

**Study on Graft Incompatibility in 'Fuyu' Persimmon
(*Diospyros kaki*) Grafted onto Different Rootstocks
in Relation to Physiological and
Morphological Behaviors**

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Chapter 1

Introduction

1.1. Situation of persimmon growing in the world

Persimmon belongs to the genus *Diospyros* and family Ebenaceae. Oriental persimmon, Japanese persimmon or kaki (*Diospyros kaki* L. f. or *D. kaki* Thunb., according to different authors) is named the “food of the Gods” (from Greek, *Dios* meaning God and *Spyros* meaning food). The species seem to have originated in China, where its culture started some centuries before Christ and it was introduced to Japan in the 7th century and to Korea in the 14th century. In Europe, it was introduced in the 17th century and later, in the 18th century, it was already known world-wide (Sugiura, 1997).

In China, it is found wild at altitudes up to 1,830-2,500 m and it was cultivated from Manchuria southward to Kwangtung. Early in the 14th Century, Marco Polo recorded the Chinese trade in persimmons. Korea has long-established ceremonies that feature the persimmon. The tree has been grown for a long time in North Vietnam, in the mountains of Indonesia above 1,000 m and in the Philippines. It was introduced into Queensland, Australia, about 1885. It has been cultivated on the Mediterranean coast of France, Italy, and other European countries, and in southern Russia and Algeria for more than a century. Europeans refer to the persimmon by the Japanese name ‘kaki’. Seeds first reached the United States in 1856 when they were sent from Japan by Commodore Perry. Grafted trees were imported in 1870 by the U.S. Department of Agriculture and distributed to

California and the southern states. In India it was introduced during 1920's (Mehta et al., 2005) and in Nepal, during 1910's, by the Nepalese students and Japanese tourists (Simkhada et al., 1999).

Oriental persimmon *Diospyros kaki* (Thunb.), otherwise known as Japanese persimmon, traces its primary center of diversity in China, followed by Japan (Zheven and Zhukovsky, 1975). Persimmon are fairly common trees in warm regions of the world, mainly in Asia and South American countries, where nearly 190 species are known. Out of the 190 species of the genus *Diospyros*, only four are commercially used for fruit production, namely; *D. kaki* Thunb., *D. lotus* L., *D. virginiana* L. and *D. oleifera* Cheng (Ito, 1980). The general description of persimmon fruit trees is shown in Table 1.

Japanese persimmon is the most important species and has numerous horticultural varieties. It was the primary fruit crop for a long time in Japan and even today, it ranks fourth in production at 265,000 t (2003), after Satsuma mandarin, apple, and pear. The total acreage of persimmon was 24,400 ha in 2003. Persimmon adapts to a warm climate with considerable rainfall, as that of Japan, where trees are widely spread in home gardens and sides of crop fields. Persimmon is now grown in many other countries, such as Brazil, Italy, Spain, Israel, Australia and New Zealand (Yamada, 2006).

In Japan, two types of non-astringent cultivars have been developed. They include the pollination-constant non-astringent (PCNA) cultivars, which are currently important in commercial production and the pollination-variant non-astringent (PVNA) cultivars. A few PCNA cultivars of Chinese

origin have recently become known. Mature fruit of non-astringent cultivars can be eaten at the firm stage, as can apples. On the other hand, for edibility, the fruit of astringent cultivars need to be treated after harvest to remove astringency using carbon dioxide gas or ethanol vapor or by drying after peeling. Without those treatments, the fruit cannot be eaten until they reach the over-ripe stage with very soft flesh. In some countries, over-ripened soft fruit are commonly consumed: however, this is rarely the case in Japan. In Japan, the fruit of astringent cultivars are commonly consumed as fresh table fruit after the astringency is removed.

Persimmon is believed to be originally astringent because most cultivars of Chinese and Korean origin are of the astringent type and even in Japan, local astringent cultivars are distributed throughout the country and show the largest variation. It is possible that two types of non-astringent genotypes mutated spontaneously or underwent a chromosomal change. Those genotypes were naturally crossed repeatedly with astringent genotypes and their descendants and thus the current local cultivars have appeared primarily as chance seedlings.

Classification based on astringency

Persimmons is classified into non-astringent and astringent cultivars depending on whether the fruit lose astringency on the tree at maturity. On the other hand, Hume (1914) developed a grouping based on flesh color, which darkens or remains unchanged under the influence of pollination: the former is called pollination-variant (PV), and the latter, pollination-constant (PC). Non-astringent persimmons naturally lose astringency on the tree, whereas their astringent counterparts necessitate artificial means of

removing astringency before they can be consumed. Strictly speaking, changes in flesh color in persimmon is related to the seed formation rather than to pollination in which the alcohol and acetaldehyde exuded by the seeds causes the tannins to oxidize resulting in darkening of the flesh. Thus, persimmon can be further subdivided into four groups based on the changes in flesh color (Yamada, 2006), namely;

- (1) Pollination constant and non-astringent (PCNA*) – tannin cells cease development at an early stage of fruit development, eventually tannins are diluted and deastringency occurs (varieties: ‘Fuyu’, ‘Jiro’, ‘Gosho’, ‘Suruga’). They usually have dark tannin spots.
- (2) Pollination variant and non-astringent (PVNA) – characterized by a large number of seeds; produces high levels of acetaldehyde and ethanol (varieties: ‘Nishimurawase’, ‘Zenjimarū’, ‘Amahyakume’, ‘Shogatsu’, ‘Mizushima’). They have dark tannin spots and may become astringent when seedless.
- (3) Pollination constant and astringent (PCA) – seeds do not exude acetaldehyde and ethanol thus flesh color is not changed by seed formation (varieties: ‘Saijou’, ‘Shakokushi’, ‘Atago’, ‘Yotsumizu’, ‘Hagakushi’, ‘Yokono’, ‘Hachiya’, ‘Gianbo’). These varieties have no dark tannin spots.
- (4) Pollination variant and astringent (PVA) – less number of seeds; produces low levels of acetaldehyde and ethanol (varieties: ‘Hiratanenashi’, ‘Rendaiji’, ‘Aizumishirazu’, ‘Koshuhyakume’, ‘Emon’). Astringent when pollinated with some dark tannin spots around the seeds.

(*PCNA is the mutant as a sweet type and PCA/PVNA/PVA are astringent type depend upon the capacity of seed to produce alcohol or aldehyde in terms of factors affecting condensation of soluble tannins.

Astringency is caused by water-soluble tannins. They are found in large special cells, “tannin cells”, which are scattered in the fruit flesh. Treatment with carbon dioxide gas, ethanol vapor and drying after peeling changes soluble tannins into insoluble forms. In PV cultivars, seeds exude acetaldehyde and ethanol. Acetaldehyde causes the soluble tannins to be condensed or coagulated and to become insoluble and oxidized: as a result, many brown specks are formed in the flesh, and the flesh color darkens. Cultivars whose fruit have a large number of seeds and produce a considerable amount of acetaldehyde are classified as PVNA, while those which produce less are classified as PVA. When seed formation is poor, a dark area develops only around the seeds and the remaining flesh is astringent, even in PVNA. In PCA, seeds scarcely produce acetaldehyde and ethanol and as a result, the flesh color is not changed by seed formation.

In PCNA, the mechanism by which astringency is naturally lost while the fruit are on the tree is different from that in PV. Tannin cells in PCNA cultivars of Japanese origin cease developing at an early stage of fruit development, while other cells continue to develop: thereby, tannins are diluted as fruit develop, and the astringency is naturally lost at maturity on the tree. The mechanism in PCNA cultivars of Chinese origin has not been clearly elucidated. However, the tannin cells in fruit at maturity are small, as are those in PCNA of Japanese origin.

PCNA vs. non-PCNA is qualitatively inherited. The trait of PCNA of Japanese origin is recessive to that of non-PCNA. The trait of PCNA of Chinese origin has been suggested to be dominant over that non-PCNA.

In recent years, cultivation of persimmon has spread to countries like Brazil, Italy, New Zealand, Israel where fruits are mainly exported (Llacer and Badenes, 2001). On the in countries where persimmons are considered traditional crops such as in Japan, China and Korea, the fruits are consumed mainly in local markets. In these countries, however, plantings, in terms of cultivar preferences are not homogeneous as each cater to the demands of the local or target market. For example, some prefer fruits with crisp pulp, as in 'Fuyu' or 'Jiro', while others prefer partly softened or fully softened pulp, which is characteristic of astringent types that have undergone deastringency treatment such as 'Rojo Brillante' and 'Tonewase'.

Area planted to non-astringent types such as PCNA cultivars 'Fuyu' and 'Jiro' have expanded in recent years. However, PCNA cultivars do not adapt well to diverse climatic conditions and have a narrow genetic variability. This narrow genetic base will eventually induce inbreeding depression thus the need to find new PCNA cultivars (Yonemori et al., 2005) such as 'Luo Tian Tian Shi' from China, which should open new strategies for persimmon breeding.

Astringent types (PVNA, PVA, and PCA) on the other hand, possess certain advantages, such as adaptability to wide range of climatic conditions, excellent taste and fruit quality (Llacer and Badenes, 2001). However, the fruits have to be subjected to treatments that remove astringency after

harvest, a procedure that entails additional cost. Aside from this, most astringent cultivars soften rapidly after harvest, a problem that is further aggravated by the deastringency treatment.

In terms of world production, according to FAO (2001), the world-wide persimmon acreage was more than 300,000 ha and production accounted for more than 2,300,000 tons. Of the total, China, Japan and Korea accounted for 95%, followed by Brazil, Italy, Israel and New Zealand at 2.61, 1.80, 0.59 and 0.05%, respectively. However, the plantings in these countries are marginal as compared to Japan, China and Korea, the seasonal differences between persimmon growing countries can be advantaged of by filling in the supply of fruits during the lean season. Japan ranks as the second largest producer of persimmon fruits next to China, with an annual production of 286, 000 t and 265, 000 t in the year 2000 and 2003 respectively. The overall production trend is stationary. The latest trend shows, Japanese production is slightly decrease whereas, Brazil, Israel, Spain, New Zealand and Australia are expected to increase.

Persimmon often regarded as a strictly temperate species, however it appears to be readily adapted to a wide range of climatic conditions (warm temperate to sub-tropical) resulting to its cultivation in unexploited areas of Asia, Europe, and Latin America. Recently, production has spread to other regions and consequently, persimmon became a familiar fruit in non-traditional countries like Nepal, India, Pakistan, Myanmar and Bhutan. The future of this fruit depends on the selection of high quality cultivars and specific marketing techniques to ensure a firm non-astringent persimmon reaching the consumer.

In Japan, in terms of production by persimmon type, PCNA cultivars represented 55% of the total production in 1998, of which 31% was 'Fuyu'. On the other hand, among the astringent cultivars, 'Hiratanenashi' and its bud sport 'Tonewase', both PVA, made up 26% of the total production on the same year. PCNA cultivars are grown mainly in areas where mean annual temperature ranges from 14 to 16°C, which favors natural removal of astringency. PVNA cultivars, on the other hand, can be grown even in colder regions because they require less warm temperatures (Yamada, 1994). In Japan, persimmon trees blossom in mid-May to early June and reach commercial maturity early October to early December, depending on variety. Minimum fruit maturity is based on skin color changes from green to orange or reddish-orange ('Rendaiji', 'Hiratanenashi'), or to yellowish-green or yellow ('Fuyu', 'Jiro').

Persimmon fruits of the non-astringent type can be readily consumed after harvest. Non-astringent cultivars such as 'Fuyu' generally store well after harvest with relatively long post harvest shelf-life. On the other hand, astringent type fruits have to be subjected to treatments that remove astringency before they can be consumed. These treatments are aimed at inducing the accumulation of acetaldehyde resulting in the polymerization of soluble tannins, and the subsequent removal of astringency. Among the popular methods include the use of warm water, spraying with 35-40% ethanol or exposure to elevated CO₂ levels through the use of dry ice (solid carbon dioxide) to liberate the gas, or the constant temperature short duration treatment (CTSD) (Yamada, 1994).

Table 2 and 3 show the area and production for those countries that according to FAO (2001) have more than 100 h and /or produce more than 1000 t per year. In 1961, only five countries had such a production: China, Japan, Korea, Italy and Brazil. In 2000, two more countries were added to the list: Israel, beginning in 1980, and New Zealand, starting from 1990. Other countries, still according to FAO (2001), are close to those numbers: Australia, Iran and Mexico. Some countries like Spain, Portugal, Turkey and others do not appear in the persimmon statistics from FAO, although at present they largely surpass the production of 1000 t, probably because persimmon is included in their statistics together with other minor fruit crops.

In the last 40 years in the world, area and production have been increased almost by 2.5 times (Tables 2 and 3). Persimmon area and production in 2000 accounted for more than 300,000 ha and 2,300, 000 t. Its increasing trend is explained by the evolution of the culture in China and always accounts for a substantial percentage of the world area and production (Figs 1 and 2). Among the main producing countries, beside China, the trend has been different, an increase in Korea and Brazil and a decrease in Japan and Italy.

Persimmon area and production from Asian countries, where the crop has been established for many years (China, Japan and Korea), have always represented more than 90% of the world totals: 97% of area (Fig. 1) and 95% of production (Fig. 2) in 2000. The contribution to the production by non Asian countries is therefore very small, even if they have a higher productivity, for instance more than 15 t/ha in Italy and Israel and less than 10 t/ha in Asian countries. Finally, the amount of persimmon exported in the

world is rather insignificant: less than 8000 t in 1999 (with a value of \$ 11,500,000) of which 82% was from Israel (FAO, 2001).

In Nepal, there are various varieties of *D. kaki* ('Haluwabed' in Nepal) being cultivated including the indigenous strains for domestic consumption. The trees are usually big and grown along the mid-hilly areas. The shape and size of the fruit also differs from each other. All the varieties grown are astringent, late-ripening, juicy (after becoming overripe), with low keeping quality, seedy and with less edible flesh. In recent years the Government of Nepal has focused on the growing of the PCNA (Non-astringent and pollination constant) type characterized by early ripening, larger fruits, less cracking, high eating and long keeping quality with high productivity. However, in the government institutions and private nurseries the problem of graft-incompatibility has hindered the mass propagation of persimmons with more desirable traits. In Nepal, the asexual propagation method of grafting is usually available using *D. kaki* (local persimmon) as both rootstock and scion with less than 60% success. The use of the more ideal rootstock, *D. kaki* or 'Hondai' and *D. lotus* or 'Mamedai' which is used as a rootstock for Japanese persimmon in Japan, has not yet introduced.

1.2 Objectives

The general objective of this research was to study possible mechanisms in scion and rootstocks, and to provide new information, which will enable the elucidation of the graft compatibility phenomenon. The objectives of this study are as follows:

1. A study on the situation of persimmon cultivation in Nepal and some factors affecting the success potential of grafting;
2. A study on the morphological observation in the graft union among different combinations between scion and rootstocks;
3. A study on the translocation and distribution of ^{13}C -photosynthates in 'Fuyu' persimmon (*Diospyros kaki*) grafted onto different rootstocks; and
4. To integrate these knowledge's aiming at referring and transferring into persimmon cultivation in Nepal.

Table 1. General description of persimmon fruit trees.

Organs	Description
X	15;
Somatic nos.	30, 60, 90, 135;
Tree	Deciduous/evergreen/shrubs;
Winter buds	Ovoid, with about 3 outer scales, the terminal bud lacking;
Flowers	Dioecious, whitish, pistillate usually solitary, staminate cymes;
Calyx/corolla	Usually 4-lobed, rarely 3-7 lobed;
Stamens	Usually 8-16;
Ovary	4-12 celled;
Styles	2-6;
Fruit	A large juicy berry, 1-10 seeded, usually with an enlarged calyx at base;
Seed	Large flattened.

Table 2. Evolution of persimmon area (ha) from the main producing countries in the last 40 years (FAO, 2001)

Countries	1961	1870	1980	1985	1990	1995	2000
World	122,381	188,611	154,432	239,055	212,057	231,712	312,974
China	76,230	140,270	110,684	191,119	162,993	169,568	242,400
Japan	36,700	35,900	29,400	29,800	29,500	28,000	30,000
Korea	2,346	5,192	6,590	9,838	13,581	25,009	30,821
Italy	4,600	3,800	3,300	3,313	3,055	2,878	2,648
Brazil	2,500	3,444	4,051	3,692	3,960	4,819	5,500
Israel			400	1,200	1,300	800	1,000
New Zealand					488	428	380

Table 3. Evolution of persimmon production (tons) from the main producing countries in the last 40 years (FAO, 2001)

Countries	1961	1870	1980	1985	1990	1995	2000
World	990,079	911,635	968,198	1,186,165	1,156,871	1,561,987	2,335,607
China	497,250	457,341	566,638	690,945	640,230	985,803	1,655,754
Japan	393,500	342,700	265,200	289,700	285,700	254,100	286,000
Korea	13,271	30,310	31,837	97,031	95,758	194,585	273,846
Italy	70,740	59,600	61,100	56,200	68,770	61,300	41,907
Brazil	15,298	21,659	39,958	43,658	46,712	51,685	61,000
Israel			3,400	8,100	17,200	11,000	13,800
New Zealand					972	1,600	1,200

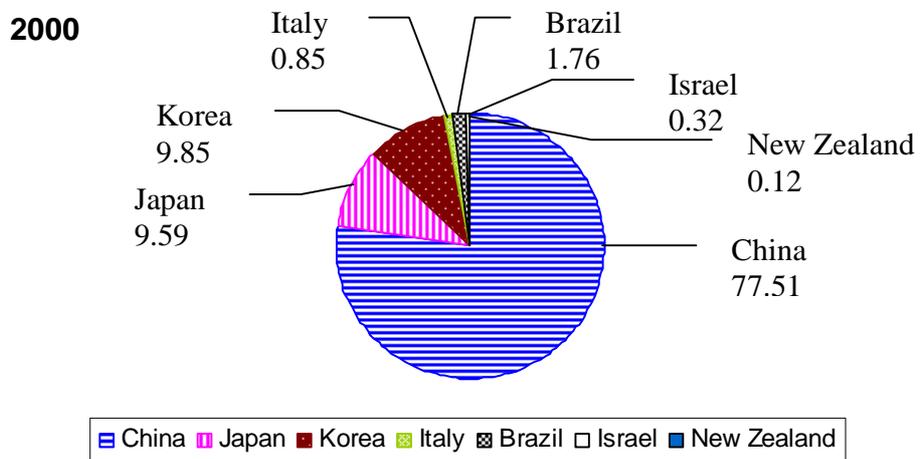
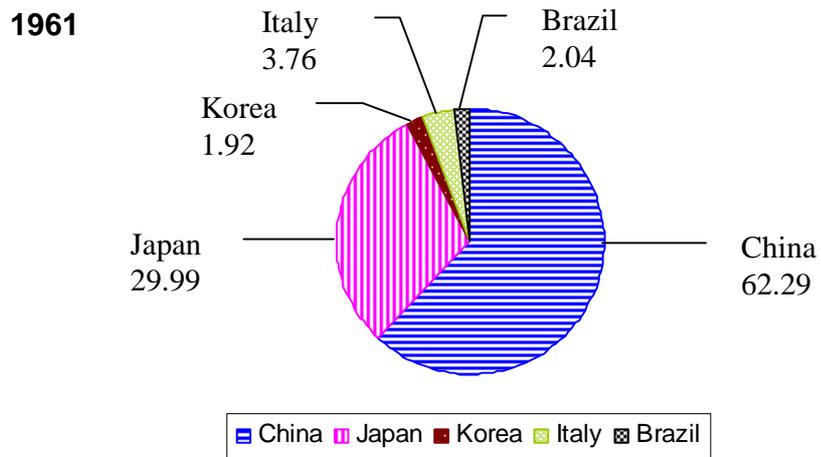


Fig.1. Percentage of persimmon area in comparison of 1961 and 2000.

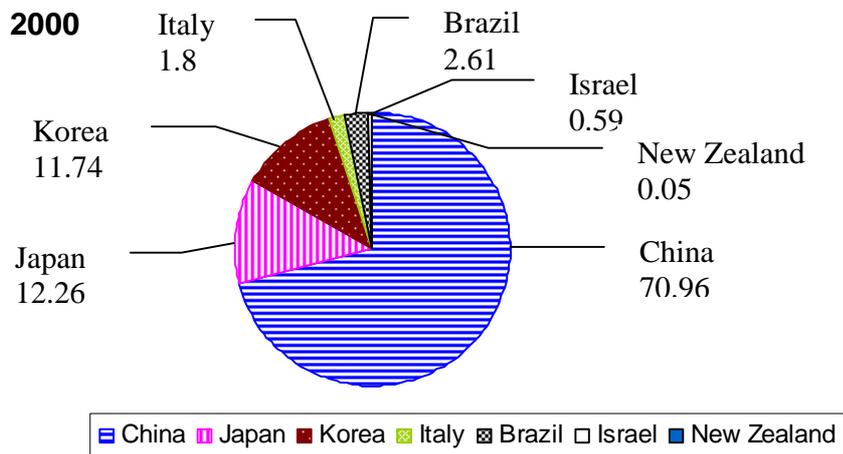
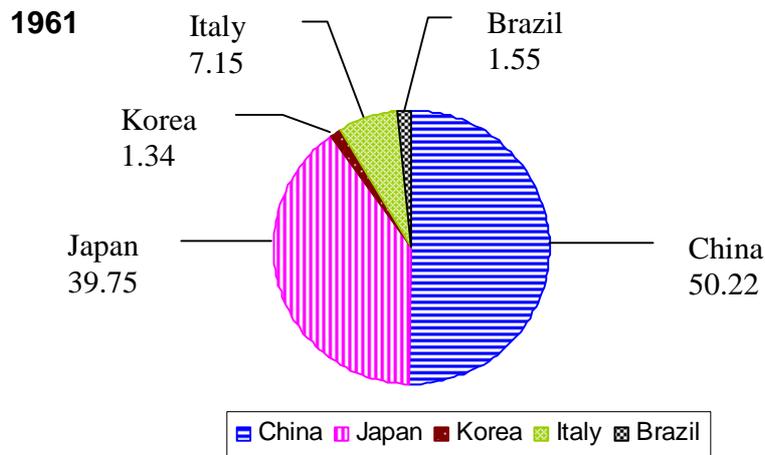


Fig.2. Percentage of persimmon production in comparison of 1961 and 2000.

Chapter 2

Review of related literature

2.1. Propagation

The propagation techniques for persimmon do not greatly vary from those employed for other fruit trees and are: reproduction from seed, propagation from grafting, budding, cutting and by micro propagation.

Reproduction from seed is employed for the production of rootstocks. In autumn, persimmon seeds are collected when the fruits are ripe. Once they are separated from the flesh, the seeds are dried and layered in constantly moist sand. Planting can take place in winter in containers in a greenhouse at 22°C. When external temperatures are higher, night-time temperatures do not fall below 5°C and soil temperature is not lower than 10°C, the young rootstocks are placed directly in the nursery of young trees at a spacing of 20 cm in the row and 100 cm between rows.

Some nurserymen plant directly in the soil of the young tree nursery at the end of winter, but generally they prefer to permit the seeds to germinate in soft soil (1:1, sand:soil) which is carefully shaded since young seedlings are very sensitive to being burned by the sun. In this case, transplanting of the young rootstocks in the young tree nursery takes place when they have reached a height of approximately 20 cm, making sure to trim the tap-root in order to favor greater development of the lateral roots. Special care is given to transplanting so as to avoid breaking the fragile roots and young plants are kept constantly moist. Generally shading is also employed in this phase in

order to favor more rapid rooting of the plantlets and to limit losses as much as possible. The rootstocks usually reach sufficient size for grafting at the end of the year when they were planted.

To favor rooting, studies have been conducted on the effects of mycorrhiza on the growth of young persimmon seedlings. The plants were inoculated with: *Gigaspora margarita*, *Glomus aggregatum*, *Glomus fasciculatum*, *Glomus mossaeae* and *Glomus* sp. R-10. Various effects were found depending on the fungal species utilized. From the obtained results, it emerged that 15 weeks after inoculation the plants showed an increase in height. In addition, there was an increase in the number of leaves, and thus leaf area; shoots and roots had greater dry weight. The fungi increased the concentration of Phosphorus both in the principal and lateral roots in nearly all the inoculated plants (Matsubara and Hosokawa, 1999).

