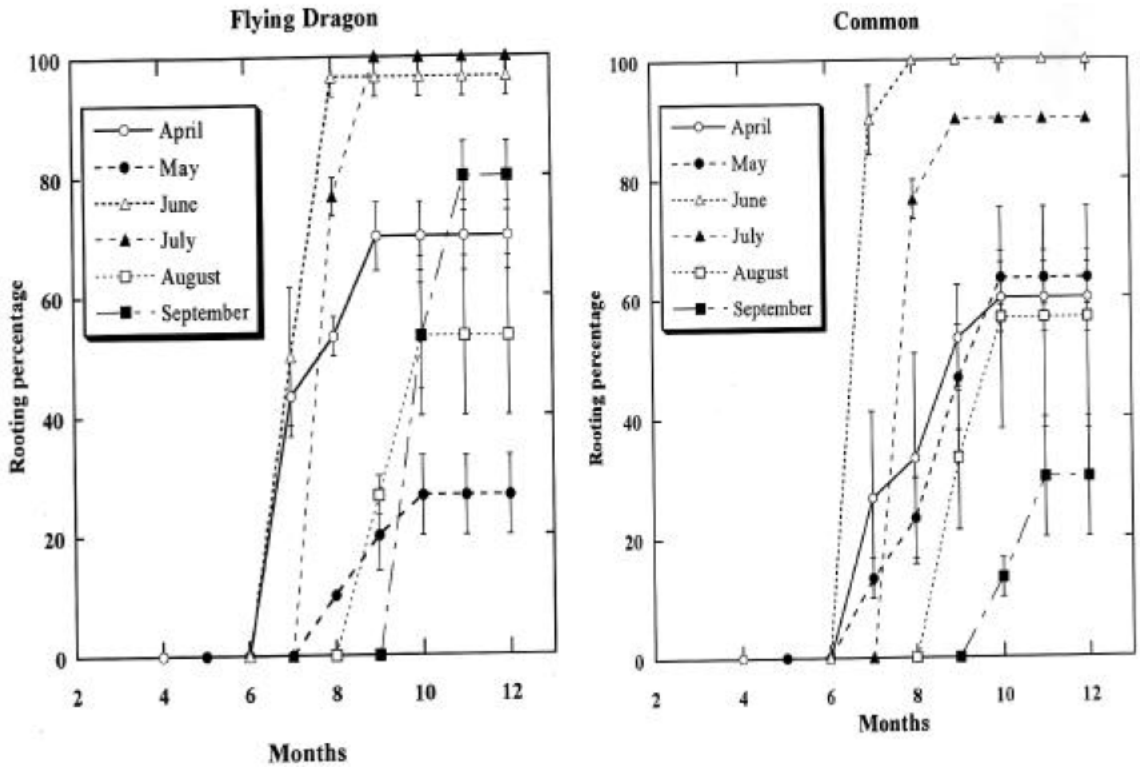

Fig. 4. Seasonal changes in rooting percentage of two trifoliate orange (Common and Flying Dragon) cuttings

cuttings collected from juvenile trifoliate orange trees have good rooting ability in suitable season, that is, June and July. Tetsumura *et al.* (2001) emphasized the juvenility, length of cuttings, planting time and environmental condition for improving the rooting of the cuttings. Rooting hormones, planting time, growth rate of suckers, maturity of the stock plants and propagation environment might be among the important factors affecting the rooting of stem cuttings. Further research is necessary to elucidate the main factors that affect rooting in stem cuttings of citrus.

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