The production of rootstocks can be obtained by cuttings. The best starting material comes from root cuttings. It has been pointed out that the best method to obtain self-rooted plantlets is to remove large sized roots from young plants and put them to root in greenhouse under mist and in controlled environment, and then place them in the young tree nursery in spring. With this method it is possible to obtain rootstocks of a caliber able to support the graft of a single year.

It is known as that micropropagation makes it possible to obtain self-rooted plantlets on a large scale and this propagation system is in development for persimmon. Micropropagated trees, compared to grafted trees, do not demonstrate significance difference with regard to productive efficiency, and there is no effect on the quality of the fruit. Moreover, with

this technique the plants do not undergo shock from transplanting and there is an increase in the length and number of shoots, where as there are scarce effects on the number of female flowers and no effects on the number of male flowers. It should be mentioned that micro propagated plants have a rate of growth in the first years of life which is greater and thus could bring advantages for commercial nurseries where vigorous vegetative growth is preferable and for orchards in poorly fertile soils cold areas.

In most cases, nurserymen graft persimmon plants when they are still in dormancy or at the beginning of vegetative growth. The most common method of grafting is by veneer graft, side graft, whip or tongue graft, and cleft graft (for top-working), in that greater percentage of success, in contrast to bud grafts (chip, T and shield budding). Generally the graft is applied at about 20 cm from the collar or, in areas which are more exposed to winter chill, at 70-100 cm in order to attenuate possible damage, using the notoriously more resistant rootstock *Diospyros lotus*. The scion are removed from healthy and well lignified plants during winter dormancy and kept under refrigeration until the time of grafting. The thinner seedlings are grafted by whip and tongue, while those with a larger diameter are cleft or wedge grafted. The diameter of the scions used is from about 6-10 mm. Some nurserymen have obtained good results also with under-bark graft, which is also done at the end of winter or the beginning of vegetative growth and with wood that has been kept under refrigeration for one year. Good results are obtained using chip budding. As for the graft, it should be mentioned that persimmon is sensitive to temperature of the grafting waxes (used to avoid fungal infection) which are too high.

2.1.1. Terminology in relation to grafting

Scion: Scion is the short piece of detached shoot containing several dormant buds, when united with the stock, comprises the upper portion of the graft and from which will grow the stem or branches, or both of the grafted plant. It should be desired cultivar and free from disease. For example in persimmon, one of the most popular non-astringent persimmon cultivars in the US is the 'Fuyu' and it is the most widely grown cultivar in the world. This is a good one to start with since it is a good performer and should be relatively easy to find. The 'Fuyu' is noted for its good yield of "flattened" fruit, and vigorous, yet manageable, upright growth habit.

Rootstock: Stock (Rootstock, Understock) is the lower portion of the graft, which develops into the root system of the grafted plant. If the grafting is done high in a tree, as in top working, the stock may consist of the roots, trunk, and scaffold branches. Rootstock can be divided into two groups: seedling and clonal (including apomictic seedlings).

Interstock: Interstock (Intermediate stem/stock, Interstock/stem) is a piece of stem inserted by means of two graft unions between the scion and the rootstock. This practice is called double-working. An interstock is used for several reasons, such as to circumvent certain kind of incompatibility, disease resistance or cold hardiness, influence on the growth of the tree.

Cambium: Cambium is a thin tissue of the plant located between the bark (phloem) and the wood (xylem). It's cells are meristematic: they are capable of dividing and forming new cells. For a successful graft union, it is essential that the cambium of the scion be placed in close contact with the cambium of the stock.

Callus: Callus is a term applied to the mass of parenchyma cells that develops from and around wounded plant tissues. It occurs at the junction of a graft union, arising from the living cells of both scion and stock. The production and interlocking of these parenchyma (callus) cells constitute one of the important steps in the healing process of a successful graft.

2.2. Rootstocks for Persimmon

Rootstock performance depends on function of root, growth control, tolerance to different soils, soil pests, yield efficiency and fruit quality.

Root function: Roots, unlike tops of plants, have no distinct period of dormancy and are able to grow whenever temperature, moisture and other conditions are favorable. Also, they are not passive organs that many suppose them to be. They do not merely anchor the tree to earth and absorb water, which then passively carries mineral elements into the plant. The twenty-odd elements essential to life are obtained mainly from the soil by means of active transport mechanism across the membranes of root cells (Epstein, 1973). This active transport of minerals from the soil solution into the roots is an important to life on earth as is the entry of carbon (in CO₂) from the atmosphere into the leaves. Metabolic energy is required for mineral uptake because the essential elements are accumulated in plants against a concentration gradient-that is, they must be transported from a low concentration in the soil solution to a higher concentration in the plant. (normal passive diffusion in nature occurs from an area of high concentration to an area of lower concentration). A large number of factors influence root function, some of which are soil O₂ and CO₂ content, soil moisture, temperature, biotoxins, chemical residues, compaction, acidity, extent of mycorrhiza, the specific genetics of the root and the special

physiology of the cultivar-rootstock compound genetic system. The genetics of the root are important because they determine the extend of growth control; tolerance to different soil types and environments; resistance to soil-borne diseases, insects and pests; graft compatibility with the scion; nutrient uptake and balance and ultimately such factors as fruit quality and yield efficiency (Chaplin, et al., 1972, Westwood, et al., 1973).

Growth control: The dwarfing rootstock does not necessarily have a shallow root system as earlier supposed. Dwarf apple stock M 9 was found to root as deeply as did vigorous stocks in the same soil. However, some dwarf trees are poorly anchored because their roots are brittle and tend to break under the pressure of wind from one side. Reciprocal grafts of vigorous and dwarfing types indicate that for every grafted system there is a proper balance of top and root that is characteristic of the whole system and not simply of the root.

Tolerance to different soils: A key factor in root growth is the extend of soil aeration at different depths. Low O₂ and high CO₂ can reduce or stop root growth and inhibit nutrient uptake. Boyton (1939) found that at soil temperatures of 12.7-21.1°C there was a marked reduction in new rootlets formed on apples when the soil O₂ fell below about 15 percent. At an O₂ level of 10 percent and a CO₂ buildup of 5-10 percent, restricted root growth markedly reduced top growth. The small rootlets need a higher level of aeration to function normally than do larger roots. Survival of trees in waterlogged soils is mainly due to a tolerance to low O₂. Plum roots tolerate water logging much better than peach or apricot, both of which are injured by hydrolytic release of hydrogen cyanide (HCN) from their own tissues under low O₂ conditions (Rowe and Catlin, 1971). Some species, such as citrus, tend to produce H₂S in their roots under anaerobic conditions,

causing injury. Apples generally tolerate wet soils better than do stone fruits and pear roots are the most tolerant of all deciduous fruits.

Soil pests: Soils contain many potential plant pathogens, among which are the Phycomycetes (such as *Pythium* and *Phitophthora* species, which cause root rot and damping off), bacteria (such as *Agrobacterium*, which cause crown gall and *Verticillium* species, which cause wilt), and pathogenic roundworms, or nematodes, which cause root knots and lesions on many plants. Soil fumigation with methyl bromide, chloropicrin and other organic biocides often improves the growth of plants during the first year or two. These fumigants are toxic to roots, however, so fumigation should be done enough in advance of planting to permit dissipation of the toxicant. Healthy plants growing in good soil are found to have abundant mycorrhiza-symbiotic fungi that grow on or in the plant root. It may be that healthy trees have mycorrhiza because they are healthy rather than the reverse. We know, for example, earthworms in are abundant in a good soil, yet putting earthworms in a bad soil does not make it into a good one. One of the best ways to cope with soil diseases and pests is to select or breed for genetic resistance. This avoids the problem of control by chemicals, which often creates the added problem of environmental pollution. Resistance to some pests has been found, for example, root aphids of apple and pear (Westwood and Westigard, 1969), nematodes of pear and stone fruits, *Phytophthora* of pears and so forth.

Yield efficiency: The rootstock type profoundly affects the performance of a given cultivar. As will be shown, there can be as much as 50 percent or more difference in yield of a given cultivar grown on different rootstocks. Not only yield per tree but yield efficiency, that is, yield per unit of tree size, varies greatly with rootstock. This has been shown for apples, pears,

cherries, plums, walnuts and other fruits and nuts. The reasons for such effects on yield are not always apparent, but they often can be traced to differences in tolerance to adverse soils, in resistance to pests, or in uptake of nutrients. Other effects on physiological balance between root and top, which affects yield are on flower initiation, some are on fruit set and others are on fruit growth and ultimate size. Efficiency is related not only to cultivar, climate and culture but to the kind of rootstock used. Specific rootstock effects have been noted for apple, pear, sweet cherry, sour cherry, prune, filbert and walnut. Being one of the fixed factors that cannot be changed without replanting the orchard or vineyard, the efficiency of a stock should be known before the choice is made.

Fruit quality: Rootstocks can greatly influence fruit quality as was mentioned above, incompatibility produces black end of pear and makes the fruit useless but usually the influence is not so dramatic. Effects of rootstock on quality are not always the same with different cultivars, For example, when *Pyrus butulafolia* root is used. Anjou pear, the resulting fruit have internal necrotic spots in the flesh (a condition called “cork spot”), which lowers the grade of fruit. When the same rootstock is used for Seckel, large, high quality fruit is produced. The most common rootstock influences on fruit quality are differences in firmness, in level of organic acids and in sugar content. The balance of these three factors tends to change the flavor and texture. Apple scion are usually propagated on rootstock of their own species (*Malus pumila*), so differences in quality are minimal. Plum and prune stocks may induce different amounts of sugar and acids, but some can affect the incidence of internal browning, stem-end shrivel and gum spot of the fruit (Westwood, 1978).

The preferred rootstock for persimmon is the Oriental persimmon, *Diospyros kaki*. Trees propagated on *D. kaki* seedlings live longer and produce fewer root suckers than trees on the American persimmon, *D. virginiana*. Seedlings of *D. lotus* are also used as rootstock for Oriental persimmon. The cultivars of these fruits are propagated by grafting on seedling rootstocks. Veneer or side grafting, Crown grafting by the whip, or tongue, method in early spring when both scion wood and stock are still dormant is a common practice, although bench grafting is also used. Budding can be done but it is less successful than grafting.

Diospyros kaki: the oldest existing rootstock, of Japanese origins. Seedlings of this rootstock produce tap-root with few lateral roots but, trimming the tap-roots of young seedlings at the time of transplant in the young tree nursery develop a good root apparatus. The roots are rather fine and easily broken and this creates some difficulties for the nurseries with regard to packing and for farmers for with regard to rooting. This is the one most favored in Japan and affinity with all cultivars grafted onto it is excellent. It is resistant to crown gall and oak root fungus but susceptible to *Verticillium* and also considerably less resistant than *D. Virginiana* and *D. lotus* to excesses of moisture in the soil. For its hardness and uniformity of plants, both in the nursery and in the orchard, it is by far the most commonly used rootstock in Japan, China, New Zealand and California. *D. kaki* has scarce resistance to cold and for this reason it is used in southern Italy and wherever there are not problems of low winter temperatures. Trees worked on it grow well and yield satisfactory in commercial crops and is good for warm regions.

Diospyros lotus: Currently the most commonly used rootstock in Italian persimmon orchards and also widely used in California and other European countries. It has notable resistance to low winter temperatures and drought, and it is able to impart to the scion elevated vigor and uniformity of development; it produces a rather fibrous type of root system which transplants easily and does not produce suckers; it has excellent affinity with pollination-variant (PV) and pollination-constant astringent (PCA) cultivars (except for a slight delay in entering into production and an accentuation of fruit drop in combination with cv. 'Hachia'); it has total or partial incompatibility with many pollination constant non-astringent (PCNA) cultivars, including 'Fuyu'; another negative aspect of this rootstock is its quite susceptibility to crown gall (*Agrobacterium tumefaciens*) and *Verticillium* and will not tolerate poorly drained soils but is highly resistant to oak root fungus. It is good for cold regions.

Diospyros virginiana: It is of American origin, easily propagated, of elevated vigor and resistant to heavy and moist soils, but excessively misshapen (for the heterogeneity of seedlings which are obtained) and used to produce suckers. It is common in Sicilian orchards of Italy where the graft in the field is still widely practiced: rooted two-year-old seedlings of *D. virginiana* are wedge grafted the year after planting.

Diospyros rhombifolia: It was created to obtain dwarf rootstocks but it exhibits problems of incompatibility; for example with the cultivar 'Fuyu', the plants are too weak and some die after four to six years.

According to Morton (1987), Indonesians propagate the tree by means of root suckers. In the Orient, selected cultivars are raised from seed or

grafted onto wild rootstocks of the same species, or onto the close relative, *D. lotus* L. In the eastern United States, the trees are grafted onto the native American persimmon, *D. virginiana*. This rootstock significantly contributes to cold-resistance. California growers have found *D. kaki* the most satisfactory rootstock, whereas *D. lotus* rootstock resulting in much lower yields. The seeds for the production of rootstocks need no pretreatment. They are planted in seedbeds or directly in the nursery row 8 to 12 in (20-30 cm) apart with 3 to 3.5 ft (0.9-1.06 m) between the rows. After a season of growth, they may be whip-grafted close to the surface of the soil, using freshly cut scions or scions from dormant trees kept moist in sphagnum moss.

Cleft-grafting is preferred on larger stock and for top-working in old trees. In India, cleft-grafting on stem has been 88.9% successful; while cleft-grafting on crown and tongue-grafting on stem have been 73.4% successful when the grafted plants were left for 2 weeks at about 25°C and relative humidity of 75% for 2 weeks before planting. The Kulu valley in India, scions are grafted onto 2-year-old *D. lotus* seedlings which are mounded with earth to cover the graft until it begins to sprout. At the Fruit Research Station, Kandaghat, 2-year-old *D. lotus* seedlings were used as rootstock for veneer and tongue grafts from cv. 'Hachiya' between late June and the third week of August. Success rates ranged from 80 to 100% (Mishra, 1982).

In Palestine, trees grafted on *D. lotus* and grown on light soil are dwarfish, fruit heavily at first, but are weak and short-lived. Those grafted on *D. virginiana* are larger and vigorous and bear heavily consistently. The only disadvantage is that the shallow root system fans out to 20 m from the base of the tree and wherever the roots are injured by cultivation, suckers spring up and become a nuisance (Morton 1987).

2.3. Scion rootstock (shoot-root) relationships

Combining two (or more, in the case of inter-stocks) genotypes into one plant by grafting one part producing the top and the other part the root can produce unusual growth patterns which may be different from what would have occurred if each component part were grown separately. Some of these have major horticultural value. Others are detrimental and are to be avoided.

These growth patterns may result the following characters: (a) incompatibility reactions. (b) because one of the graft partners possesses one or more specific characteristics not found in the other. Examples are resistance to certain diseases, insects or nematodes or tolerance of certain adverse weather or soil conditions. Other growth patterns following grafting may result (c) from specific intersections between the stock and the scion which would alter size, growth, productivity, fruit quality or other horticultural attributes. In practice it may be difficult to separate which of the three kinds of influencing factors is dominant in any given graft combination growing in a particular environment.

The performance of both scion and rootstock depends upon the compatibility of the two components. In general, closely related cultivars and species are compatible, some genera are compatible, but more distantly related plants are almost always graft-incompatible. Some confusion exists about the meaning of the term “compatible”. As here used, it means a satisfactory union in the horticultural sense, which takes into account both the physical connection at the graft union and the physiological harmony of the genetic system. Bartlett pear on most quince rootstocks is weak and

tends to break off at the union. Bartlett on Oriental sand pear (*Pyrus pyrifolia*) root, while physically strong at the union, cause a severe disorder of the fruit in which the calyx end turns black (this condition is called “black end”). In horticultural terms, both rootstocks are incompatible with Bartlett.

Some incompatibilities have delayed effects; the black-line condition of English walnut grafted on black walnut root appears only after twenty or more years of satisfactory performance. Most incompatibilities of orchard trees, however, are apparent at an early age. A number of incompatibilities in trees are induced by either a virus or a mycoplasma. Sweet cherry infected with buckskin on *Prunus mahaleb* root and pear infected with pear decline on *Pyrus pyrifolia* root are example of induced incompatibilities (Westwood, et al., 1971).

Graft unions after a few years growth may be of three types: (1) both scion and stock are of equal size, (2) the scion overgrows the stock, (3) the stock over grows the scion. Grafts where the scion and stock are unequal in size are often thought to be incompatible. This notion stems largely from the fact that many incompatible unions show a scion overgrowth. However, many unions with scion over growth are perfectly compatible and the overgrowth simply reflects the genetic tendency of the scion to lay down wood growth by increased cambial activity, for example, Comic pear overgrows all rootstock types, even those with which it is completely compatible (Westwood, 1978).

2.3.1. Effects of rootstock on scion growth

Size control and sometimes an accompanying change in tree shape, is one of the most significant rootstock effects. This seems to be largely because the rootstock causes the vigor of a given scion cultivar to be altered. By proper rootstock selection in apples, the complete range-from very dwarfed to very large-of tree size has been obtained with a given scion cultivar grafted to the rootstock series. Rootstock effects on tree size and vigor are also recognized in citrus, pear and other species (Bitters 1950, Gardner and Horanic, 1963).

Vigorous, strongly growing rootstocks in some cases result in a larger and more vigorous plant which produces greater crops over a long period of years. On the other hand, trees on dwarfing stock may be more fruitful, and if closely planted, produce higher yields per acre, especially in the early years of bearing. The presence of the graft union itself may stimulate earlier and perhaps heavier bearing. For instance, in studies with citrus (Hodgson and Cameron, 1935) five rootstocks- sour orange, sweet orange, trifoliate orange, grape fruit and rough lemon, all came into bearing two seasons earlier when budded to themselves than when un-budded, although in each case the trees were about the same size. If there is an imperfect graft union, as with a partial incompatibility as occurs in some combinations, a reduction in translocation at the graft union can have a girdling effect and thus lead to increased fruitfulness.

Rootstock influence can vary greatly with different kinds of plants. In growing Oriental persimmon (*D. kaki*), the rootstock seem to have a direct effect on flower production and fruit set. In tests (Schroeder, 1947) using the 'Hachiya' persimmon trees on *D. lotus* roots produced more flowers but matured fewer fruits than similar trees on *D. kaki* roots, where trees on *D.*

virginiana roots produced so few flowers that crops were very poor. In grapes, where yield is dependent upon the vigor of the current season's growth, the rootstock used can be a strong influencing factor (Harmon, 1949).

There is considerable variation among plant species in regard to the effect of the rootstock on fruit characteristics on the scion cultivar. No carry-over of characteristics of the fruit which the stock would produce is encountered in fruit of the scion cultivar. For example, quince, commonly used as a pear rootstock, has fruits with a pronounced tart and astringent flavor, yet this flavor does not appear in the pear fruits. The peach is often used as a rootstock for apricot, yet there is no indication that the apricot fruits have taken on any characteristics of peach fruits.

2.3.2. Effects of scion on rootstock growth

There is a tendency to attribute all cases of dwarfing or invigoration of a grafted plant to the rootstock, the effect of the scion on the behavior of the composite plant may be fully as important as that of the rootstock. Unquestionably, the scion, the interstock, the rootstock and the graft union itself all interact to influence each other and determine the overall behavior of the plant. In certain combinations, a particular member of the combination could have a marked influence no matter what part of the plant it becomes. For instance, a dwarfing stock will exert a dwarfing influence on the entire plant whether used as rootstock, intermediate stock or scion.

If a strongly growing scion cultivar is grafted on a weak rootstock, the growth of the rootstock will be stimulated so as to become larger than it would have been if left un-grafted. Conversely if a weakly growing scion

cultivar is grafted on a vigorous rootstock, the growth of the rootstock will be lessened from what it might have been if left un-grafted. In citrus, for example, when the scion cultivar is less vigorous than the rootstock cultivar, it is the scion cultivar rather than the rootstock which determine the rate of growth and ultimate size of the tree (Hodgson, 1943).

The cold hardiness of a rootstock can be influenced by the particular scion cultivar grafted on it. This effect is not due necessarily to a winter-hardy scion imparting hardiness to the rootstock rather, it is probably related to the degree of maturity attained by the rootstock, certain scion cultivars tending to prolong growth of the roots long into the fall so that insufficient maturity of the root tissues is reached by the time killings low winter temperatures occur. The rootstock, if left un-grafted, or grafted to a scion cultivar which stops growth in early fall, may mature its tissues sufficiently early so as to develop adequate winter-hardiness. Cold-hardiness, of citrus roots is affected by the scion cultivar. Sour orange seedlings budded to 'Eureka' lemon suffered much more from winter injury than un-budded seedlings. The lemon tops were killed, as well as a portion, several inches deep, of the sour orange stock, but un-budded sour orange seedlings suffered only slight foliage injury (Webber, 1926).

2.3.3. Effects of an intermediate rootstock on scion and rootstock

1. Produce a dwarfed trees
2. Early-bearing of fruits
3. Compatibility

Yano et al. (2003) reported that, the use of compatible peach interstock to overcomes the graft-incompatibility and enhances the nitrogen allocation to scions and thereby extends the productive lives of dwarf peach

trees. The ability of certain dwarfing clones, inserted as an interstock between a vigorous top and vigorous root, to produce a dwarfed and early-bearing fruit trees has been known for centuries. It is reported that use of the paradise apple as an interstock to induce precocity in apple trees grown on crabapple rootstocks. An intermediate dwarfing stem piece seems to have a built-in mechanism that causes reduced growth in the rootstock as well as in the scion top (Parry and Rogers, 1968). Dwarfing of apple trees by the use of a dwarfing interstock, such as ‘Malling 7’, 8 or 9, has been widely used commercially for many years. This method has the advantage of allowing the use of well-anchored dwarfing clone. However, excessive suckering from the roots may occur due to the dwarfing interstock, even in rootstock types that normally do not sucker freely. This interstock effect could, in some cases, be due to the introduction of an additional graft union with the possibility of translocation restrictions. Imperfect graft unions are indicated as a cause of the dwarfing exerted on orange trees by a lemon interstock, the lemon itself is strong growing. On the other hand, there is evidence that the observed effects of the interstock are due directly to an influence of the interstock piece rather than to abnormalities at the graft union (Sax, 1953).

2.3.4. Possible mechanism for the effects of rootstock on scion & scion on rootstock

The nature of the rootstock-scion relationship is very complex and probably differs among genetically different combinations. The fundamental mechanism by which stock and scion influence each other has yet to be adequately determined. Some of the explanations offered for the observed effects are speculative, often conflicting, and sometimes not well

substantiated. Several theories have been advanced as possible explanations for the interaction between stock and scion.

One suggested mechanism is that the rootstock influences are the result of translocation effects rather than the absorbing ability of the root system. The stem portion of the tree has a definite influence (Blair, 1938) in which commonly used rootstock were used as intermediate stocks between a vigorous root system and the scion cultivar. The expected effects were still present, although to a lesser degree, even though the materials were used as inter-stocks rather than as the entire root-absorbing system. This same influence on the tree was noted if the intermediate stock tissue was reduced to just a ring of bark (Roberts, 1949).

Differences in growth rate were suggested by Vyvyan (1934) as a possible explanation of some of the observed reciprocal effects of stock and scion, especially the influence on vigor. He pointed out that for a given scion cultivar on a given soil, the stem/root ratio in any given scion stock combination is remarkably constant regardless of tree size or age. This constant ratio between stem and root means that both grow at the same rate. If two different cultivars with markedly different growth rates are combined as stem and root by grafting into one plant which would subsequently have a constant growth rate, there must be some alteration in the growth rate of the components. Either the slower growing cultivar must speed up or the faster growing one must decrease its rate. Thus the stock and the scion would be expected to mutually affect each other's growth rate.

Chandler (1925) and Gardner et al, (1939), discussing the subject in general terms, asserted that the effects of stock on scion and scion on stock can be explained by physiological factors, chiefly the influences due to changes in vigor. Chandler (1925) pointed out that when the scion is the more vigorous part of the combination, the carbohydrate supply to the roots should be greater. Since certain roots supply and are supplied by certain branches, it would be expected that the branching habit of the top would influence the branching habit of the roots, thus explaining the different root types can be obtained in different scion cultivars. If trees of different cultivars were pruned to exactly the same number and distribution of branches, then the difference in rootstock growth associated with the different scion cultivars might not occur.

Tukey and Brase (1933) concluded from their studies that no one part of a grafted tree could be considered to have complete control, but that all rootstock, inter-stock and scion-influenced the growth of the whole, although generally the rootstock had the dominant role. Although no completely satisfactory explanation of how the three genetically different components of a grafted plant-rootstock, inter-stock, and scion-interact to influence the growth, flowering and fruiting responses of the composite plant, three approaches can be considered:

- (1) nutritional uptake and utilization,
- (2) translocation of nutrients and water, and
- (3) alterations in endogenous growth regulators.

2.4. Methods of grafting

According to Hartmann and Kester (1975), grafting is the art of joining parts of plants together in such a manner that they will unite and continue their growth as one plant. In another words, grafting is the process and operation of inserting a part of one plant into another or placing it upon another in such a way that a union will be formed and the combination will continue to growth. Any method that permits cambial contact between plant parts is a means of grafting. The part of the graft combination, which is to become the upper portion, is termed as scion, and the part, which becomes the lower portion, is termed as rootstock, or under-stock, or just the stock. All methods of joining plants are properly termed grafting, but when the scion part is only a small piece of bark containing a single bud, the operation is termed as budding.

2.4.1. Formation of the graft union

A number of detailed studies have been made on the healing of graft unions, mostly with woody plants. Briefly the usual sequence of events in the healing of a graft union is as follows:

1. Freshly cut scion tissue capable of meristematic activity is brought into secure, intimate contact with similar freshly cut stock tissue in such a manner that the cambial regions of both are in close proximity. Temperature and humidity conditions must be such as to promote growth activity in the newly exposed and surrounding cells.
2. The outer exposed layers of cells in the cambial region of both scion and stock produce parenchyma cells, which soon intermingle and interlock; this is called callus tissue.

3. Certain cells of this newly formed callus which are in line with the cambium layer of the intact scion and stock differentiate into new cambium cells.
4. These new cambium cells produce new vascular tissue, xylem toward the inside and phloem toward the outside, thus establishing vascular connection between the scion and stock, a requisite of a successful graft union.

For any successful grafting operation there are five important requirements;

(a) The stock and scion must be compatible: They must be capable of uniting to closely related plants, such as two apple cultivars, can be grafted together. Distantly related plants, such as an oak and an apple tree cannot be grafted together.

(b) The cambial region of the scion must be in intimate contact with that of the stock: The cut surfaces should be held together tightly by wrapping, nailing, or some other such method. Rapid healing of the graft union is necessary so that the scion may be supplied with water and nutrients from the stock by the time the buds start to open.

(c) The grafting operation must be done at a time when the stock and scion are in the proper physiological stage: Usually this means that the scion buds are dormant. For deciduous plants, dormant scion wood is collected during the winter and kept inactive by storing at low temperature. The rootstock plant may be dormant or in active growth, depending upon the grafting method used.

(d) Immediately after the grafting operation is completed, all cut surfaces must be carefully protected from desiccation: This is done either by covering with grafting wax or by placing the grafts in moist material or in a covered grafting frame.

(d) Proper care must be given the grafts for a period of time after grafting: Shoots coming from the stock below the graft will often choke out the desired growth from the scion. In some cases, shoots from the scion will grow so vigorously that they break off unless staked and tied or cut back.

As anyone experienced in grafting or budding knows, the results obtained are often inconsistent, an excellent percentage of “takes” occurring in some operations, whereas in others the results are discouraging. There are a number of factors which can influence the healing of graft unions. One of the symptoms of incompatibility in grafts between distantly related plants is a complete lack, or a very low percentage, of successful unions. Grafts between some plants known to be incompatible, however, will initially make a satisfactory union, even though the combination eventually fails.

Some plants are much more difficult to graft than others even when no incompatibility is involved. In top grafting apples and pears, even the simplest techniques usually give a good percentage of successful unions, but in top grafting certain of the stone fruits, such as peaches and apricots, much more care and attention to details are necessary. Strangely enough, top-grafting peaches to some other compatible species, such as plums or almonds, are more successful than reworking them back to peaches. Many times one method of grafting will give better results than another, or budding

may be more successful than grafting, or vice versa. For example, in top working the native black walnut (*Juglans hindsii*) to the Persian walnut (*Juglans regia*) in California, the bark graft method is more successful than the cleft graft. Some species, such as the Muscadine grape (*Vitis rotundifolia*), mango (*Mangifera indica*) and *Camellia reticulata* are so difficult to propagate by the usual grafting or budding methods that “approach grafting” in which both partners of the graft are maintained for a time on their own roots, is often used. This variation among plant species and cultivars in their grafting ability is probably related to their ability to produce callus parenchyma, which is essential for a successful graft union.

There are certain environmental requirements that must be met for callus tissue to develop. Temperature has a pronounced effect on the production of callus tissue. As reported by Shippy (1930) previously, in apple grafts little, if any, callus is formed below 0°C or above about 40°C. Even around 4°C, callus development is slow and meager, and at 32°C or higher, callus production is retarded, with cell injury becoming more apparent as the temperature increases, until death of the cells occurs at 40°C. Between 4°C and 32°C, however, the rate of callus formation increases directly with the temperature. In such operations as bench grafting, callusing may be allowed to proceed slowly for several months by storing the grafts at relatively low temperature 7 to 10°C, or if rapid callusing is desired, they may be kept at higher temperature for a shorter time. Following bench grafting of grapes, a temperature of 24 to 27°C is about optimum; 29°C or higher results in profuse formation of a soft type of callus tissue, which is easily injured during the planting operations. Below 70° F callus formation is slow, and below 5°C it almost ceases.

Since the parenchyma cells comprising the important callus tissue are thin-walled and tender, with no provision for resisting desiccation, it is obvious that if they are exposed to drying air for very long, they will be killed. This was found to be the case in studies of the effect of humidity on healing of apple grafts (Shippy, 1930). Air moisture level below the saturation point inhibited callus formation, the rate of desiccation of the cells increasing as the humidity dropped. In fact, the presence of a film of water against the callusing surface was much more conducive to abundant callus formation than just maintaining the air at 100 percent relative humidity.

Highly turgid cells are more likely to give proliferation of callus than those in a wilt condition. *In vitro* studies (Doley, 1970) of stem pieces of ash (*Fraxinus excelsior*) have shown that callus production on the cut surfaces was markedly reduced as the water potential (turgidity) decreased. Unless a completed graft union is kept by some means at a very high humidity level, the chances of successful healing are rather remote. With most plants through waxing of the graft union, which retain the natural moisture of the tissue, is all that is necessary. Damp peat moss or wood shavings are good media for callusing, providing adequate moisture and aeration.

It has been shown that oxygen is necessary at the graft union for the production of callus tissue. This would be expected since rapid cell division and growth are accompanied by relatively high respiration, which requires oxygen (Shippy, 1930). For some plants, a lower percentage of oxygen than is found naturally in air is sufficient, but for others healing of

the union is better if it is left unwaxed but placed in a well-moistened medium. This may indicate that the plants have a high oxygen requirement for callus formation. Waxing restricts air movement and oxygen may become a limiting factor, thus inhibiting callus formation. This situation seems to be true for the grape, usually the union is not covered with wax or other air-excluding materials during the callusing period. There is evidence that light will inhibit callus development. *In vitro* callus cultures of black cherry (*Prunus serotina*) became much larger in darkness than in light for some clones (Caponetti et al., 1971).

Some propagation methods, such as T-budding and bark grafting, depend upon the bark “slipping,” which means the cambium cells are actively dividing, producing young thin-walled cells on each side of the cambium. These newly formed cells separate readily from one another, so the bark “slips.” Initiation of cambial activity in the spring results from the onset of bud activity since, shortly afterwards; cambial activity can be detected beneath each developing bud, with a wave of cambial activity progressing down the stems and trunk. This stimulus is due to production of auxin and gibberellins originating in the expanding buds (Wareing, 1964).

In budding seedlings in the nursery in late summer it is important that they have an ample supply of soil moisture just before and during the budding operation. If they should lack water during this period, active growth is checked, cell division in the cambium stops, and it becomes impossible to lift the bark flaps to insert the bud.

There is evidence to show that callus proliferation essential for a successful graft union occurs most readily at the time of year just before and during “bud break” in the spring, diminishing through the summer and into fall. Increasing callus proliferation takes place again in late winter, but this is not dependent upon breaking of bud dormancy. Sometimes the techniques used in grafting are so poor that only a small portion of the cambial regions of the stock and scion are brought together. Although healing occurs in this region and growth of the scion may start, after a large leaf area develops and high transpiration rates occur, sufficient movement of water through the limited conducting area can not take place, and the scion subsequently dies. Other errors in grafting technique, such as poor or delayed waxing, uneven cuts, or use of desiccated scions can, of course, result in grafting failure. Poor grafting techniques, although they may delay adequate healing for sometime-weeks or months-do not in themselves cause any permanent incompatibility. Once the union is adequately healed, growth can proceed normally.

Using virus-infected propagating materials in nurseries can reduce bud “takes” as well as the vigor of the resulting plant (Posnette, 1966). In stone fruit propagation the use of bud wood free of ring spot virus has consistently given improved percentages of “takes” over infected wood. Sometimes bacteria or fungi gain entrance at the wounds made in preparing the graft or bud unions. For example, it was found that a rash of failures in grafts of *Cornus Florida* ‘Rubra’ on *C. Florida* stock was due to the presence of the fungus *Chalaropsis thielavioides* (Collins, 1958). Chemical control of such infections materially aids in promoting healing of the unions (Doesburg, 1962). In South and Central America, rubber (*Hevea*) trees are propagated by a modification of the patch bud. A major cause of budding

failures in these countries has been infection of the cut surfaces by a fungus, *Diplodia theobromae* but control of this infection can be obtained by fungicidal treatments (Langford, 1954).

Trials with the use of growth substances, particularly auxin, applied to tree wounds or to graft unions have not given consistent results in promoting subsequent healing; consequently such materials are not generally used for this purpose. In tissue culture studies, however, a definite relationship exists between callus production (which is essential for graft healing) and the levels of certain applied growth substances, particularly kinetin and auxin (Doley and leyton, 1970; Fujii and Nito, 1972). There is some evidence too, abscisic acid stimulates callus production, especially when applied to tissues in combination with auxin or kinetin (Blumenfield and Gazit, 1969).

As previously postulated by (Dave, 2002), reasons for graft failure would be thought as the followings:

1. Stock and scion were not compatible.
2. The cambiums were not meeting properly.
3. Scions were upside down.
4. Grafting was done at the wrong time.
5. Under-stock or scion were not healthy.
6. Scions were dried out or injured by cold.
7. Scions were not dormant.
8. The graft was not properly covered.
9. The scion was displaced by storm, birds, or other means.
10. The graft was shaded too much by other growth.
11. The graft was attacked by insects or diseases.
12. The graft union was girdled because tape was not cut or released in time.

2.5. Graft incompatibility

The compatibility refers to as the ability of two different plants, grafted together, to produce a successful union and to develop satisfactorily into one composite plant is termed as compatibility. The opposite, of course would be incompatibility. The distinction between a compatible and incompatible graft union is not clear-cut. On one hand, stock and scion of closely related plants, unite readily and grows as one plant. On the other hand, stock and scion of unrelated plants grafted together are likely to fail completely in uniting. Characterization of incompatibility, however, is not that distinct, for many graft combinations lie between these two extremes. They may unite initially, with apparent success, but gradually develop incompatibility symptoms with time, due either to failure at the union or to the development of abnormal growth patterns.

Graft incompatibility in fruit trees has been classified by Mosse (1962) into two types: (1) translocated incompatibility and (2) localized incompatibility.

(1) Translocated incompatibility: includes those cases in which the incompatible condition is not overcome by the insertion of a mutually compatible inter-stock because, apparently, some labile influence can move across it. This type involves phloem degeneration, and can be recognized by the development of a brown line or necrotic area in the bark. Consequently, restriction of movement of carbohydrates occurs at the graft union—accumulation above and reduction below. Reciprocal combination may be compatible. In the various combinations in this category the range of bark tissue breakdown can extend from virtually no union at all, to a mechanically weak union with distorted tissues, to a strong union with

tissues normally connected. An example of this category is the combination of 'Nonpareli' almond on 'Mariana 2624' plum shows complete phloem breakdown, although the xylem tissue connections are quite satisfactory. However, another cultivar, 'Texas' almond on 'Mariana 2624' plum produces a good, compatible combination. Inserting a 6-in. piece of 'Texas' almond as an interstock between the 'Nonpareli' almond and the 'Marianna' plum stock fails to overcome the incompatibility between these two components (Kester et al., 1965).

(2) Localized incompatibility: includes combinations in which the incompatibility reactions apparently depend upon actual contact between stock and scion; separation of the components by insertion of a mutually compatible inter-stock overcomes the incompatibility symptoms. In the incompatible combination the union structure is often mechanically weak, with continuity of cambium and vascular tissues broken, although there are cases in which the union is strong and the tissue joined normally. Often external symptoms develop slowly, appearing in proportion to the degree of anatomical disturbance at the graft union. Root starvation eventually results, owing to translocation difficulties across the defective graft union. An example of this category is that of 'Bartlett' pear grafted directly on quince rootstock. With the use of a "compatible bridge" of 'Old Home' pear as an interstock, the three part combination is completely compatible, and satisfactory tree growth takes place (Mosse, 1958). Masses of parenchymatous or bark tissue, or both, rather than normally differentiated tissues are commonly found at the union in this type of incompatibility, interrupting the normal vascular connection between stock and scion.

2.5.1. Symptoms of incompatibility

Graft union malformations resulted from the incompatibility can usually be correlated with certain external symptoms. Below are some symptoms that have been associated with incompatible graft combinations:

1. Failure to form a successful graft or bud union in a high percentage of cases.
2. Yellowing foliage in the latter part of the growing season, followed by early defoliation. Decline in vegetative growth, appearance of shoot dieback, and general ill health of the tree.
3. Premature death of the trees, which may live only a year or two in the nursery.
4. Marked differences in growth rate or vigor of scion and stock.
5. Differences between scion and stock in the time at which vegetative growth for the season begins or ends.
6. Overgrowths at, above, or below the graft union.

An isolated case of one or more of the above symptoms does not necessarily mean the combination is incompatible; some of these symptoms can also result from unfavorable environmental conditions, such as lack of water, essential nutrient, attacks by insects, diseases, poor grafting or budding techniques (Argles, 1936). Incompatibility is indicated by the breaking off of trees at the point of union, particularly when they have been growing for some years and the break is clean and smooth, rather than rough or jagged. This may occur in a year or two or even later stages, after the union is made-for instance, in apricot on almond roots. Scion overgrowth at the graft union is often believed to be a certain indication of incompatibility.

In some instances the stock-scion combination grows in an apparently normal fashion for varying period of time perhaps for many years then difficulties arise. A good example of “delayed incompatibility” is the so called “black line” of walnut; this occurs in certain Persian walnut orchards in Oregon, California, and France where cultivars of *Juglans regia* are grafted onto seedling rootstock of *J. hindsii* or onto Paradox.

Compatibility relationship between clones may change with time (Luckwill, 1962). For example, the pear variety ‘Bristol Cross,’ when it was introduced in 1932 was known to make a strong union when grafted directly on quince roots. Thirty years later, this variety when worked on quince roots required a mutually compatible inter-stock such as ‘Beurre hardy’ in order to produce an acceptable union. Likewise, ‘Conference’ pear on quince in 1937 developed strong unions and vigorous trees but 20 years later many individual trees of this combination showed weak, incompatible unions (Williams, 1956).

Although incompatibility is clearly related to genetic differences between stock and scion, the mechanisms by which particular cases are expressed are not clear. With the large number of genetically different plant materials that can be combined by grafting, a wide range of different physiological, biochemical, and anatomical systems are brought together, with many possible interactions, both favorable and unfavorable. Several proposals have been advanced in attempts to explain incompatibility, but generally the evidence supporting most of them is inadequate and often conflicting.

One possible mechanism is that different growth characteristics of the stock and scion occur. That is, if marked differences occur in vigor or in the time of starting or completing vegetative growth for the season, incompatibility may be expected. Cambial activity in the spring, which originates with swelling of the scion cultivar buds, and spreads downward through the stem and through the graft union, will activate the cambium of the rootstock equally well in compatible and incompatible combinations. A study in which growth rates of scion and rootstock were measured close to the graft union in incompatible pear-quince combinations failed to show that the incompatibility was associated with differences in growth rates or time of cambial activity. Although differences in growth rates may influence the severity of the symptoms, it is unlikely that is a basic cause of incompatibility.

Another possible mechanism is physiological and biochemical differences between stock and scion. This is supported by studies with incompatible combinations of certain pear cultivars on quince rootstock (Gur, 1957). The experimental evidence supports the following conclusion:

1. When certain pear cultivars are grafted onto quince roots, a cyanogenic glucoside, prunasin –normally found in quince, but not in pear, tissues- is translocated from the quince into the phloem of the pear. The pear tissues break down the prunacin in the region of the graft union, with hydrocyanic acid as one of the decomposition products. This enzymatic breakdown is hastened by high temperatures. In addition, different pear cultivars vary in their ability to decompose the glucoside.
2. The presence of the hydrocyanic acid leads to a lack of cambial activity at the graft union, with pronounced anatomical disturbances in the phloem

and xylem at the union resulting. The phloem tissues are gradually destroyed at and above the graft union. Conduction of water and materials is seriously reduced in both xylem and phloem.

3. A reduction of the levels of the sugar reaching the quince roots leads to further decomposition of prunasin, liberating hydrocyanic acid and killing large areas of the quince phloem.
4. A water-soluble and readily diffusible inhibitor of the action of the pear enzyme (which breaks down the glucoside) occurs in the various pear cultivars, although they differ in their content of this inhibitor. This may explain why certain pear cultivars are compatible and others incompatible with quince rootstock.

To predict in advance, by some reliable tests, whether or not a certain scion-rootstock combination would be incompatible or not would be of considerable economic value. This could be particularly important in the development of new clonal rootstocks. Several methods have been tried for making such predictions, none with complete success.

Various laboratory methods have been developed for evaluating stock-scion compatibility in young nursery trees without growing them on to maturity (Evans and Hilton, 1957). These includes water conductivity measurements through the graft union, macroscopic evaluation of the external appearance of the graft union, microscopic evaluation of the graft union and breaking strength tests.

If a graft combination known to be incompatible has been made and is discovered before the tree dies or breaks off at the union, it is possible, in

graft combinations of the localized type, to correct this condition by bridge grafting with a mutually compatible stock, if such exists. If a tree has been mistakenly propagated on a rootstock known to show symptoms of incompatibility with the scion cultivar, with the probability that the tree will eventually break off at the graft union, it is possible to correct this condition by inarching with seedlings of a compatible rootstock. If breakage does not occur until the inarches are strong enough to support the tree, it may be possible to save it, the inarched seedlings becoming the main root system (Hartmann and Kester (1975)).

Chapter 3

Situation of persimmon cultivation in Nepal and some factors affecting the success potential of grafting

3.1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is being cultivated in different parts of Nepal are mostly of the astringent type and are consumed only after they have fully ripened when the fruits become soft and sweet. In spite of the fact that persimmon was introduced in Nepal during the 1910's, it did not gain importance until the turn of the century. Some of the major reasons for its non-adoption are: less familiar, limited choice of cultivars, lack of suitable methods of propagation, inadequate vegetatively propagated plant material, lack of suitable compatible rootstocks, pollination behavior and lack of suitable pollinizer varieties, lack of proper practices for cultivation and unawareness regarding astringency. Besides these, lack of sufficient knowledge regarding fruit maturity, post harvest handling of the fruits and its consumption also mired its cultivation. Processing of fruits into other products is currently non-existent. The fruit is used for festivities during September and October. It is famous in the surroundings of the Kathmandu valley and has a potential to be one of leading fruits in mid hill regions (Fig.3). All the cultivars are grown in east-west range of mid-hill regions, ranging from 1000m to 1800m in elevation where agro-climatic conditions are suitable for its cultivation (Simkhada et al., 1999).

With the efforts being made in recent years to overcome the problems and diversification in fruit culture, the horticulture development project (supported by JICA: Japan International Cooperation Agency) has contributed to the promotion of commercially important fruits in Nepal including persimmon from 1992-1999. The project activities were concentrated in generating and developing improved fruit cultivation techniques and their practical dissemination to the farmer's field through extension and training program (Fig.4 and 5). Under the follow-up program, the extension activities are still ongoing. (HDP, Annual report, 1995-1999).

Propagation in persimmon has been proved the difficulty as compared to other commercial fruit trees due to the problem of low success potential of grafting. The availability of good grafted plant material is a major factor in the commercial cultivation of persimmon. In Nepal, the most common method for persimmon propagation is tongue grafting in the month of January-February. However, incompatibility or low success rates of grafting has hindered the mass propagation of persimmon. While grafting is usually performed using *D. kaki* (seedlings of local persimmon) as both rootstock and scion, the average rate of success is less than 60%. Bellini (1982) reported some incompatibility problems between 'Fuyu' and *D. lotus*. 'Fuyu' scion grafted onto *D. lotus* rootstock often grow slowly, bear fruit early and die within a few year (Hodgson, 1940) while astringent cultivars normally form good growth but excessive fruit dropping is sometimes a problem (Schroeder, 1947). This topic focuses on the situation of persimmon cultivation in Nepal and factors affecting low success potential of grafting.

3.2. Materials and Methods

Seedlings of two-year-old local *D. kaki* persimmon such as ‘Baluwatar’, ‘Dhading’, ‘Dhaura’, ‘Khawa’, ‘Khopasi’, ‘Kirtipur’, ‘Nuwakot’, ‘Teku’, and some Japanese persimmon cultivars were grown at the Horticulture Development Project in Kirtipur, Kathmandu, and some private nurseries in Nepal for rootstock selection and quality measurement. In addition, two-year old *D. kaki* and *D. lotus* trees grown in plastic containers at the orchard of the Agriculture and Forestry Research Center, University of Tsukuba, Japan were used in this experiment. The buds and scions of Nepalese astringent and Japanese non-astringent/astringent types were budded and grafted onto local and improved cultivars in order to observe the effect of cultivar, grafting method and graft union development. The methods used in this experiment were T-budding, side and tongue grafting. Visual evaluation was done at 1, 3, 5, 7, 9, 12 months and 1.5 and 2.5 years after grafting.

For anatomical observation under Light Microscope (Olympus BH2-RFCA), samples of graft unions from each rootstock/scion combination were taken and excised samples were fixed in FAA; formalin–acetic acid–ethanol (60%) in (5:5:90 v/v) solution, using vacuum infiltration to ensure complete absorption of the solution. After washing with water, the sections were cut from the fixed portions at 20 μm thickness using a sliding MICROM-GmbH, HM 330, and coated with glycerin before observation under the microscope.

3.3. Results

3.3.1. A case study in Nepal (part 1)

3.3.1.1. Selection of ideal combination

Rootstocks play a vital role in plant growth, fruit quality, maturity period, occurrence of diseases and pests and other characteristics of persimmon plant. A study was conducted during a two-year period from 1997-1999 to identify better rootstocks which are compatible with non-astringent or astringent type. Different scions and rootstocks were used in this study. The method used in this experiment were tongue, side, and cleft grafting (top-working) and T-budding. As regards total percentage of success with different grafting methods, tongue grafting found to be the preferred method over side-grafting. The practice of budding is not yet popular in Government institution and also private nurseries. The success rate was lower in budding as compare to tongue and side grafting (Table 4).

It was observed that *in-situ* grafting is the preferred method over grafting in transplanted rootstocks. With respect to scion/rootstock used, seedling of local persimmon ‘Dhaura’ as rootstock with seedlings of ‘Kirtipur’, ‘Hiratanenashi’, and Japanese non-astringent cultivar ‘Fuyu’, and ‘Jiro’ as scion yielded favorable results. Total percentage of graft success was higher in *in-situ* grafting (62.8 %) over transplanted rootstocks (54.2 %) as shown in Table 5. Using ‘Fuyu’ as a scion on different local seedlings, ‘Dhaura’, ‘Kirtipur’ and ‘Dhading’ gave better results as compared to other rootstocks in *in-situ* and transplanted rootstocks with 59.3 and 49.2 % respectively (Table 6).

3.3.1.2. Top-working (changing cultivars of established plants)

A fruit trees, or an entire orchard, may be of a cultivar that is undesirable. It could be an old cultivar no longer in demand, or an unproductive one, or one with poor growth habits, or possibly one that is susceptible to prevalent diseases or insects. As long as a compatible type is used, the top may be re-grafted to a more desirable cultivar, if such exists. Provision for adequate cross-pollination in fruit trees can be obtained by top-working scattered trees throughout the orchard to the proper pollinizing cultivar. Or, if a single tree is unfruitful owing to lack of cross-pollination, a branch of the tree may be grafted to the proper pollinizing cultivar. According to their interest, several cultivars of almost any fruit species can be grown on a single tree of that species by top-working each scaffold branch to a different cultivar. In a few cases, different species can be worked on the same tree. For example, on a single citrus rootstock it would be possible to have oranges, lemons, grapefruit, mandarins and limes or on a peach root system could be grafted plum, almond, apricot and nectarine.

D. kaki cultivar 'Fuyu' and 'Jiro' as scion and various local seedlings as a rootstock had been demonstrated good positive impact for cultivar change (astringent to non-astringent) in the village areas of Kathmandu valley (Fig.4.5). Top-worked plant had higher percentage success than others. Scions of *D. kaki* cultivar 'Fuyu' and 'Jiro' top-worked with *D. kaki* local seedlings 'Dhaura', and 'Kirtipur' and Japanese non-astringent cultivar 'Zenjimar' were better in comparison with other rootstocks used. Comparing the total successful percentage, 'Fuyu' showed higher rate of success (83.1 %) than 'Jiro' (80.4 %) as indicated in Table 7.

3.3.1.3. Growth and quality measurement

The growth of the trunk in 'Fuyu' from 1997 to 2000 was distinct. When grafted with 'Dhaura' the maximum growth was recorded at 31.7mm while the minimum growth of 22.1 mm was observed when grafted with 'Baluwatar' (Table not presented).

Table 8 reports the measurement of various fruit characteristics and quality. Full bloom in all cultivars begins in April while maturation starts from early September to late October. Common fruit shape is rounded square to ovoid. 'Fuyu' and 'Jiro' had higher average weight than others. Astringent types yielded higher total soluble solids content (°Brix) after deastringency treatments than non-astringent types.

Management of tree shape and size were important factors to consider in order to obtain quality fruits. Regular training and pruning of the Japanese persimmon trees had been done each year during winter. Tree branches were tied with ropes and pulled out to obtain a spreading type of tree shape. This helps the plants get good aeration and to intercept sunlight and also helps in producing fruits of good shape and size. However, Nepalese local astringent types had never performed such practice. The common tree shape in most of the Japanese cultivars were modified or improved center leader system while Nepalese astringent type has over sized trees creating problem in management and harvesting and had almost upright habit and growth vigor of the tree is semi- vigorous to vigorous.

Thinning is an important operation to achieve better quality fruits. Heavy fruiting in one year may cause very low production in the succeeding

year. Moreover, over-bearing may reduce the size and quality of the fruits. A study conducted to determine the suitable leaf to fruit ratio observed the ideal results when the ratio is 25-30 leaves per fruit.

3.3.2. Observation of graft union (part 2)

The research conducted in the University of Tsukuba in relation to tissue development in heterograft combination showed a defective union, characterized by the presence of necrotic cells, perhaps due to callus unresponsiveness to the stimulus for differentiation. Other main differences at the early stages were the presence of parenchymatous tissues without lignin coexisting with vascular tissues and necrotic cells in heterograft combination. At the later stages, adhesive phenomenon is reduced and the tree weakens in 'Fuyu'/*D. lotus* combination. Visual observations of *D. kaki* and *D. lotus* at the union region reveal that in *D. lotus*, a distinct malformation at the graft union that results in mechanical weakness which eventually leads to breakdown of the union is observed (Fig. 6).

In case of homograft combinations, initial response to grafting was the development of callus cells along the cut surfaces between the rootstock and scion. The xylem elements near the cut surfaces were discolored and the vessels were filled with gum; also necrotic tissues were observed in the old xylem of the scion. The initial results from peaches are similar to the findings reported by Salvatierra et al. (1998). After three months, new generating and connective tissues were observed in the transverse sections, and the new xylem elements had a perpendicular orientation with respect to the stem axis. At seven months, the longitudinal sections across the graft

union revealed necrotic cells in the callus at the base of the scion. After 12 months necrotic cell layers were observed surrounding the xylem of rootstocks (Fig. 7).

Some useful *Diospyros* species for temperate areas, distribution and chromosome numbers (Table 9), some recognized characteristics of *D. kaki* and *D. lotus* (Table 10), tree and bearing characteristics (Table 11), leading varieties in acreage, recently released new PCNA cultivars with dried and fresh use in Japan (Table 12) and fruit characteristics of persimmon cultivars grown in Japan (Table 13).

3.4. Discussion

Differences in development of the union region between scion and rootstock in the case of ‘Fuyu’ on *D. kaki* had higher sap plug, more live cells with cellular activity and uniform growth and vigor. In contrast, ‘Fuyu’ grafted on *D. lotus* showed lesser sap plug inside the wounded graft which resulted in lowering the graft adhesion. Success of grafting is directly related to the living cells, cellular activity and proper alignment of cambial layer between stock and scion. Rapid formation of callus prevents excessive accumulation of resinous material, which is thought to interfere with the successful graft adhesion (Suriyapananont and Suriyapananont 1997). Suriapananont and Suriaya-pananon (1997) reported that even if ‘Fuyu’ on ‘Kluairusi’ stock showed callus proliferation and extension of new cambium from the stock side, it failed to produce a successful union. The critical structural event in the formation of a successful graft is the unification of the vascular elements of both components (Yeoman, et al., 1978) while vascular

continuity between the components provided the most efficient system for the transport of water and nutrients. The cultivar stability of most fruit tree species must be ensured by grafting selected rootstock and scion genotypes. The grafted partners often belong to the same species or genus, but the use of genetically divergent genotypes is also common. In these cases, the stock and scion do not always constitute a successful graft and the result is low success potential of grafting.

This graft incompatibility or low success potential of grafting observed in some scion-rootstock combinations have been classified into two categories (Herrero, 1951; Mosse, 1962); the so-called translocated and localized incompatibility. The translocated incompatibility is associated to symptoms that resemble leaf aging and alterations in the redicular system. In this type of incompatibility, a degeneration of the sieve tubes and companion cells of the phloem just in the graft has been observed. This causes problems in the translocation of some compounds and results in their accumulation above the union. The localized incompatibility is associated to malformation at the graft union that results in mechanical weakness and subsequent breakdown of the union. A number of morphological and physiological alterations are associated to the incompatibility process preventing the development of a successful union between scion and rootstock. In this type of incompatibility, the alteration range from disorders in the functionality of the newly formed cambium and the lack of differentiation of the new tissues to the imperfect lignifications and lack of vascular continuity that causes the breakdown of the tree at the graft union.

Once the two components of graft, stock and scion, are in intimate contact, the cambial region capable of meristematic activity produce parenchymatic cells which soon intermingle and interlock, producing the callus tissue that fills the space between the two components connecting the scion and rootstock (Hartmann et al., 1990; Errea et al., 1994; Wang and Kollmann, 1996). Several researchers consider this step essential for the development of future vascular connections and have suggested that the primary recognition events occur at the point of cell to cell contact (Yeoman et al., 1978). From these point of view, the further morphological and physiological studies, i.e. the survey of partitioning of photosynthates into each organ and tissue in case of different persimmon scion /rootstock combinations must be needed.

3.5. Conclusion

Comparison of existing major grafting methods, non of them crosses the more than 60% success. However, top-worked plant had higher percentage success than others. *In-situ* grafting yielded better results than grafting done on transplanted rootstocks. Initial response to grafting was the development of callus cells along the cut surfaces between the rootstocks and scion. Necrosis of the xylem elements near the cut surfaces were observed and after three to twelve months, new generating and connective tissues were seen in the transverse sections with necrotic cells in the callus at the base of the scion. After one to three years of grafting, the graft union zone of *D. lotus* showed more necrotic points than *D. kaki* and adhesive phenomenon was reduced which resulted in tree weakening in Fuyu / *D. lotus* combination.

In my idea, these low success rate were due to the lack of deep research within existing cultivars to find out the compatible scion/rootstock partners, appropriate budding/grafting time and methods. For example, T-budding depend upon the bark “sleeping”, which means the cambium cells are actively dividing, producing young thin-walled cells on each side of the cambium. These newly formed cells separate readily from one another, finally the bark “ slips”. This stimulus is due to the production of auxin and gibberellins originating in the expanding buds. The other possible reason were long drought after the grafting and improper nursery management led to the low success of budding and grafting.

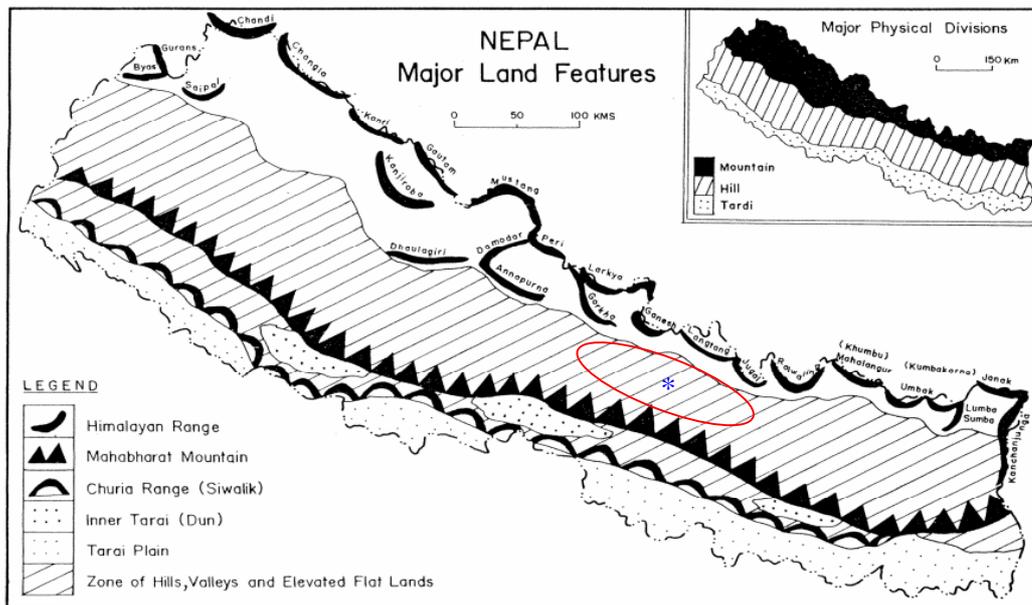


Fig. 3. The major land features of Nepal showing mainly three regions: Mountain, Hill and Plain areas. *Circle: pocket area of persimmon.



Fig. 4. Horticulture Development Training and Extension Project/Center located in Southern part of Kathmandu Valley.



Fig. 5. Underway to developing new commercial orchards in countryside (A) performing Top-working (B). 'Fuyu' and 'Jiro' as scion and various local *D. kaki* seedlings as a rootstock had been demonstrated good positive impact for cultivar change (astringent to non-astringent) in the village areas. Circle: Top-worked zone.

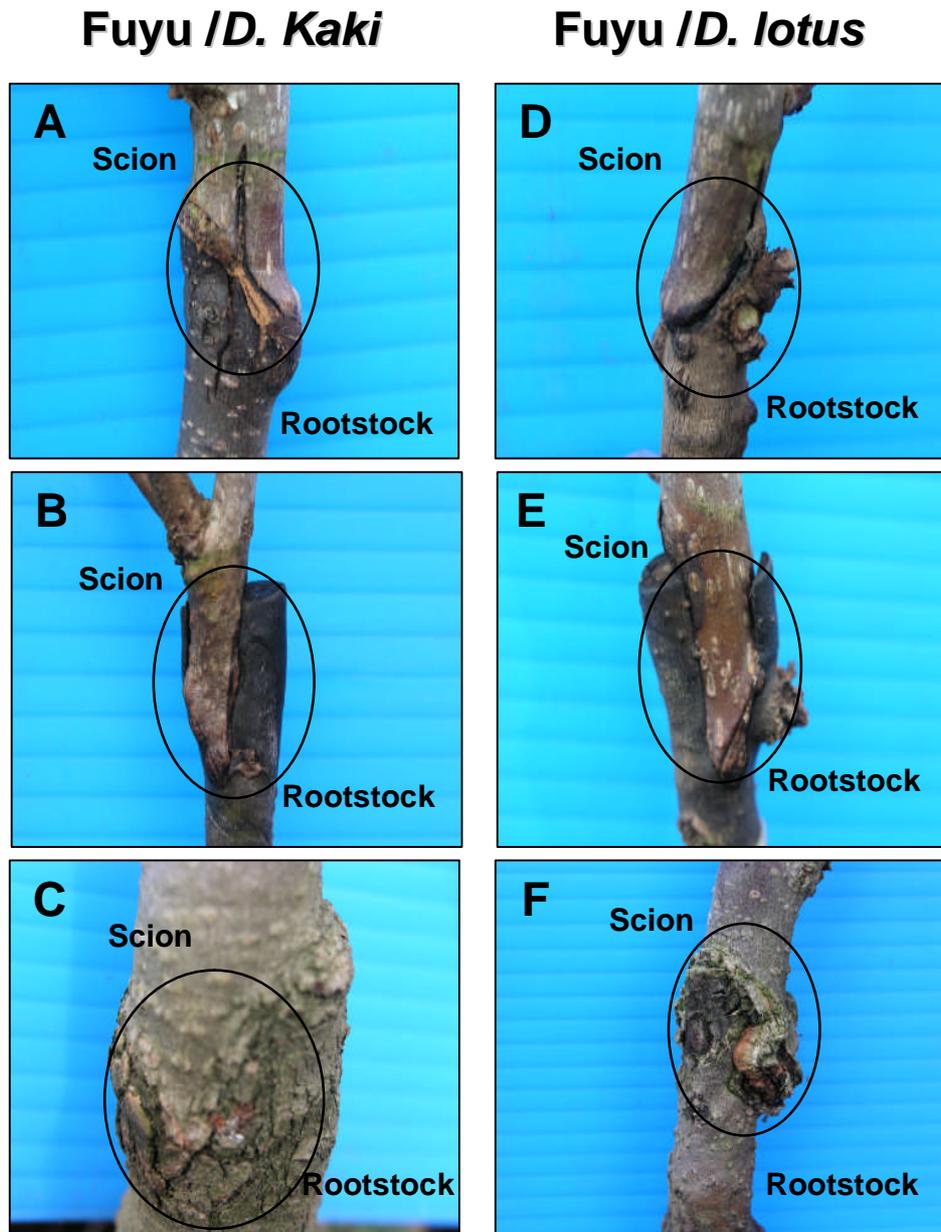


Fig. 6. Photographs showing the graft union development with different scion and rootstock combinations: Five months after tongue grafting (A.D) Five months after veneer grafting (B.E) and 2.5 years after veneer grafting (C.F). Circle: graft union.

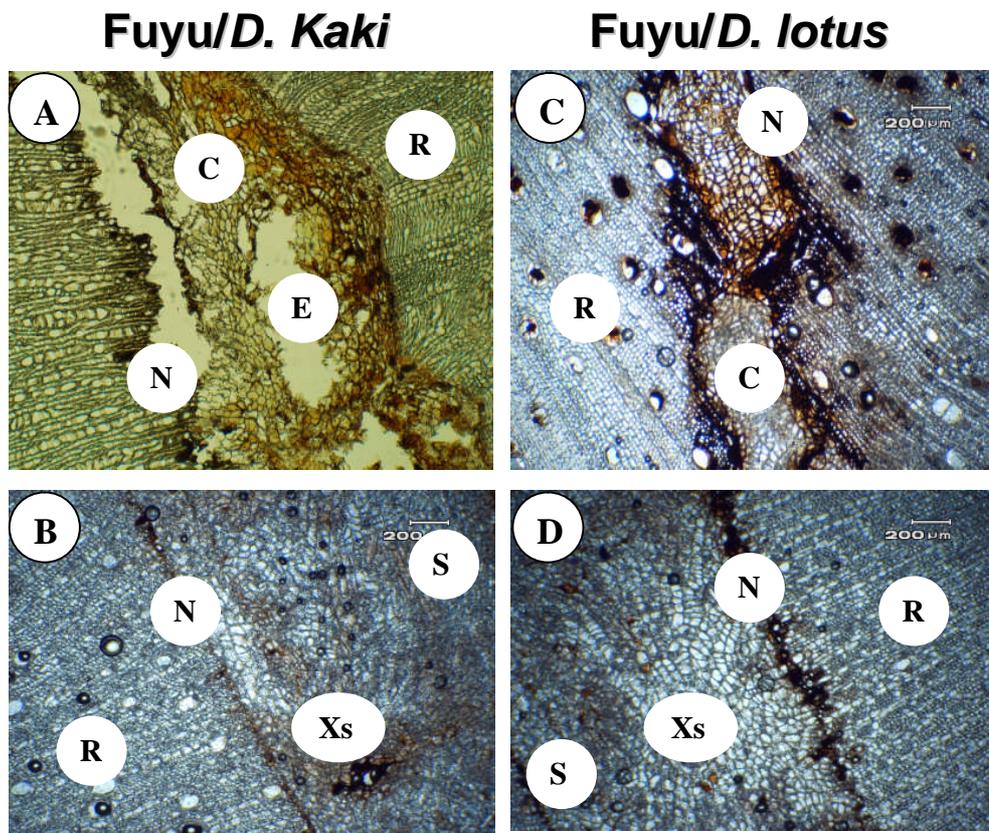


Fig. 7. Observation on transverse sections of the graft union under light microscope. One-month old (A); seven-month old (B); and 2.5-year-old (C, D); R: rootstock; S: scion; C: callus; N: necrosis; Xs: secondary xylem.

Table 4. Comparison of grafting method on the percent graft union success in 'Fuyu' grafted onto 'Dhaura' local seedlings.

Propagation methods	Type of nursery	Grafted no.	Success no.	Percent of graft union success
Tongue grafting	outdoors	1825	1087	59.5
Side grafting	outdoors	643	378	58.7
T-budding	outdoors	290	132	45.5
Total/ <i>Average</i>	-	2737	1597	<i>54.5</i>

Table 5. Success of tongue-grafting as affected by different scions onto (*D. kaki*) 'Dhaura' local seedlings.

Scion	<i>In-situ</i> grafting			Transplanted rootstocks		
	Grafted no.	Success no.	Percent of graft union success	Grafted no.	Success no.	Percent of graft union success
'Hiratanenashi'	70	48	68.5	120	71	59.1
'Fuyu'	90	58	64.4	250	132	52.8
'Jiro'	82	51	62.2	95	55	57.8
'Zenjimaruru'	29	15	51.7	25	10	40.0
'Suruga'	48	30	62.5	20	8	40.0
'Kirtipur'	55	41	74.5	140	87	62.1
'Baluwatar'	48	26	54.1	25	11	44.0
'Teku'	42	26	61.9	25	12	48.0
'Dhading'	66	41	62.1	110	53	48.1
'Banepa'	46	26	56.5	75	41	54.6
Total/ <i>Average</i>	576	362	<i>62.8</i>	885	480	<i>54.2</i>

Table 6. Success of tongue grafting in different graft combinations: 'Fuyu' as scion and various local seedlings as a rootstock.

Rootstock	<i>In-situ</i> grafting			Transplanted rootstocks		
	Grafted no.	Success no.	Percent of graft union success	Grafted no.	Success no.	Percent of graft union success
'Kirtipur'	210	132	62.8	165	85	51.5
'Khopasi'	78	41	52.5	35	13	37.1
'Nuwakot'	32	18	56.2	30	15	50.0
'Khawa'	36	19	52.7	40	19	47.5
'Dhaura'	105	69	65.7	120	71	59.1
'Baluwatar'	45	23	46.6	30	12	40.0
'Teku'	26	14	53.8	30	13	43.3
'Dhading'	145	89	61.3	80	39	48.7
'Banepa'	150	86	57.3	110	48	43.6
Total/ <i>Average</i>	827	491	<i>59.3</i>	640	315	<i>49.2</i>

Table 7. Success of top-working in different graft combinations: 'Fuyu' and 'Jiro' as scion and various local seedlings as a rootstock.

Rootstock	'Fuyu'			'Jiro'		
	Top-worked no.	Success no.	Percent of graft union success	Top-worked no.	Success no.	Percent of graft union success
'Kirtipur'	32	29	90.6	26	22	84.6
'Khopasi'	13	10	76.9	18	14	77.7
'Nuwakot'	12	9	75.0	16	13	81.2
'Khawa'	16	12	75.0	19	15	78.9
'Dhaura'	24	23	95.8	31	29	93.5
'Baluwatar'	12	9	75.0	17	13	76.4
'Teku'	18	15	83.3	12	9	75.0
'Dhading'	14	11	78.5	25	19	76.0
'Banapa'	11	8	72.7	13	10	76.9
'Atago'	23	18	78.2	24	18	75.0
'Suruga'	26	22	84.6	23	17	73.9
'Zenjimarū'	13	12	92.3	16	14	87.5
Total/Average	214	178	83.1	240	193	80.4

Table 8. Characteristics of some Japanese and Nepalese persimmon cultivars grown in Nepal (HDP, 1995-1999).

A. Japanese non-astringent type grown in Nepal.

Cultivar	Date of full bloom	Harvest time	Fruit quality					Tree shape	Tree growth vigor
			Shape	Weight (g)	Color	Hardness (kg)	Brix (%)		
'Jiro'	April early	Sept. mid	Rounded square	225	Orange yellow	7.4	14.5	Upright	Semi-vigorous
'Maekawa-Jiro'	April mid	Sept. early	Rounded square	230	Red yellow	6.1	15.5	Upright	Semi-Vigorous
'Matsumotowase-Fuyu'	April mid	Sept. late	Rounded square	230	Red yellow	4.3	15.0	Spreading	Semi-vigorous
'Fuyu'	April early	Oct. early	Rounded square	250	Orange yellow	4.8	16.0	Upright	Vigorous
'Hanagosho'	April early	Oct. early	Ovoid	220	Orange yellow	4.6	16.5	Upright	Vigorous
'Zenjimaruru'	April late	Sept. early	Ovoid	175	Red orange	7.0	12.2	Slight Spread	Vigorous

B. Japanese astringent type grown in Nepal.

'Hiratanenashi'	April mid	Oct. early	Flat square	170	Orange yellow	5.0	14.5	Spread	Semi-Vigorous
'Aizumishirazu'	April mid	Sept. late	Ovoid	160	Orange yellow	4.8	15.0	Slight Spread	Vigorous
'Atago'	April mid	Sept. late	Ovoid	150	Orange yellow	5.1	14.0	Upright	Vigorous
'Hachiya'	April mid	Sept. mid	Ovoid	184	Yellow	4.9	15.0	Slight Spread	Medium
'Mompei'	April late	Oct. early	Ovoid	158	Orange yellow	5.3	17.0	Upright	Vigorous

C. Nepalese astringent type grown in Nepal.

'Teku'	April mid	Oct. early	Ovoid	150	Yellow	5.6	19.2	Upright	Vigorous
'Dhaura'	April mid	Oct. early	Ovoid	175	Orange yellow	4.8	18.7	Upright	Vigorous
'Banepa'	April late	Oct. late	Ovoid	115	Yellow	4.2	19.3	Upright	Vigorous
'Dhading'	April late	Oct. mid	Ovoid	110	Yellow	5.2	17.4	Upright	Vigorous
'Baluwatar'	April late	Oct. late	Rounded square	120	Yellow	4.9	17.8	Upright	Vigorous
'Khawa'	April mid	Oct. mid	Rounded square	105	Yellow	5.3	19.2	Upright	Vigorous
'Khopasi'	April late	Oct. mid	Ovoid	115	Yellow	4.8	18.3	Upright	Vigorous
'Kirtipur'	April late	Oct. early	Ovoid	190	Orange yellow	5.1	19.5	Upright	Vigorous
'Nuwakot'	April mid	Oct. mid	Ovoid	125	Yellow	4.8	18.5	upright	vigorous

Table 9. Some useful *Diospyros* species for temperate areas and their distribution with chromosome number*.

Species	Distribution	Chromosome number	Utilization
<i>D. kaki</i> Thunb.	China, Japan, Korea and other countries of introduction	90,135	Fruit production, rootstocks
<i>D. lotus</i> L.	Europe and America	30	Fresh, rootstocks
<i>D. virginiana</i> L.	North America	60, 90	Fresh, rootstocks

*Giordani, 2001.

Table 10. Some recognize characteristics of *D. kaki* and *D. lotus*.

Description	<i>D. kaki</i>	<i>D. lotus</i>
Popularity	oldest rootstock, Asia	Europe and California
Root	produce tap/lateral roots	produce fibrous roots
Affinity	almost good for all	good for PCA/PVA type
Resistant	crown gall, oak root fungus	oak root fungus
Susceptible	<i>Verticillium</i> spp.	crown gall, <i>Verticillium</i> spp.
Hardness	hardy, uniform plants	low winter, drought resistant
Adoption	warm regions	cold regions
Yield	satisfactory	satisfactory

Table 11. Tree and bearing characteristics of persimmon cultivars grown in Japan*.

Cultivar	Tree vigor	Number of female flowers	Number of male flowers	Fruit drop at the early developmental stage of the fruit	Yield
Soshu	Medium	Many	None	Medium	Medium
Nishimarawase	Medium	Medium	Medium	Little	Medium
Tonewase	Vigorous	Many	None	Little	High
Izu	Not-vigorous	Many	None	Medium	Low
Saijo (early ripening)	Vigorous	Medium	None	Little	Medium
Maekawa-Jiro	Vigorous	Medium	None	Little	High
Hiratanenashi	Vigorous	Many	None	Little	High
Matsumotowase-Fuyu	Medium	Many	None	Little	High
Yoho	Vigorous	Many	None	Little	High
Taishu	Medium	Medium	Medium	Little	Medium
Fuyu	Vigorous	Many	None	Little	High
Jiro	Vigorous	Medium	None	Little	High
Atago	Vigorous	Many	None	Little	Very high

*Yamada, 2006.

Table 12. Leading varieties in acerage, recently released new PCNA cultivars with dried and fresh use in Japan*.

Leading varieties in acerage	New PCNA cultivars	Leading cultivars for dried purpose	Leading cultivars for fresh use
Fuyu	Taishu	Hachia	Fuyu
Hiratanenashi	Soshu	Ichidagaki	Jiro
Tonewase	Yoho	Hiratanenashi	Matsumotowase-Fuyu
Matsumotowase-Fuyu	Yubeni	Sanja	Maekawa-Jiro
Hachia	Shinshu	Hagakushi	Hiratanenashi
Nishimurawase	Kanshu		Tonewase
Jiro-early ripening	Kishu		
Jiro			
Saijo			
Atago			
Izu			
Saijo-early			

*Yamada, 2006.

Table 13. Fruit characteristics of persimmon cultivars grown in Japan*.

Cultivar	Astringent type	Fruit ripening time	Fruit weight (g)	Fruit Skin color	Quantity Of brown specks in flesh	Flesh firmness	Flesh texture	Juiciness	Soluble solids content (%)	Fruit cracking at calyx end	Fruit cracking at stylar end	Stained Fruit skin	Shelf life
Soshu	PCNA	Late Sept- Early Oct.	250	Red- orange	Few	Medium	Dence	Much	15	None	Very few	Little	Semi-short
Nishimarawase	PVNA PVA	Late Sept- Early Oct.	230	Red- orange	Many	Firm	Coarse	Medium	16	Few	None	Little	Long
Tonewase	(Seedless)	Early Oct.	230	Yellow- orange	None	Soft	Dence	Much	14	None	None	Very little	Medium
Izu	PCNA	Mid Nov.	240	Red- orange	Few	Medium	Dence	Medium	15	Medium	None	Many	Short
Saijo (early ripening)	PCA	Late Oct.	180	Yellow	None	Medium	Dence	Much	18	None	Very few	Many	Short
Maekawa-Jiro	PCNA PVA	Early Nov.	270	Red- orange	Few	Medium	Dence	Medium	16	None	Medium	Little	Medium
Hiratanenashi	(Seedless)	Early Nov.	240	Yellow- orange	None	Soft	Dence	Much	14	None	None	Very little	Medium
Matsumotowase-Fuyu	PCNA	Early Nov.	270	Red- orange	Medium	Medium	Medium	Much	16	Medium	None	Little	Semi-long
Yoho	PCNA	Early Nov.	280	Red- orange	Few	Firm	Dence	Medium	16	Medium	Few	Little	Semi-long
Taishu	PCNA	Early Nov.	400	Orange	Few	Soft	Coarse	Much	18	Few	Few	Medium	Medium
Fuyu	PCNA	Late Nov.	280	Red- orange	Medium	Medium	Medium	Much	16	Medium	None	Little	Long
Jiro	PCNA	Mid Nov.	280	Red- orange	Few	Medium	Dence	Medium	16	None	Many	Little	Medium
Atago	PCA	Early Dec.	270	Orange	None	Medium	Medium	Medium	15	Few	None	Little	Long

*Yamada, 2006.

Chapter 4

Morphological observation of the graft union among different combinations between scion and rootstocks

4.1. Introduction

Graft incompatibility occurs due to anatomical, physiological and genetic reasons (Hartmann et al., 1997). Moore (1983) suggested that graft incompatibility may be caused by lack of vascular redifferentiation and regeneration of phloem, or by cellular necrosis at the graft interface. These abnormalities result from anatomical mismatching between scion and rootstock (Yeoman, 1978), lack of lignification of cells interlocked at the graft union (Hartmann et al., 1997). The earliest methods used to detect graft incompatibility relied on the external symptoms such as graft union malformations, yellowing of foliage, decline in vegetative growth and vigor, marked differences in growth rate of scion and rootstock (Hartmann et al., 1997), or anatomical abnormalities after grafting. This method requires waiting until the symptoms are visible, which could take years. Additionally, early anatomical observations may not always correlate with long-term graft survival (Andrews and Marquez, 1993).

Propagation in persimmon has proved difficult as compared to other commercial fruit trees due to the problem of graft incompatibility. Fruit trees are usually formed by a combination of scion and rootstock. For that combination to be successful, a good union between a scion and rootstock is

necessary (Errea et al., 2001; Feucht, 1988). The grafted partners often belong to the same species or genus but the use of genetically divergent is also common and graft incompatibility frequently occurs (Errea et al., 2001). Different reasons may have an influence on graft success: inherent system of cellular incompatibility, formation of plasmodesmata, vascular tissue connections and the presence of growth regulators and peroxidases. Macromolecules (phloem proteins, RNA, hormones) that are present in the sap phloem might be also important during vascular differentiation in compatibility process (Pina and Errea, 2005). In incompatible grafts differentiation is not complete (Errea et al., 1994c). The presence of phenols was generally associated with small cells in incompatible combinations, which did not form successful unions (Errea et al., 2001). Auxin (IAA) is involved in the development of compatible unions and induces the differentiation of vascular tissues. The activity of IAA oxidase (Aloni, 1987) and transport of IAA transport (Stenlid, 1976) can be altered by naturally occurring substances such as phenols. *p*-Coumaric acid is an IAA antagonists or auxin-oxidase cofactors (Lockard and Schneider, 1981), but phloroglucinol is an IAA oxidase inhibitor (DeKlerk et al., 1999). Truetter and Feucht (1988) found an accumulation of pruning above the incompatible union of *Prunus avium* grafted to *Prunus cerasus*. From *p*-Coumaric acid pruning can be synthesized under stress conditions. Prunin enhances oxidative decarboxylation of IAA (Truetter and Feucht, 1988).

Comparative time, cultivar, methods and anatomical studies of persimmon cultivar 'Fuyu' and 'Hiratanenashi' as a scion on *D. kaki* and *D. lotus* as rootstocks, showed visible differences. Differences in species within the genera and between scion and rootstock sometimes cause abnormal

growth within the graft union (Hodgson, 1940; Suriyapananont et al., 1997). These workers reported some incompatibility problems between ‘Fuyu’ grafted onto *D. lotus* rootstock often grow slowly, bear fruit early and die within a few years.

The aim of the research was to determine the possible mechanisms that could be related with adhesive phenomenon of ‘Fuyu’ persimmon grafted onto *D. kaki* and *D. lotus* and to provide new information which will help to elucidate the possible mechanisms of graft compatibility phenomenon.

4.2. Materials and methods

The trees were planted in plastic containers at the orchard of the Agriculture and Forestry Research Center, University of Tsukuba (Fig.8). The buds and scions of cultivars ‘Fuyu’ and ‘Hiratanenashi’ were budded and grafted onto *D. kaki* and *D. lotus* in July, August, September of 2002 and March, April, May of 2003, respectively, in order to observe graft union development. The methods used in this experiment were chip and T-budding, and veneer and tongue grafting (Fig.9). The success of bud and graft union was performed using the following arbitrary index.

- 10 = Excellent: perfect union
- 7 = Good: graft union well formed but with callus tissue visible
- 5 = Fair: union imperfectly formed with callus visible
- 3 = Poor: deformed or swollen union and different diameters
between stock and scion
- 0 = Blight: dead scion due to incompatibility

The process of cutting, mounting and preparation of callus cross-sections were done following the procedure as described below. Samples of 10 graft unions from each rootstock/scion combination were taken and excised samples were fixed in FAA; formalin–acetic acid-ethanol (60%) in (5:5:90 v/v) solution, using vacuum infiltration to ensure complete absorption of the solution. After washing with water, the sections were cut from the fixed portions at 20 µm thickness using a sliding MICROM-GmbH, HM 330, and coated with glycerin before observation under the microscope. Visual evaluation was done at 1, 3, 5, 7, 9, months and one, two and half years and four after grafting as well as anatomical observation with LM (Olympus BH2-RFCA).

4.3. Results

Results revealed positive interaction of time, cultivars and grafting methods, in relation to graft compatibility. Results show that with respect to time, method and cultivar, late July to August was the ideal time for budding, chip budding was preferred over T-budding and ‘Fuyu’ budded on *D. kaki* showed the highest compatibility as compared to ‘Fuyu’ budded on *D. lotus* and Hiratanenashi with *D. lotus* respectively. Based on these findings, combination with *D. kaki* yielded the best results (Fig.10).

The results also confirm that, with respect to time, April was the best time for grafting. While with respect to method, veneer grafting was preferred over tongue grafting and with respect to cultivar, based on these findings, combination with *D. kaki* gave the best results (more than 80%

'take'). After the mid part of May, rootstocks start to release water thereby resulting in graft incompatibility reducing percent of graft success (Fig.11).

4.3.1. Observation of bud and graft union development

The cross cut images of *D. kaki* 'Fuyu', and 'Hondai' and *D. lotus* was shown in Fig.12. The tissues of *D. kaki* 'Fuyu' and 'Hondai' were whitish whereas *D. lotus* had yellow. The development of bud and graft union was studied as shown in Figs 13-34 herein after. Callus formation began within 7 days after budding/grafting. The tissues proliferated more from the rootstock than from the scion. In rootstocks, callus tissue proliferated more from cells in the area around the cortex, phloem and xylem tissue, cambium and pith. The area of callus proliferation in the scion was the same as that found in rootstock but notably slower in the latter.

4.3.1.1. One and three month after grafting

The response of the tissues to grafting is the development of callus cells, which occurred on the cut surfaces between rootstock and scion. The parenchymatous cells produced from cells at the cut end of ray xylem formed homogeneous masses, which were stained strongly. These masses originated from the edge of the cut portion. A necrotic layer was observed surrounding the callus. The xylem elements near the cut surfaces showed rusted cells and resin inside the vessels. The callus cells lying between the cut end of the cambium of scion and rootstock differentiated into cambium cells, bridging the remaining callus. The new cambium cells were aligned horizontally joining the cambium of stock and scion (Fig.13). Three months after grafting, observation of the transverse sections showed that the new generating and connective vascular cambium had a sinuous shape, the

elements of xylem had an orientation perpendicular to the axis, and larger vessels were observed in zone near to the union. The new xylem developed in the both combination zone showed necrotic lines and isolated pocket of necrotic cells (Fig.14).

The observation of development of the bud union and subsequent development of vascular tissues of the bud combinations; 'Fuyu' budded onto both *D. kaki* and *D. lotus*, and 'Hiratanenashi' budded onto *D. lotus*. These combinations were compared in order to observed compatibility between them through visual observation of the outer structure in the inter-graft zone (Fig. 15).

4.3.1.2. Seven and nine month after grafting

The new xylem showed presence of larger vessels than original vessels in the xylem of the rootstock. Based on these observations of graft union it may be concluded that *D. kaki* and *D. lotus* revealed similar development of callus and cambial cells. The initial stages of tissue development appeared normal, vascular cambium from the callus formed between the stock and scion to unite the two components. Based on these observations of graft union it may be concluded that *D. kaki* and *D. lotus* revealed similar development of callus and cambial cells. The initial stage of tissue development appeared normal, vascular cambium from the callus formed between stock and scion to unite the two components (Figs.16-18).

4.3.1.3. One to three-years after grafting

Observation of one to two and half year old unions revealed that *D. lotus* rootstocks showed necrotic points at the xylem in the cutting zone of the graft union. This symptom was consistently observed through the samples of these rootstocks compared with the *D. kaki*. The cambium connection between rootstock and scion in both graft combination after two and half year was completed (Figs. 19-24).

The formation of new xylem on the portions of scion was lower in proportion to the formation of new xylem on the side of the rootstock. Although it was observed in both rootstocks, in *D. kaki* rootstocks this decreased with age, showing concentric growth rings around rootstock and scion. Vessels in swirling shape were observed in the callus in the zone of union with scion, the vessels being larger than those of rootstock. A necrotic cell layer was also observed between the rootstock and scion. (Figs.19, 23).

The visual observation between one and half to two and half year after veneer grafted 'Fuyu'/*D. kaki* and 'Fuyu'/*D. lotus* plants had the remarkable differences observed in the graft union zone. The poor performance of 'Fuyu'/*D. lotus* combination (especially in Fig. 24, B) may be caused by partial blockage at the graft union or reduction in movement of water or nutrient materials or both through the graft union ultimately, malfunctioning of the graft union which adversely affected assimilate translocation (Figs. 20, 21, 22, 24 and 25).

4.3.1.4. Four to five-years after grafting

In four to five-year-old graft union a systematic presence of necrotic points at the xylem was observed in transverse/longitudinal section of both the combination but the amount was higher in 'Fuyu'/*D. lotus* combination. The symptoms was observed both above and below the cut zone of graft union (Figs. 26, 27). The outer and inner cut sections observation shows the differences between to them regarding necrotic and abnormal growth patterns. This could indicate that some substances from the rootstock or accumulated substance in this zone produced this abnormal characteristics (Figs. 28, 29, 30). The different sizes and lower number of callus and developing new vessels were observed in longitudinal cut sections of *D. lotus*, the xylem elements originated from the callus were oriented horizontally (Fig. 31).

Xylem is a complex tissue, composed of the conducting or tracheary elements, fibres and parenchyma. Xylem fibres are elongated elements with pointed ends and are thought to have evolved from tracheids. They usually have thicker walls and the pits may have smaller borders than the tracheids of the same species (Esau, 1964). Tracheids originate from single cells, are normally elongated and pointed at both ends, and are imperforate, i.e. the primary wall is always continuous, pit-closing membranes being present in the region of pits.

The tracheids of above/below the graft union zone of *D. kaki* and *D. lotus* were presented in Figs. 32 and 34. Tracheids are present in all groups of vascular plants. By contrast, vessel membranes are perforate elements aggregated longitudinally into files of cells connected through the pores or

perforations. These chains of cells are vessels, and may vary from two cells to an indefinite but considerable length, perhaps several feet. Vessels of the primary and secondary xylem are thus formed from a longitudinal file of procambial or cambial cells respectively. In the case of *D. lotus*, the xylem elements orientation lost a normal pattern with respect to old xylem and vessels of different sizes were observed. The shape and size of vessels were also varied to each other (Fig. 33). Comparing the distribution of developed vessel and tracheid elements between the different rootstocks, more or less similar pattern was observed in this study.

In general, the developmental steps can be summarized as follows.

1. **1st step:** (pre callus stage) - discolored and production of callus tissue by both graft components (stock and scion) in the cambium region.
2. **2nd step:** (callus formation stage) - intermingling and interlocking of parenchyma cells
3. **3rd step:** (formation of cambium bridge stage) - cambium layer extended and differentiation of certain parenchyma cells of the callus into new cambium cells connecting with the original cambium in the stock and scion.
4. **4th step:** (formation of vascular tissue stage) - production of new vascular tissues by the new cambium, permitting passage of nutrients and water between the stock and scion.

4.4. Discussion

Comparative formation of the graft union was studied on the basis of effect of time, cultivar and grafting method of persimmon cultivars ‘Fuyu’ and ‘Hiratanenashi’ as scion and *Diospyros kaki* and *Diospyros lotus* as rootstocks showed visible differences between them. Based on the results, late July to August was the ideal time to perform budding operations while the best method to use was chip budding. Fuyu budded on *D. kaki* showed the highest compatibility in contrast to other combinations tested. It was appeared that the best time was April for grafting using veneer (shoot) grafting. Fuyu grafted on *D. kaki* showed highest compatibility than others.

The tissue development in heterograft combination showed a defective union, characterized by the presence of necrotic cells, perhaps due to callus unresponsiveness to the stimulus for differentiation. The callus cells showed an irregular appearance and the cell walls seemed to be thinner than the callus cells on the homograft union. Other main differences at the early stages were the presence of parenchymatous tissues without lignin coexisting with vascular tissues and necrotic cells in heterograft combination. At the later stages in Fuyu/*D. lotus* combinations, adhesive phenomenon is reduced and the tree weakens eventually (Fig. 24).

In the case of homograft combinations, initial response to grafting was the development of callus cells along the cut surfaces between the rootstock and scion. The xylem elements near the cut surfaces were discolored and the vessels were filled with gum; also necrotic tissues were observed in the old xylem of the scion. After three months, new generating and connective

tissues were observed in the transverse sections, and the new xylem elements had a perpendicular orientation with respect to the stem axis. After seven months, the longitudinal sections across the graft union revealed necrotic cells in the callus at the base of the scion.

Once the two components of the graft, stock and scion, are in intimate contact, the cambial region capable of meristematic activity produce parenchymatic cells which soon intermingle and interlock, producing the callus tissue that fills the space between the two components connecting the scion and the rootstock (Hartman et al., 1990; Errea et al., 1994; Wang and Kolimann, 1996). Several researchers consider this step essential for the development of future vascular connections and it has been suggested that the primary recognition events occur at the point of cell-to-cell contact (Yeoman et al., 1978). The basis of this recognition system would be protein molecules released from the plasmalemma that move into and across the wall from opposite cells, forming a complex with catalytic activity resulting in the formation of a successful graft. When this complex is not formed, due to differences between the cells in contact, a special kind of protein called lectins, produce a mutual rejection of the opposing graft cells leading to the formation of an incompatible graft (Yeoman and Brown, 1976).

The cambium connection performed at least in one of both cut edge, showed the variation along the graft union. Ermel et al. (1997), did histocytological study of pear-quince grafts at few days after grafting and found that neocambial junction was delayed. It would be the first structural symptoms of incompatibility response. The relative position of scion and

stock strongly influenced the cambial union. The adjustment of scion and stock is suggested (Esau, 1965; Errea et al., 1994) as the cambial formation starts from the cut edges of stocks and scion, the misalignment or dysfunction could delay the function up to three months as conifers (Santamour, 1989). It seemingly the 'Fuyu'/*D. lotus* was affected by the irregular position of the scions more than the 'Fuyu'/*D. kaki* providing a defective union. Thus, it is important to find a most appropriate method of graft that improves the alignment and adhesion between scion and rootstock. The proportion of new xylem and size of vessels produced in the 'Fuyu'/*D. lotus* combination was lower than 'Fuyu'/*D. kaki* and also, the existence of un lignified parenchymatous cells would provide a weaker union in 'Fuyu'/*D. lotus* graft union. These results are coincident with the finding by Errea et al. (1994), who established the similarities and differences on the graft union of *Prunus* (apricot).

According to Simons and Chu (1985), the orientation of the newly formed vascular tissues between the rootstock and scion is necessary for a successful union or this would affect the growth of scion. New vascular elements require 2-3 years of growth to assume a normal longitudinal orientation. Tissues malfunctioned and necrosis were apparent in M-26 apple rootstock, concluding that if necrosis persists and becomes accentuated, vascular continuity would be impaired. A number of factors can influence the healing of graft unions, such as temperature, moisture and status and availability of oxygen during and following grafting.

Temperature has a pronounced effect on the production of callus tissue. In apple grafts little, if any, callus is formed below 0°C or above

about 40°C. Even around 4°C, callus development is slow and merger, and at 32°C and higher, callus production is retarded, with cell injury becoming more apparent as the temperature increases, until death of the cell occurs at 40°C. Between 4° and 32°C, however, the rate of callus formation increases directly with the temperature (Shippy, 1930). Unless a completed graft union is kept by some means at a very high humidity level, the chances of successful healing are rather remote. With most plants, through waxing of the graft union, which retains the natural moisture of the tissue, is all that is necessary. Damp peat moss or wood shavings are good media for callusing, providing adequate moisture and aeration. It has been shown that oxygen is necessary at the at the graft union for the production of callus tissue (Shippy, 1930).

It is well known that auxin is involved in the vascular development, resulting in limiting and controlling both phloem and xylem differentiation (Aloni, 1989). The abnormal xylem differentiation observed here could be related to auxin level since this symptom was also observed in 'Fuyu'/*D. lotus* combination. Other factors as a physical barrier or toxins would be affecting the vascular differentiation and the growth of scion.

The necrosis which was found in the interphase between stock and scion could act as either physical barrier producing resin in the vessels and impeding a normal flux of hormones and nutrients. The polar transport of hormones is impeded by harmful metabolites such as cyanides or phenols (Rubery, 1989). It has been proposed that bark of different rootstocks may influence the quantities of auxin passing down to the roots in such a way as

to produce different effects on roots development and cytokinin synthesis (Jones, 1984).

On the other hand, cyanide, a harmful compound, has been related to some specific graft combinations. Pear/quince (Gur et al., 1968) and peach almond (Gur and Blum, 1973) combinations showed incompatibility which was supposed to be caused by cyanide compounds released to the medium after tissue disruption for the hydrolysis of cyanogenic compound (prunasin). Cyanogenic plants are able to release cyanide by the hydrolysis of cyanogenic glucoside. The most highly cyanogenic plant tissues are cherries, peaches and plums. These plants have mechanism of detoxifying the effects of cyanide thus CN is not the end product of metabolism but an active turnover takes place.

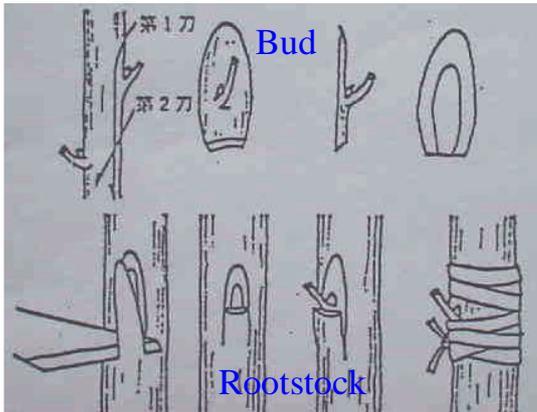
4.5. Conclusions

The result presented in this study show that with respect to timing of grafting and budding operations, late July to August was the ideal time to perform budding with chip budding as the recommended method. Fuyu budded onto *D. kaki* showed the highest compatibility in contrast to other combinations tested. Likewise, April was the ideal time to perform grafting using veneer grafting, which was a more superior compared to tongue grafting. Fuyu grafted on *D. kaki* showed highest compatibility than others. The callus cells showed an irregular appearance and the cell walls seem to be thinner than the callus cells on the homograft union. At the later stages, adhesive phenomenon was reduced and the tree weakened in Fuyu grafted onto *D. lotus* combinations.

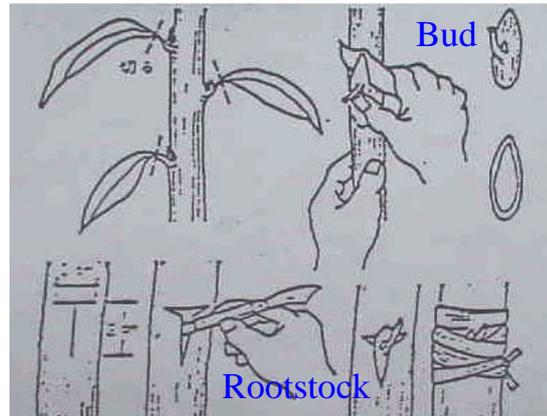
In my idea, the resulted April was the ideal time to perform grafting is the cambium cells are active for callus proliferation which is essential for a successful graft union occurs most readily at the time of year just before and during ‘Bud break’ in the spring, diminishing through the summer and into fall. The veneer grafting method was superior is due do the whole portion of cambial regions of rootstock and scion are brought together as compare to tongue grafting where it is difficult to perform grafting operations. The higher rate of physiological and anatomical results shows the ‘Fuyu’ *D. kaki* combination had the higher and faster rate of healing process appeared which resulted the production of new vascular tissues by the new cambium, permitting passage of nutrients and water between rootstock and scion as compare to the ‘Fuyu’ *D. lotus* combinations.



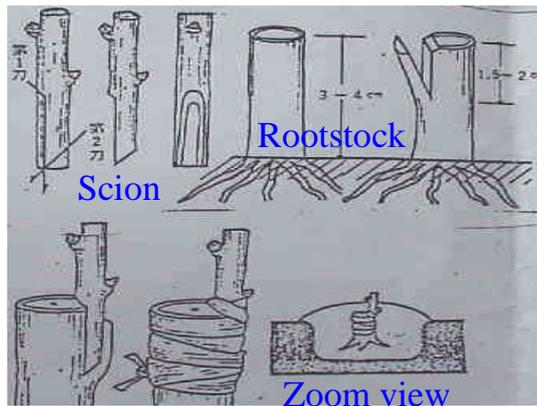
Fig. 8. Buds for budding (A), scions for grafting (B) with *D. kaki* (C) and *D. lotus* (D) rootstocks before budding and grafting respectively.



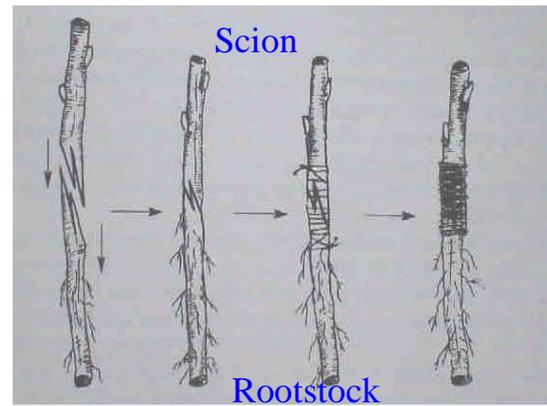
Chip budding



T-budding



Veneer grafting



Tongue grafting

Fig.9. Used methods and steps in making the budding and grafting. These methods were widely used and quite successful if the scions were match properly to the rootstocks. (Source: National Institute of Fruit Tree Science (NIFTS), Tsukuba, Japan.

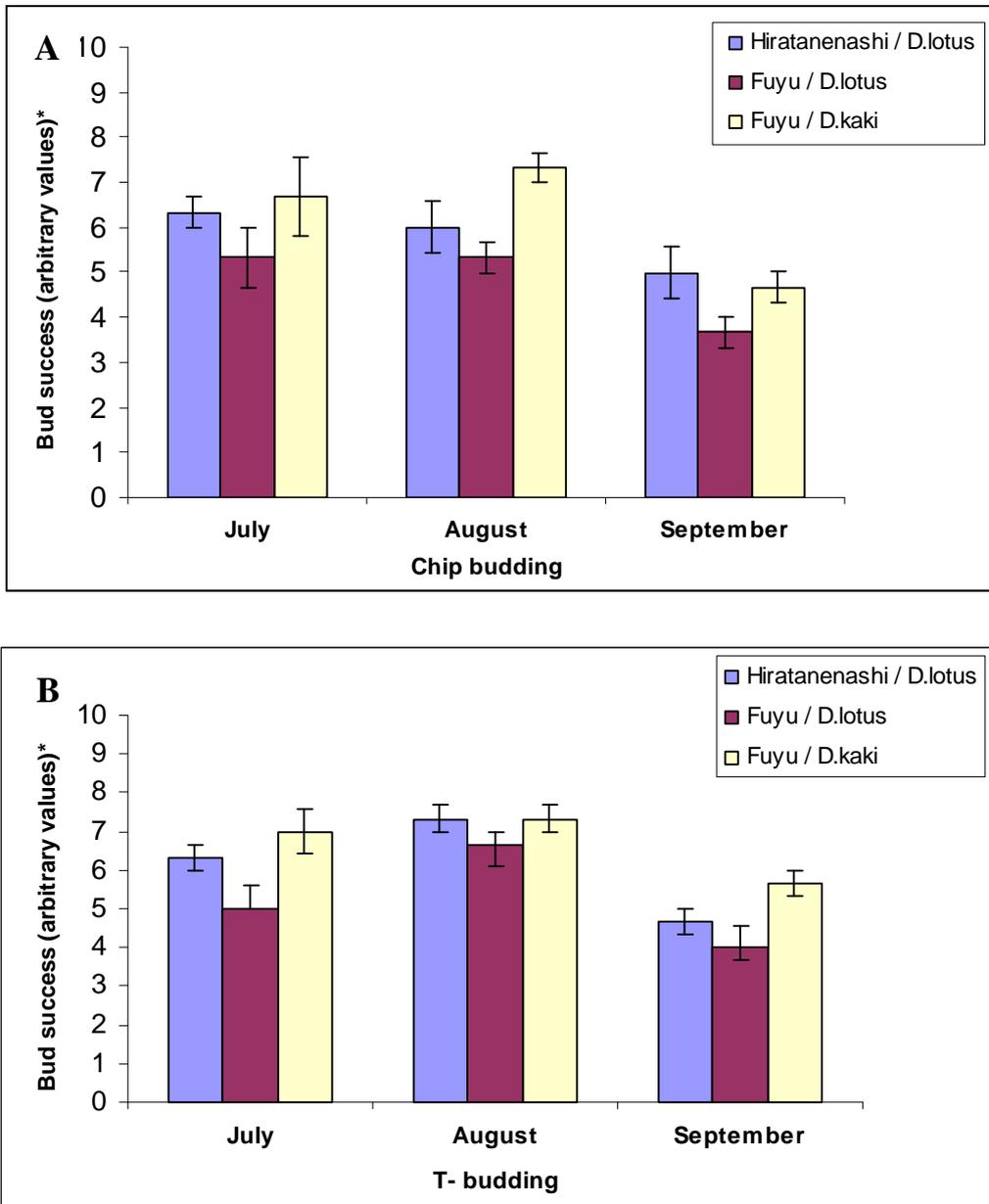


Fig. 10. Comparison of compatibility in various chip-budded (A) and T-budded (B) bud/rootstock combinations. Vertical bars denote SE. *Arbitrary values represent 10 = excellent ; 7 = good; 5 = fair; 3 = poor; 0 = dead.

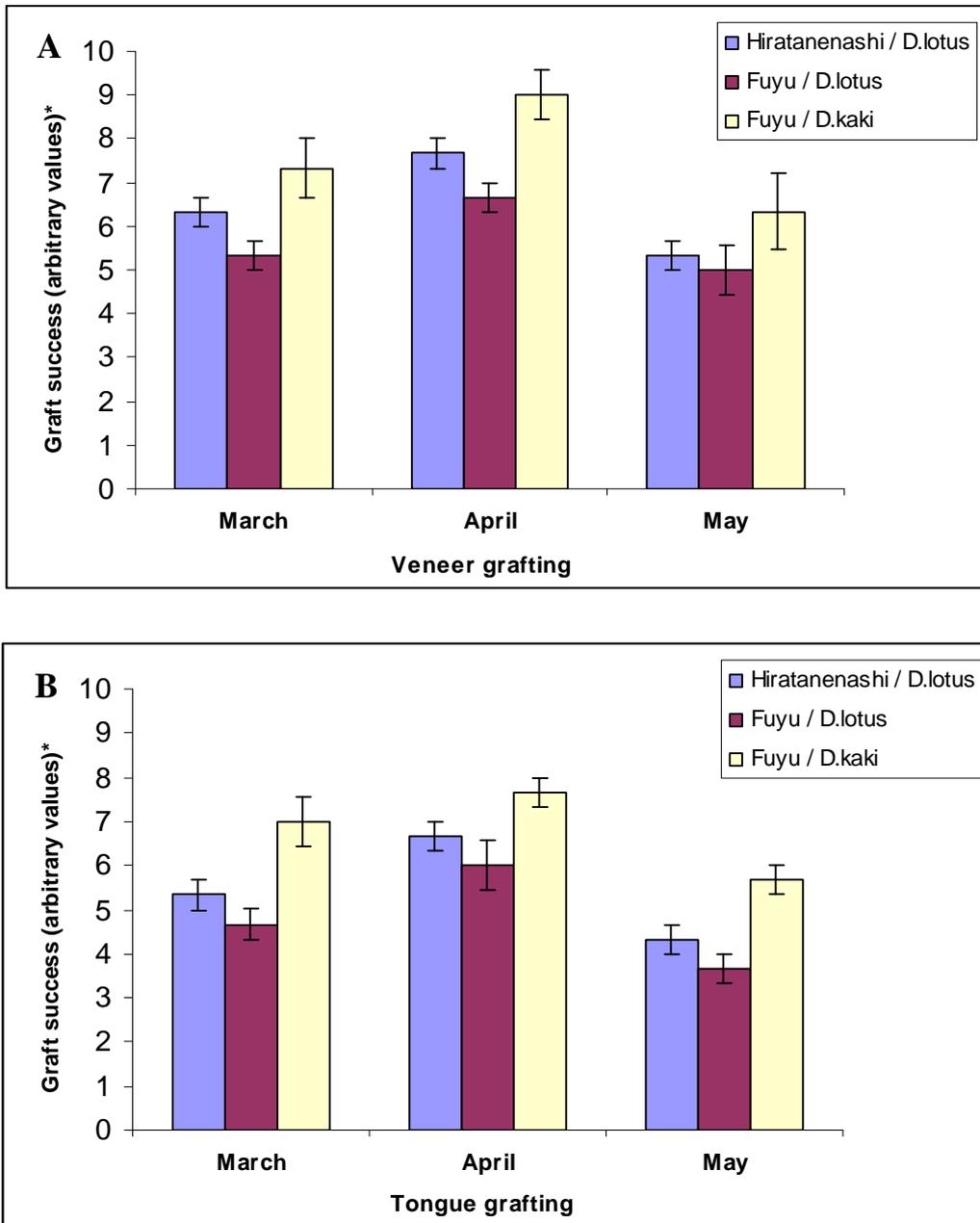


Fig. 11. Comparison of compatibility in various veneer-grafted (A) and tongue-grafted (B) scion/rootstock combinations. Vertical bars denote SE. * Arbitrary values represent: 10 = excellent; 7 = good; 5 = fair; 3 = poor; 0 = dead.

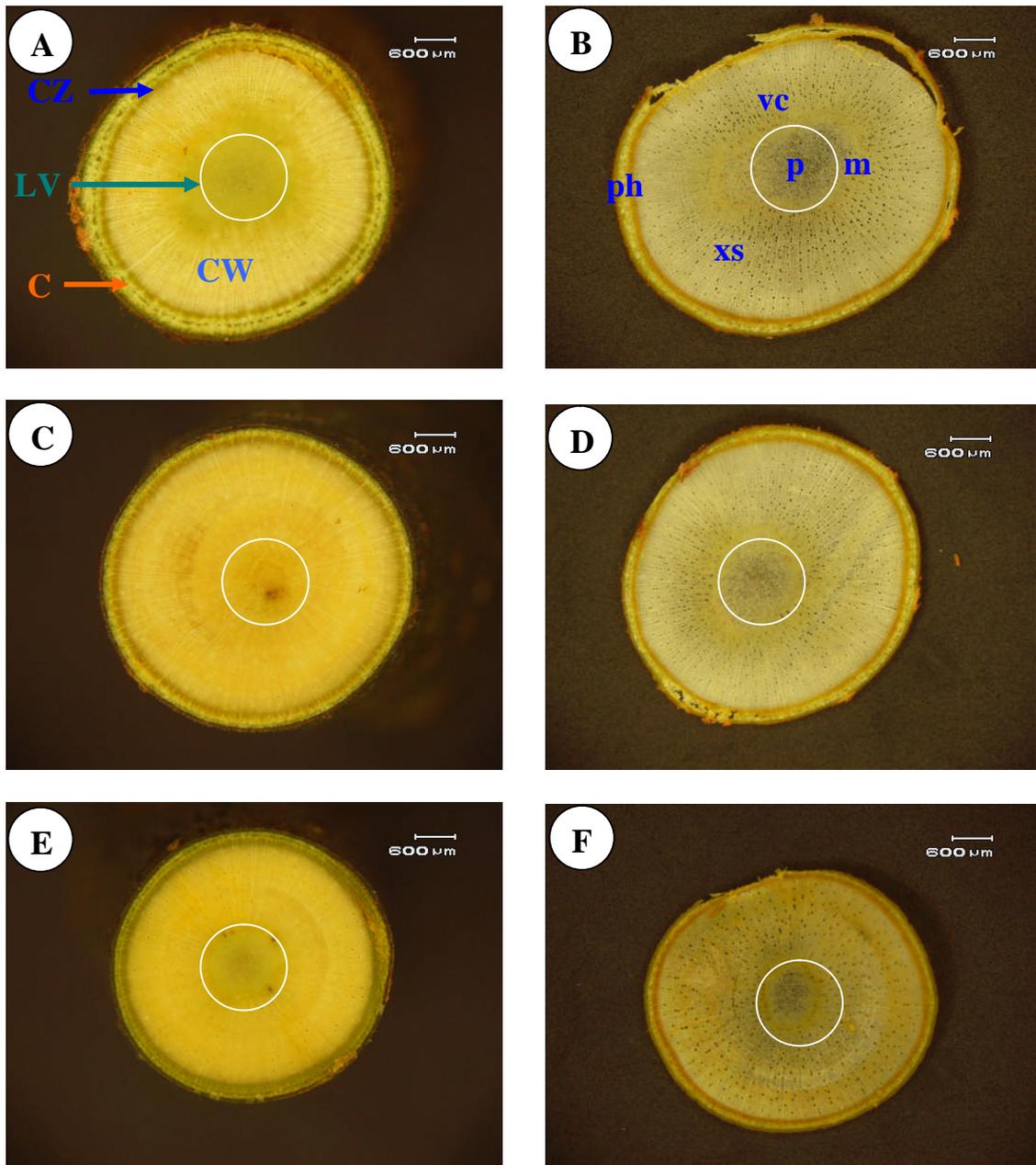


Fig. 12. Cross cut images of *D. kaki* 'Fuyu' (A, B.); *D. kaki* 'Hondai' (C, D.) and *D. lotus* 'Mamedai' (E, F.). CW: compression wood; C: cortex; LV: large vessels; CZ: cambial zone; PH: phloem; XS: xylem; VC: vascular cylinder; P: pith and M: medulla.

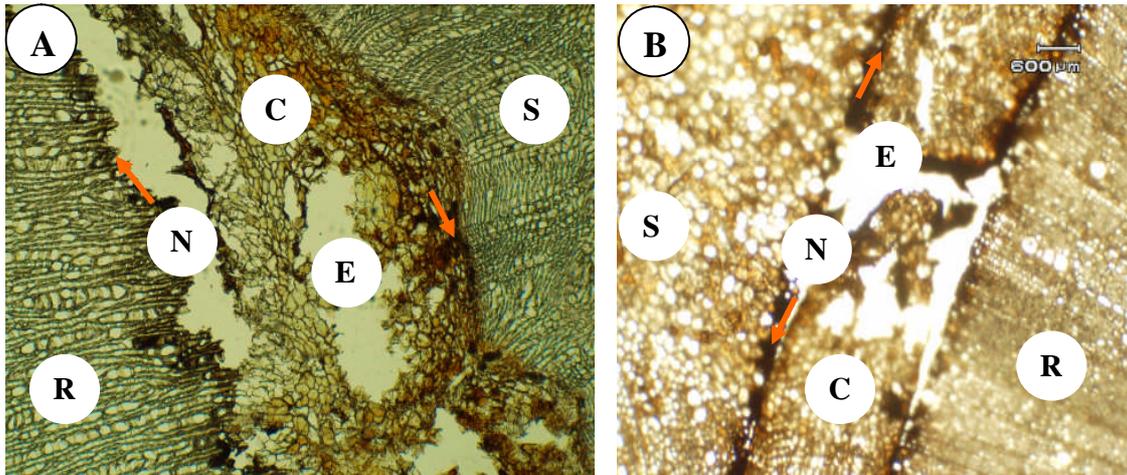


Fig. 13. Transverse section of veneer grafted 'Fuyu'/*D. kaki* (A) and 'Fuyu'/*D. lotus* (B) one and two months after veneer grafting respectively. S: scion; R: rootstock; C: callus; E: empty N: necrosis; Orange arrows: pointed to necrotic region.

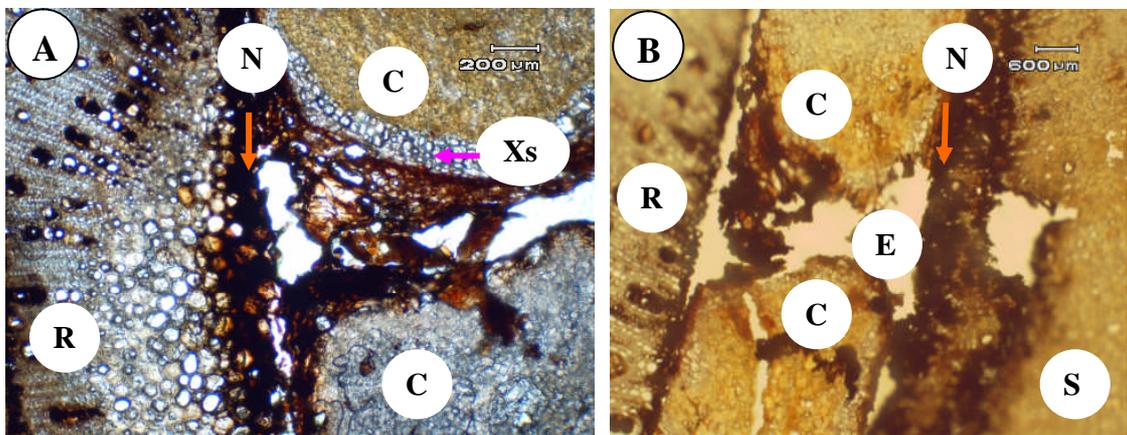


Fig. 14. Transverse section of three months after tongue grafted 'Fuyu'/*D. kaki* (A) and 'Fuyu'/*D. lotus* (B). R: rootstock; S: scion; C: callus; E: empty; Xs: secondary xylem; N: necrosis; Orange arrows: pointed to necrotic region.



Fig. 15. Field comparison between chip budded 'Fuyu'/*D. kaki* (A), 'Fuyu'/*D. lotus* (B), 'Hiratanenashi'/*D. lotus* (C) and T-budded 'Fuyu'/*D. kaki* (D). Circle: budded area.

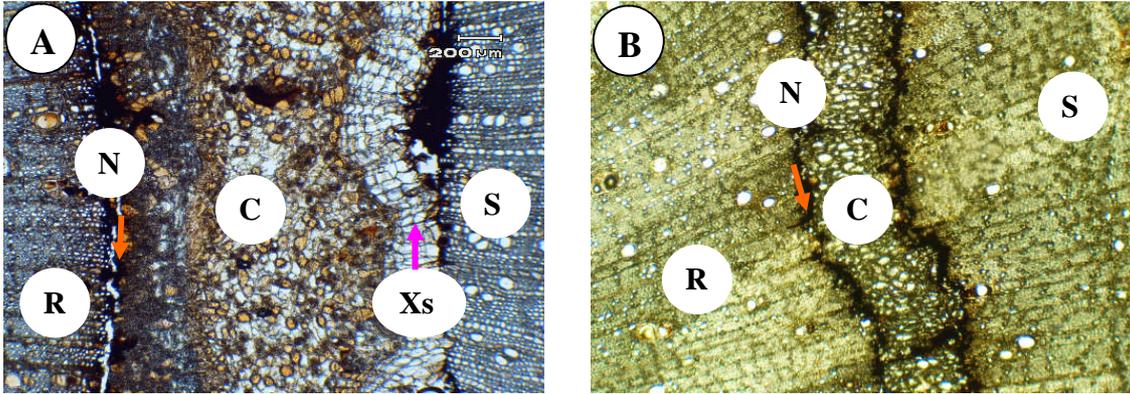


Fig. 16. Transverse section of 'Fuyu' with *D. kaki* seven months after chip budding (A) and veneer grafting (B). R: rootstock; S: scion; C: callus; Xs: secondary xylem; N: necrosis; Orange arrows: pointed to necrotic region.

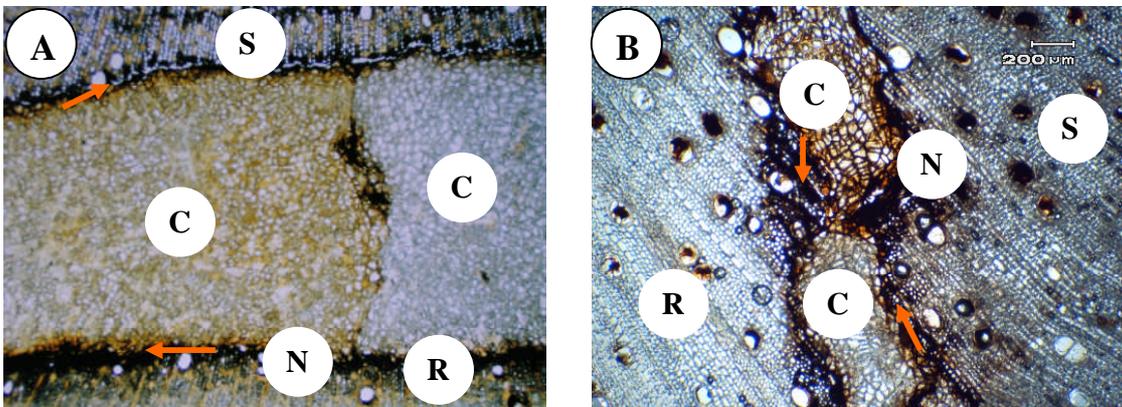


Fig. 17. Transverse section of seven months after veneer grafted 'Fuyu'/*D. kaki* (A) and 'Fuyu'/*D. lotus* (B). R: rootstock; S: scion; C: callus; Xs: secondary xylem; N: necrosis; Arrows: pointed to necrotic region.

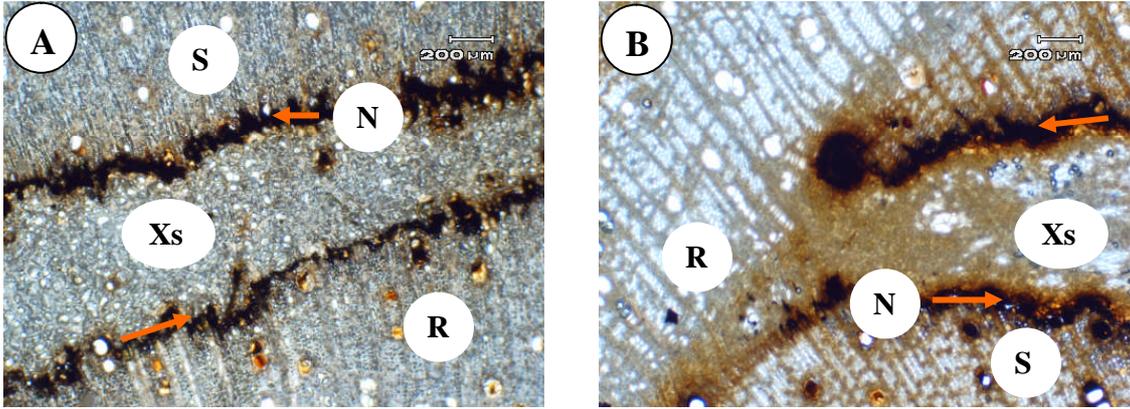


Fig. 18. Transverse section of nine months after veneer and tongue grafted 'Fuyu'/*D. kaki* (A) and 'Fuyu'/*D. lotus* (B), respectively.

R: rootstock; S: scion; C: callus; Xs: secondary xylem; N: necrosis; Arrows: pointed to necrotic region.

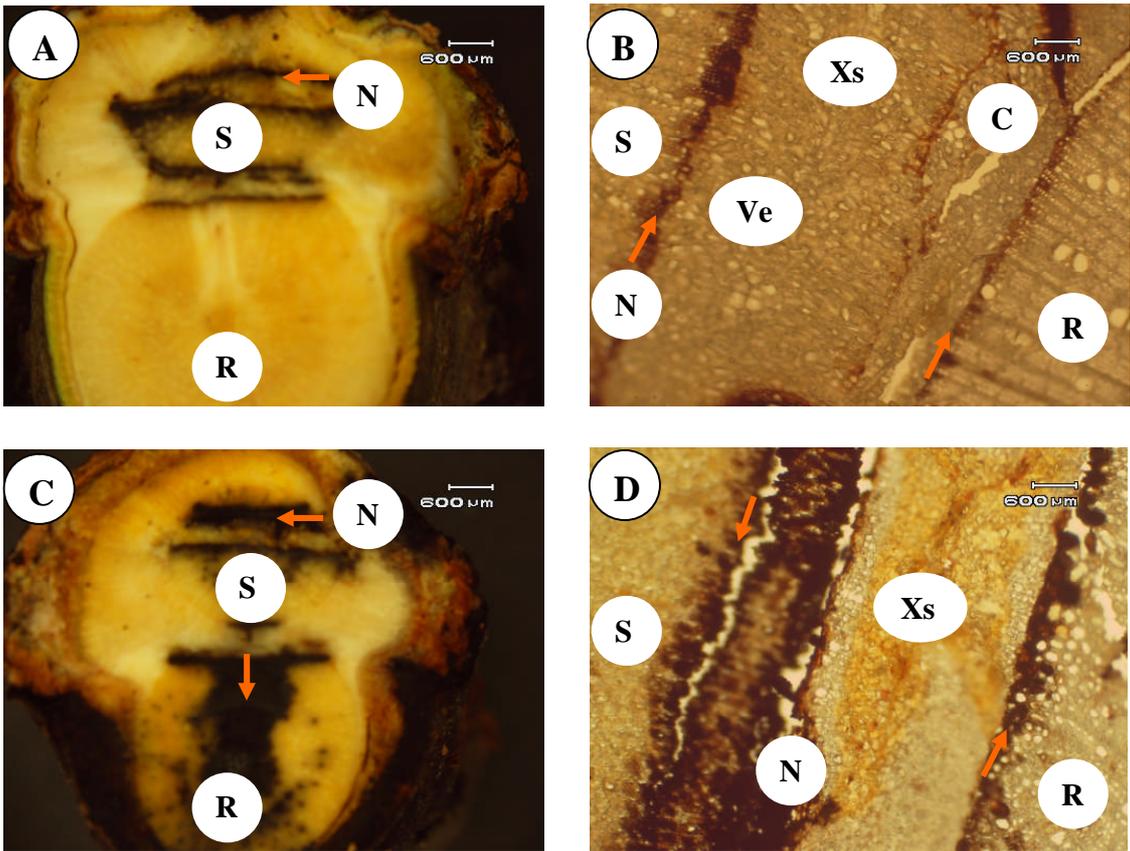


Fig. 19. Transverse section of one-year after veneer grafted 'Fuyu'/*D. kaki* (A, B), and 'Fuyu'/*D. lotus* (C, D).

R: rootstock; S: scion; C: callus; Xs: secondary xylem; Ve: vessels; N: necrosis.

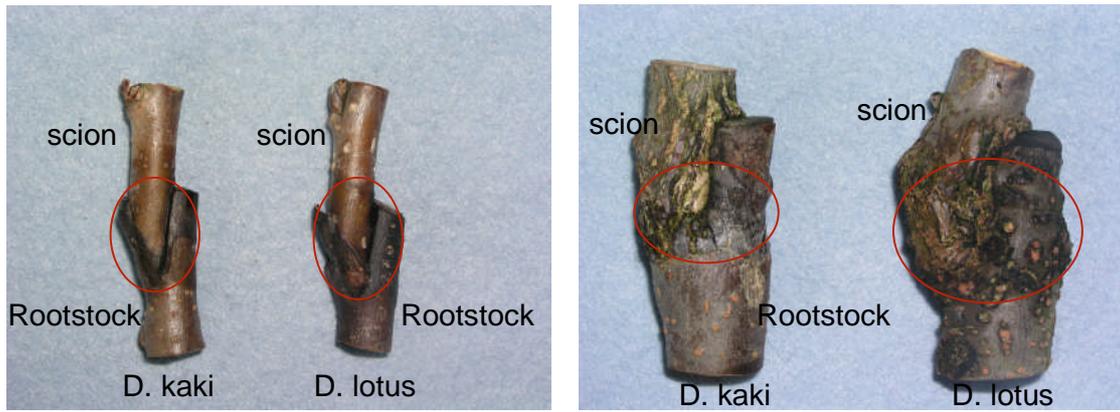


Fig. 20. Visual differences of veneer grafted one-year-old (A), and two-year-old (B), 'Fuyu'/*D. kaki*/*D. lotus* combinations. Circle: grafted area.

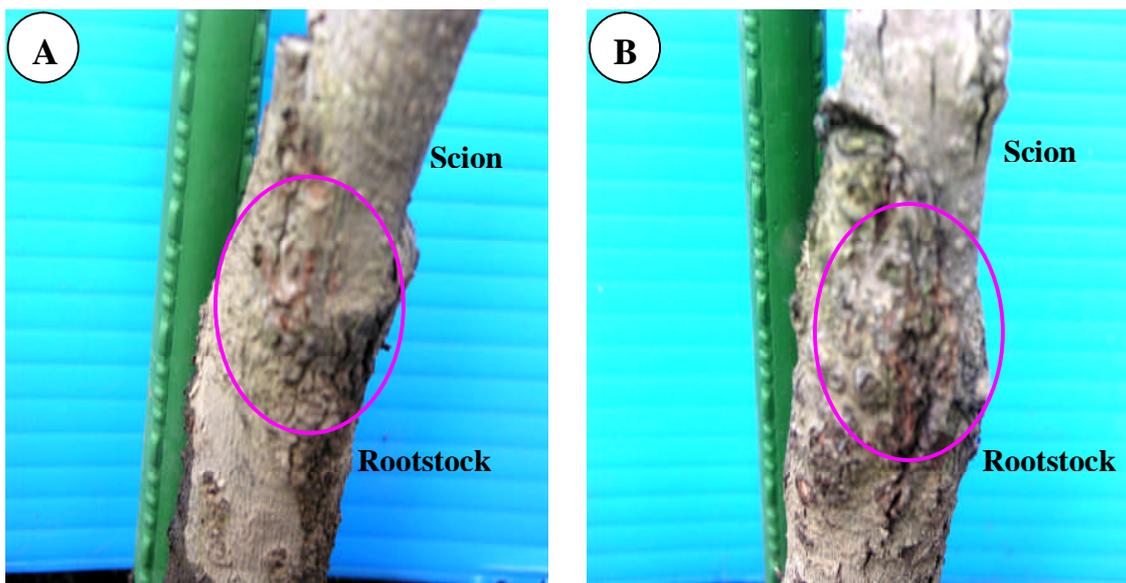


Fig. 21. Visual differences of one and half years after veneer grafted 'Fuyu'/*D. kaki* (A), and 'Fuyu'/*D. lotus* (B). Circle: grafted area.

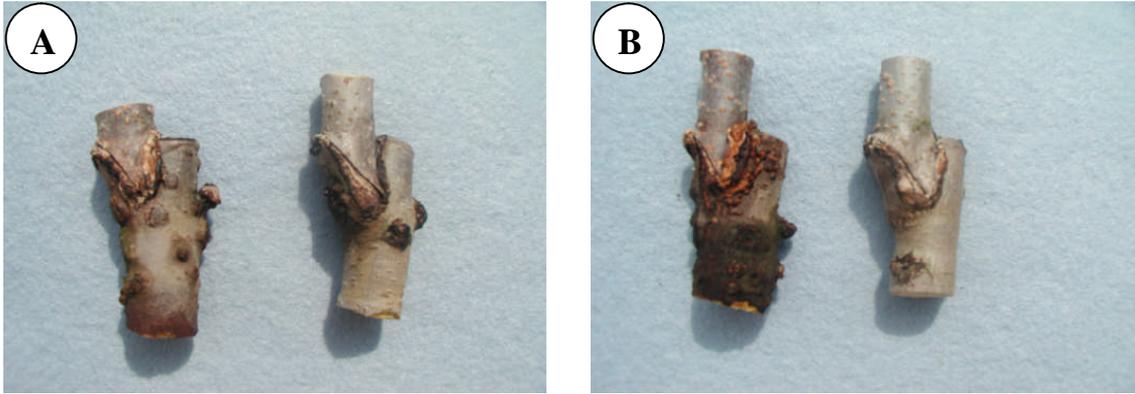


Fig. 22. Visual differences of two years after veneer grafted 'Fuyu'/*D. kaki* (A), and 'Fuyu'/*D. lotus* (B).

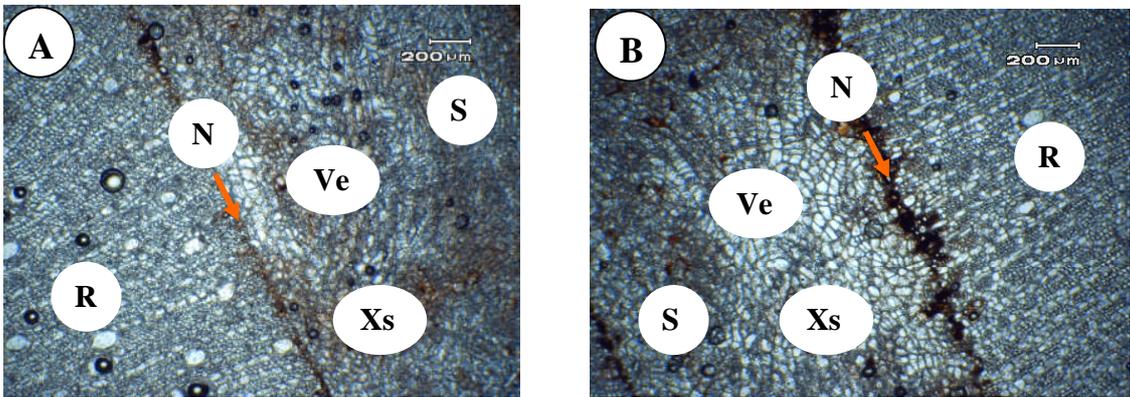


Fig. 23. Transverse section of two and half years after veneer grafted 'Fuyu'/*D. kaki* (A), and 'Fuyu'/*D. lotus* (B). R: rootstock; S: scion; Xs: secondary xylem; N: necrosis; Arrows: pointed to necrotic region.

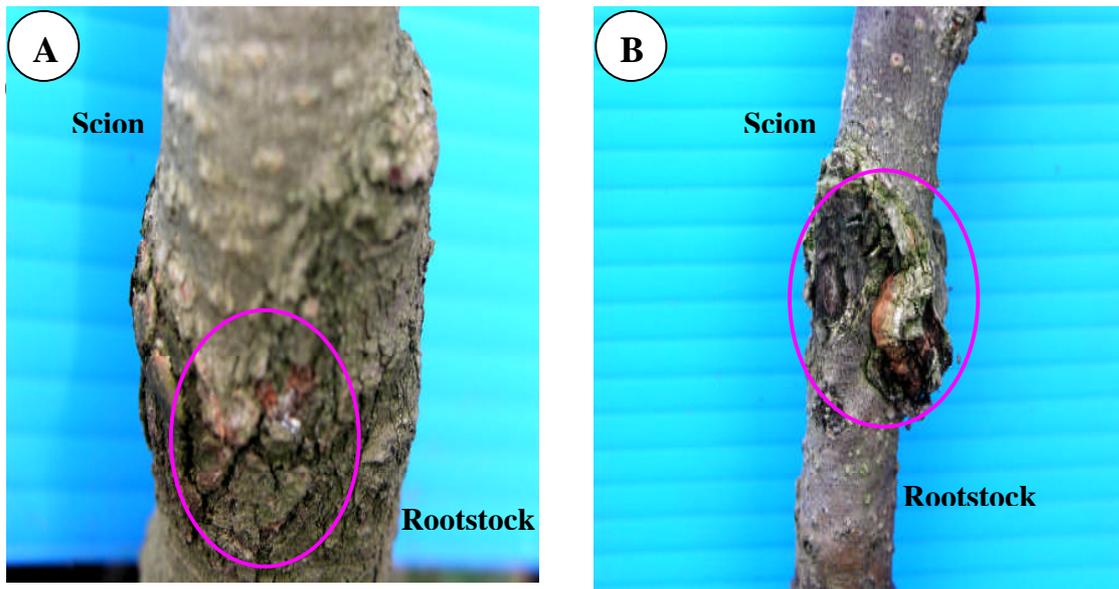


Fig. 24. Visual differences of two and half years after veneer grafted 'Fuyu'/*D. kaki* (A), and 'Fuyu'/*D. lotus* (B). Circle: grafted area.

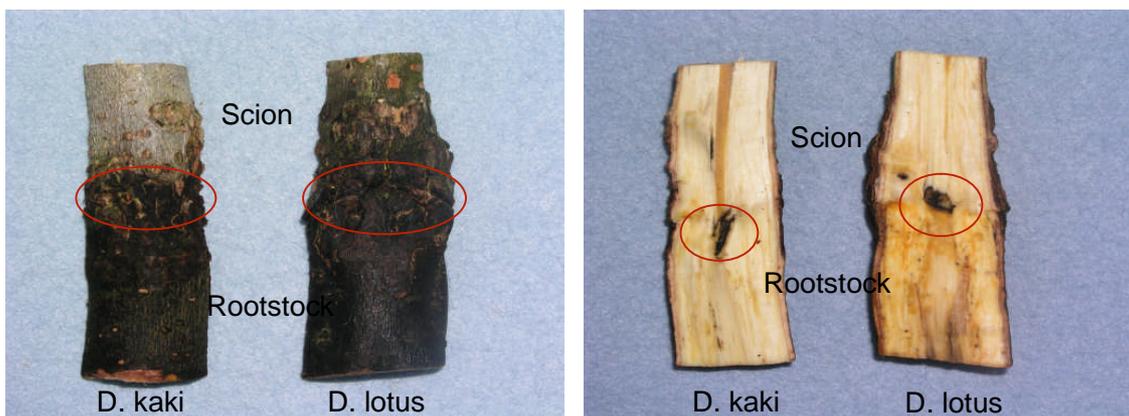


Fig. 25. Visual differences of veneer grafted three-year-old 'Fuyu'/*D. kaki*/*D. lotus* combinations. Circle: grafted area with necrotic spots.

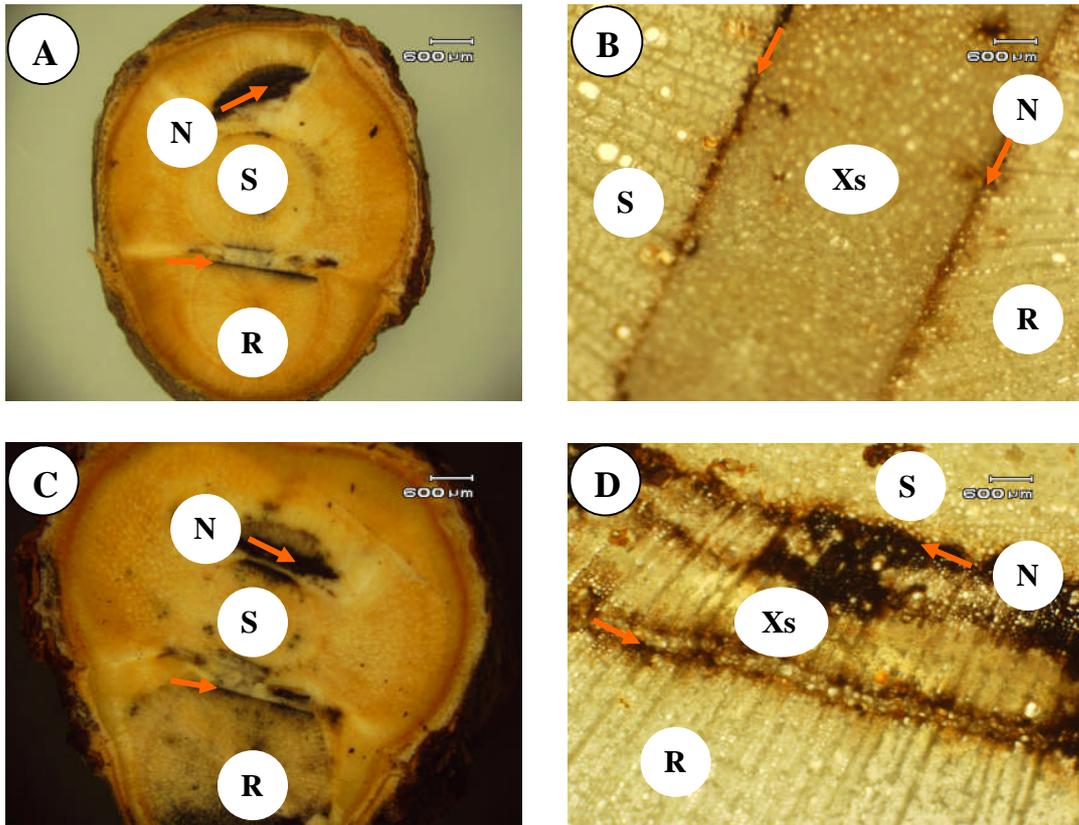


Fig. 26. Transverse section of four years after veneer grafted 'Fuyu'/*D. kaki* (A, B), and 'Fuyu'/*D. lotus* (C, D). R: rootstock; S: scion; Xs: secondary xylem; N: necrosis; Arrows: pointed to necrotic region.

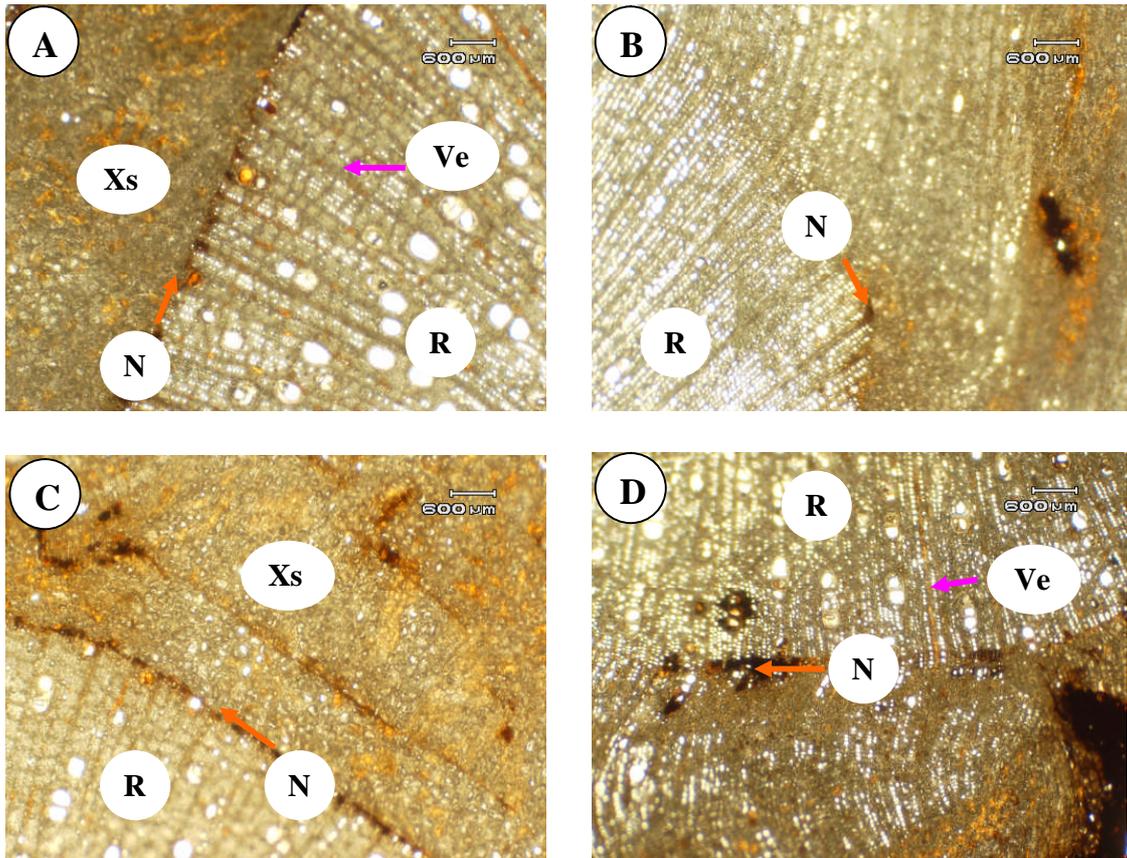


Fig. 27. Transverse section of four years after veneer grafted 'Fuyu'/*D. kaki* (A, B), and 'Fuyu'/*D. lotus* (C, D). R: rootstock; Xs: secondary xylem; Ve: vessels; N: necrosis; Orange arrows; pointed to necrotic region.

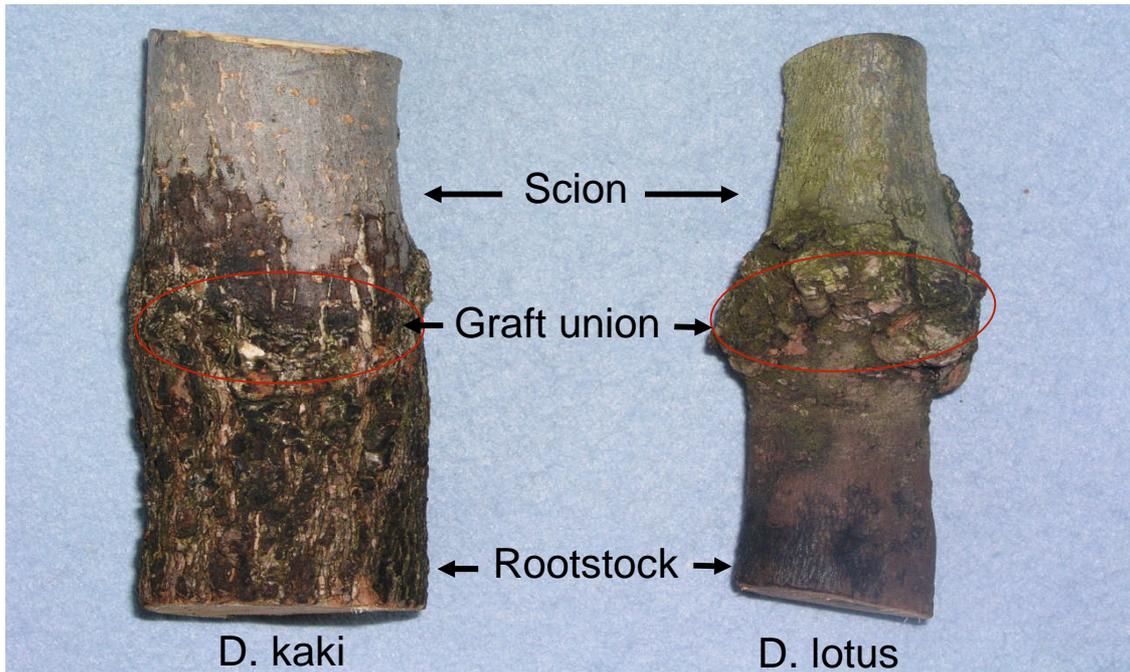


Fig. 28. Visual differences on outer section of veneer grafted five-year-old 'Fuyu'/*D. kaki*/*D. lotus* combinations. Circle: grafted area with necrotic spots.

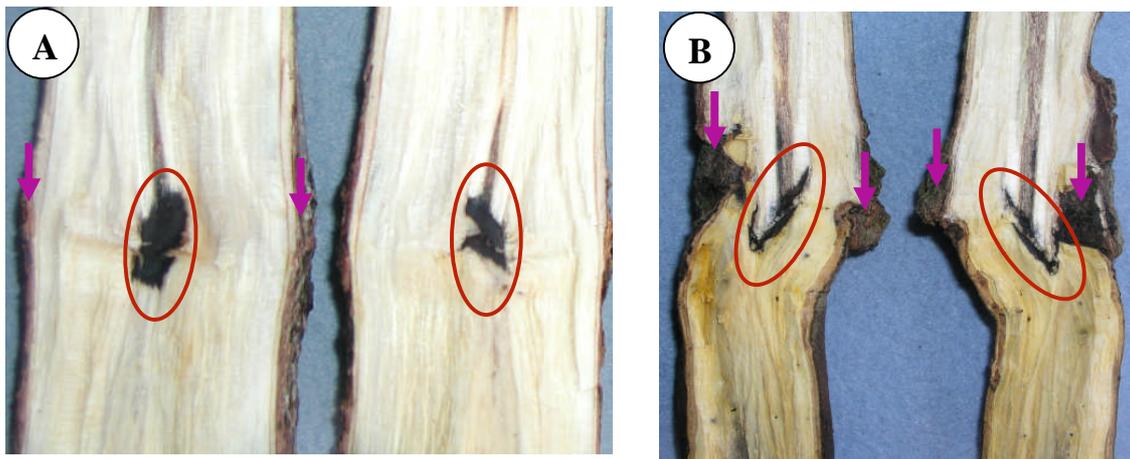


Fig. 29. Visual differences on inner section of veneer grafted five-year-old 'Fuyu'/*D. kaki* (A) and 'Fuyu'/*D. lotus* (B) combinations. Circle: grafted area with necrotic spots, Arrows: over growth areas.

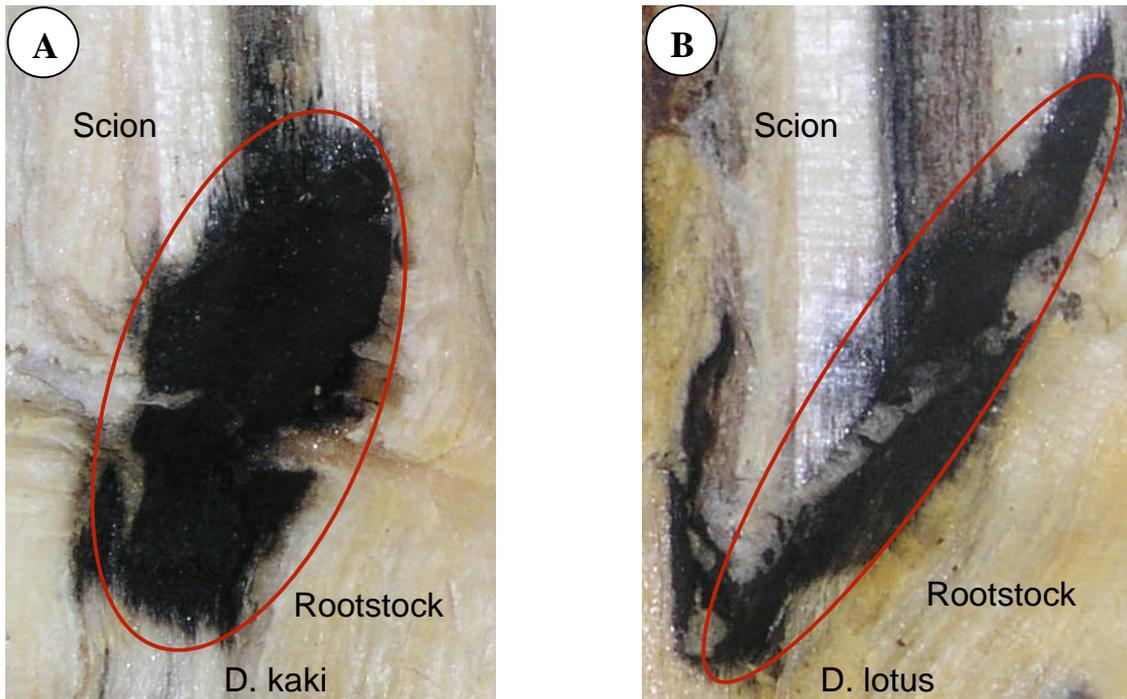


Fig. 30. Visual differences on inner section of veneer grafted five-year-old 'Fuyu'/*D. kaki* (A) and 'Fuyu'/*D. lotus* (B) combinations. Circle: grafted area with necrotic spots.

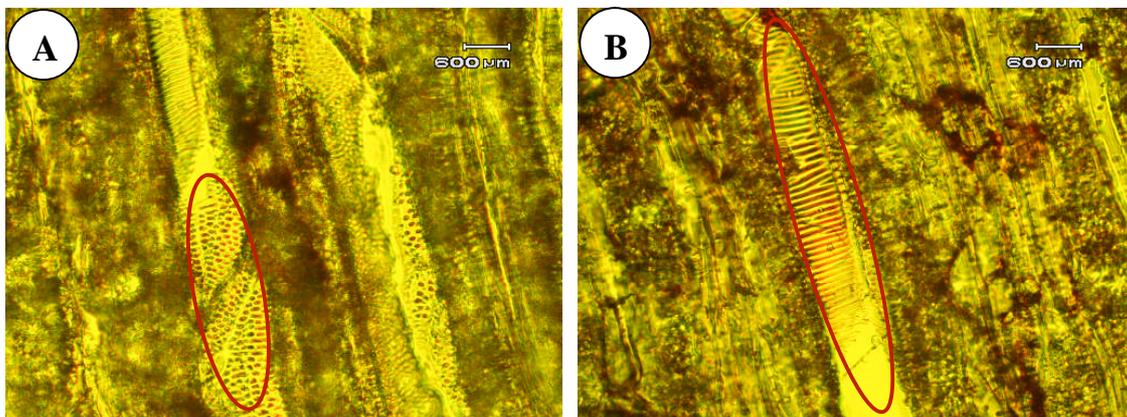


Fig. 31. Longitudinal section of veneer grafted five-year-old 'Fuyu'/*D. kaki* (A) and 'Fuyu'/*D. lotus* (B) combinations. Circle: callus with developing vessels.

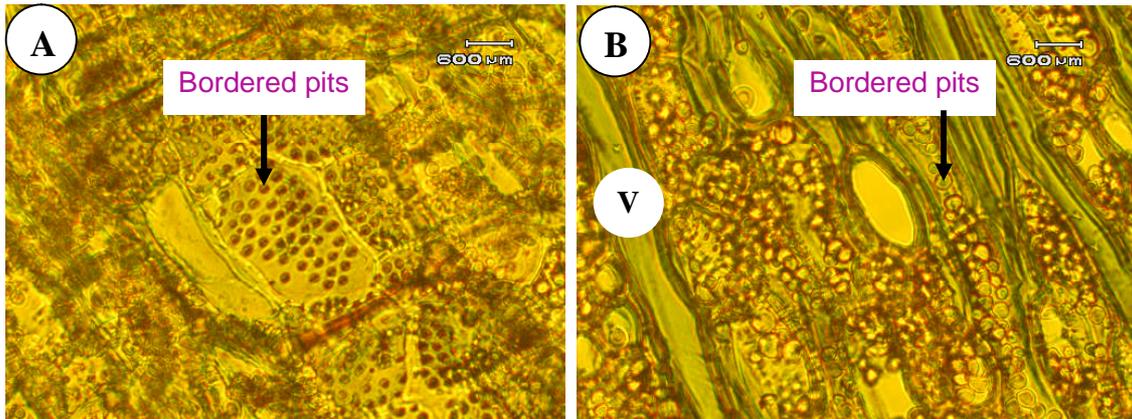


Fig. 32. Longitudinal section of veneer grafted five-year-old 'Fuyu'/'*D. kaki*' (A) and 'Fuyu'/'*D. lotus*' (B) combinations. V: vessels; Arrows: showing the tracheids.

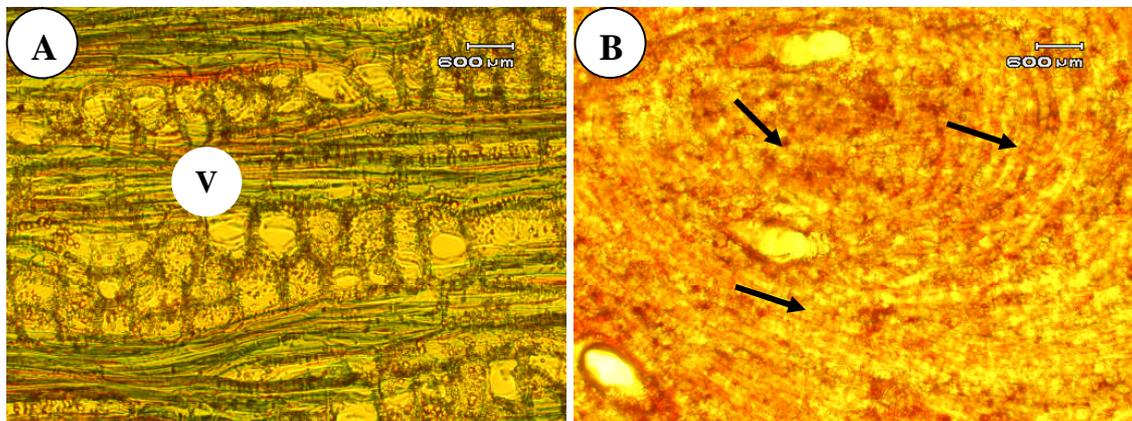


Fig. 33. Visual differences of veneer grafted five-year-old 'Fuyu'/'*D. kaki*' (A) and 'Fuyu'/'*D. lotus*' (B) combinations. V: vessels; Arrows: showing circled vascular tissues (xylem elements) near the necrotic areas of the graft union zone.

D. kaki

D. lotus

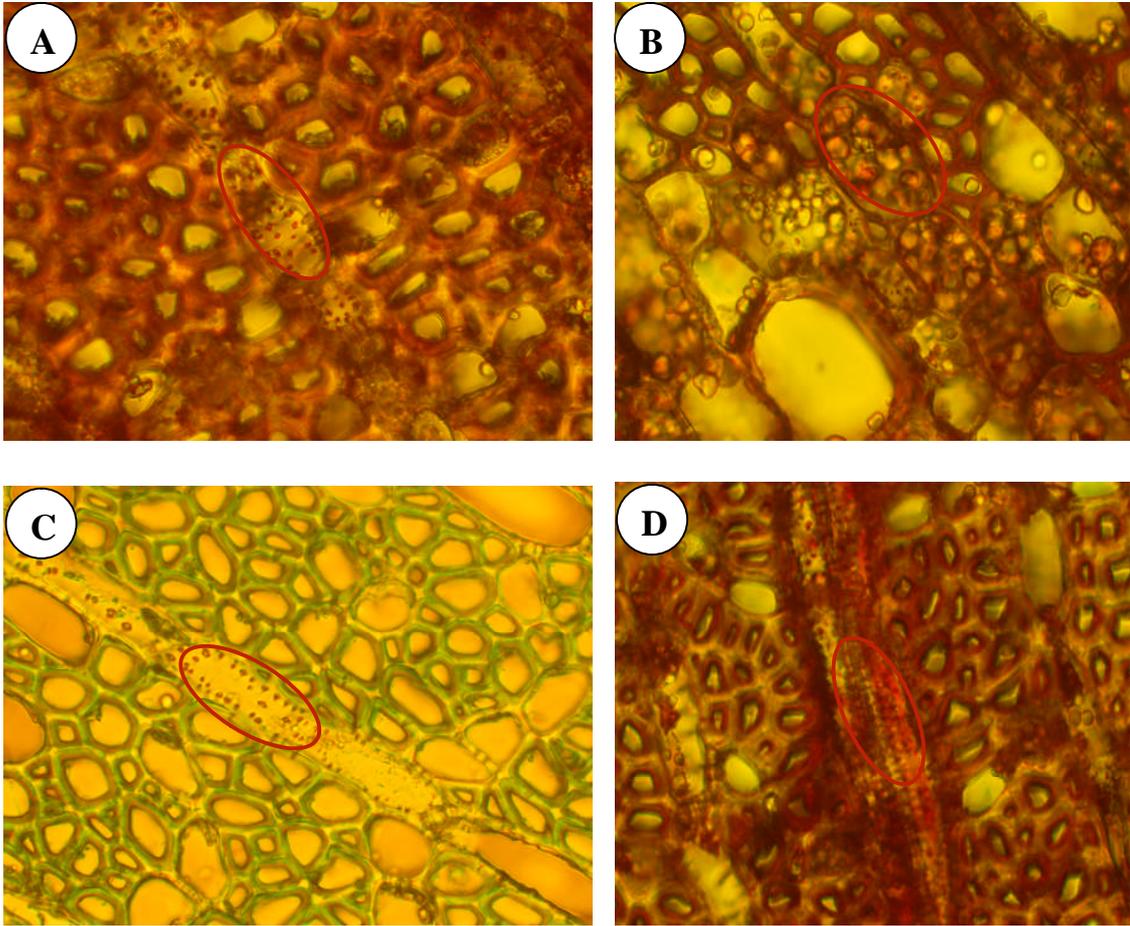


Fig. 34. Transversal section of veneer grafted five-year-old *D. kaki* and *D. lotus* combinations, just above graft union (A, B) and Just below graft union (C, D) zone. Circle showing the tracheids.

Chapter 5

Translocation and distribution of ^{13}C -photosynthates in ‘Fuyu’ persimmon (*Diospyros kaki*) grafted onto different rootstocks

5.1. Introduction

The persimmon cultivar ‘Fuyu’ is one of the most popular, remarkably superior, commercially leading, economic, non-astringent and widely grown cultivars in the world. It is noted for its good yield of “flattened” fruit and for its vigorous, manageable and upright growth habit. However, relatively low success rate of grafting has hindered the mass propagation of persimmon with more desirable traits. Overcoming these problems could strengthen the persimmon industry and diversify the cultivation in other areas.

Carbohydrate reserve in deciduous trees is very important for reproductive development in the initial growth stages (Gaudillere et al., 1992; Lockwood and Sparks, 1978; Loescher et al., 1990; Quinlan, 1969; Teng et al., 1999). Furthermore, Moing and Gaudillere (1992) reported that, not only carbohydrate content but also the total amount of carbohydrates and nitrogen components in each part is important for maintaining tree vigor. In grafted vines of kiwifruit, however, low hydraulic conductance in rootstock reduced water transport to the shoots and ultimately decreased stomatal conductance, photosynthetic rate and shoot growth for a given investment in root biomass (Clearwater et al., 2004). In young prune trees, carbohydrate content was

affected by rootstock but this was dependent on the carbohydrate type and on the seasons (Gaudillere et al., 1992). The distribution of carbohydrates within young prune plant involved production of carbohydrates in photosynthetic organs (source), phloem loading and subsequent translocation and unloading at the region of growth or storage (sink). In the case of combination between peach and plum as a rootstock, carbon availability in the roots and nitrogen assimilation by the scions played very important role in graft incompatibility (Yano et al., 2002). In the compatible system *Lycopersicon esculentum* on *Solanum tuberosum* as well as in the autografts, ¹⁴C-labelling techniques revealed that assimilate transport occurred from the apical callus of the scion, to the basal callus of the stock. As expected, a close interrelation between transport and phloem restitution in the graft union could be demonstrated (Schoning and Kollmann, 1995, 1997). On the contrary, in the incompatible system *Vicia faba* on *Helianthus annuus*, an increase in ¹⁴C-transport to the stock during all stages of graft union development did not occur and there was no correlation between phloem regeneration and assimilate transport across the graft interface. 5-6 carboxyfluorescein translocation experiments confirmed non-functional phloem connections in this heterograft (Schoning and Kollmann, 1997). The study of tissue development in apricot with incompatible combination showed clear differences in the level of differentiation of the callus produced where the differentiation was not complete and remnants of parenchyma cells coexisted with differentiated vascular tissues in incompatible combination (Errea et al., 1994). The use of compatible peach inter-stocks with different rootstock improved translocation of photosynthetic products and consequently overcomes the mobilization of substances at the graft union. Thus, graft compatibility and rootstock are

crucial to utilization of carbohydrate reserves, which determine the ultimate plant vigor and economic yield. Some incompatibility problems between *D. kaki* cv. 'Fuyu' and *D. lotus* have been observed (Bellini, 1982). 'Fuyu' scion grafted onto *D. lotus* rootstocks often grow slowly, bear fruit early, and die within a few years while astringent cultivars normally form good growth but excessive fruit dropping might be affected by certain rootstock (Hodgson, 1940). However, detailed information about the utilization of carbohydrate reserves in persimmon is not available. The objective of this study is to elucidate the effect of rootstocks on translocation and partitioning patterns of photosynthates from shoots to different parts of the plants.

5.2. Materials and Methods

This study was conducted at the Agricultural and Forestry Research Center, University of Tsukuba. Two-year-old 'Fuyu' persimmon trees veneer-grafted on *D. kaki* and *D. lotus* rootstocks were grown in 2005 in 40-liter black plastic containers with loamy soil on open field. Trees were administered with 60 g mixed fertilizer (NPK at a rate of 12:12:12) per pot annually in March, and pest and disease were controlled by usual practices. The physiological parameters were measured randomly in three matured leaves per plant on the periphery of the canopy with the LI-6400 System (Li-Cor, INC, USA), a portable closed gas-exchange photosynthesis meter equipped with LED lamp (PPF:1600 $\mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{sec}^{-1}$). The measurement was done at monthly interval at midday from June to August, 2005.

In order to observe the translocation of carbohydrates, thirty-six 2-year-old vigorous trees grown in plastic containers were selected and fed $^{13}\text{CO}_2$ at the matured leaves in August for ^{13}C tracer experiment. In specific branch $^{13}\text{CO}_2$ was administered with one vial containing 1 g of $\text{Ba}^{13}\text{CO}_3$ having 99% ^{13}C atom as shown in Fig. 35. A branch in the middle part of the tree was put into a 20×30 cm transparent plastic bag and $^{13}\text{CO}_2$ was generated by injecting 50% lactic acid into the vial using the syringe. The hole was immediately sealed and the feeding began at 10 AM under clear or mostly sunny conditions which lasted 6 hrs. After feeding, the bags were removed from the chamber and the trees were separated into the following parts at 6, 24, 48, 72, 96 and 120 hr after feeding: $^{13}\text{CO}_2$ exposed leaves and branches, leaves and branches on upper, middle, and basal parts of tree, trunk shoot, graft union, tap root, and roots more than 2mm in thickness (roots $\geq 2\text{mm}$) and roots less than 2mm in thickness (roots $< 2\text{mm}$). The samples were dried in an oven at 60°C and ground into powder with a vibration mill. The ^{13}C abundance was determined by an infrared $^{13}\text{CO}_2$ analyzer (EX-130S, Japan Spectroscopic Co. Ltd., Tokyo) after combustion of sample at 900°C in an O_2 stream, according to the method by Okano et al. (1983), and Kouchi and Yoneyama (1984).

In addition, girdling treatment was carried out in order to elucidate the effectiveness of rootstock on photosynthate partitioning as shown in Fig. 38. After 24 hrs $^{13}\text{CO}_2$ feeding was performed only on the parts above girdling.

Data analysis

The ^{13}C abundance in the sample was expressed as atom % excess, which refers to the difference between the ^{13}C atom % recovered in the labeled sample minus the ^{13}C atom % in the control (about 1.1 % in untreated plants). The absolute amount of ^{13}C recovered in each organ was calculated as follows: dry matter of each organ (g) \times carbon ratio in the sample (%) \times ^{13}C atom excess (%) (Kubota et al., 1990). The percentage of ^{13}C partitioning for each organ, which is related to the sink activity was obtained by dividing the ^{13}C content in each organ by the dry matter of the organ. Student's t-test was used to determine if there are significant differences between different rootstocks. Results were expressed as average of three trees.

5.3. Results

All growth parameters, except tree diameter, were significantly affected by the type of rootstock (Table 14). The total number of leaves and branches, shoot growth, tree height and chlorophyll content were much higher in *D. kaki* than in *D. lotus* while percentage stunted shoots was significantly lower in the former than in the latter. Similarly, all physiological characteristics measured as photosynthetic rate, stomatal conductance and transpiration rate were significantly higher in *D. kaki* than in *D. lotus* (Table 15).

Table 16 shows the distribution of dry matter and ^{13}C in individual organs at 120 hrs after feeding. The total DW was higher (191.00g) in *D. kaki* than in *D. lotus* (150.09g), especially on the trunk shoot, tap root, and both roots ≥ 2 mm and < 2 mm. The ^{13}C content per unit dry matter and the relative

value in each organ in relation to the whole tree did not widely vary with rootstock. However, the total amount of ^{13}C was substantially higher (65.18mg) in *D. kaki* than in *D. lotus* (39.27mg).

There were differences in the partitioning patterns of the ^{13}C toward the organs with respect to the time after feeding. The total content of ^{13}C in whole tree at each sampling time were higher in *D. kaki* than in *D. lotus*. The changing pattern in ^{13}C amounts in individual organs is presented in Fig. 36.A-E. Uptake of ^{13}C in *D. kaki* was greater than in *D. lotus*. The translocation into each organ begun even after 6 hrs. The partitioning of ^{13}C in fed leaves and branches were substantially higher in *D. kaki* than in *D. lotus* (Fig.36.A). ^{13}C partitioning was relatively higher in leaves than in branches. The amount of ^{13}C partitioned to leaves was significantly higher in middle parts followed by upper and bottom parts, and this was consistently manifested up to 24 hrs, after which it decreased with time in both rootstock combinations (Fig.36.B). The partitioning of ^{13}C among different parts of the branches was similar to that in leaves. It increased with time reaching maximum level at 24 hrs after feeding (Fig.36.C). The highest amount of ^{13}C was partitioned to trunk shoot followed by tap root and graft union. In case of graft union and tap root, the ^{13}C partitioning increased slightly with time (Fig.36.D). The translocation of ^{13}C toward roots $\geq 2\text{mm}$ and $< 2\text{mm}$ of *D. kaki* consistently increased with time in contrast to that in *D. lotus* wherein ^{13}C seemed to hardly move. The amount of ^{13}C was highest at 120 hrs after feeding in both combinations. In roots $\geq 2\text{mm}$ and $< 2\text{mm}$, ^{13}C levels were significantly higher in *D. kaki* than in *D. lotus* at each sampling period

(Fig.36.E). Taken together, the amount of ^{13}C partitioned to parts above and below the graft union was substantially higher in *D. kaki* than in *D. lotus*. The partitioning percentage on the parts above and below the graft union of *D. kaki* were 53.4 and 46.6, while in *D. lotus*, 68.5 and 31.5, respectively (Fig. 37).

The girdling treatment was conducted to assess the direct effect of rootstock on ^{13}C assimilation. The distribution of DW and ^{13}C in each organ at 120 hrs after feeding is presented in Table 17. The ^{13}C content per unit dry matter and the relative value in each organ in relation to the whole tree were slightly higher in *D. kaki* than in *D. lotus*. In terms of the total amount of ^{13}C , it was higher in *D. kaki* (24.77mg) than in *D. lotus* (16.88mg). The middle part of leaves and branches in *D. kaki* accumulated significantly higher amount of ^{13}C as compared to those in *D. lotus* (Table 17).

5.4. Discussion

The *Diospyros* is a large genus consisting of more than 400 species, which is mainly distributed in warm regions of the world (Spongberg, 1979). Grafting is one of the popular methods of asexual propagation in persimmon. *D. kaki* is commonly used as rootstock for persimmon in Asia. Seedlings of *D. lotus* are also used as rootstock. The cultivars of persimmon fruits are propagated by grafting on these seedling rootstocks. Trees propagated on *D. kaki* seedlings live longer and produce fewer root suckers than trees on American persimmon, *D. virginiana*. Graft union malformation is one of the major problems in this propagation method. The relatively low success rate of grafting and its incompatibility have limited the mass propagation of

persimmon. Incompatibility is clearly related to genetic differences between rootstock and scion. The mechanism of graft incompatibility is not yet fully understood and many works focused on this problem in order to understand the mechanism of graft development. Physiological, biochemical and anatomical differences between rootstock and scion have been implicated in graft incompatibility. This is supported by studies with incompatible combinations of certain pear cultivars on quince rootstock (Gur, 1957). That the new vascular connections could be not well differentiated or weakly established has been postulated as the main reason for incompatibility in woody plants (Mosse, 1962; Errea et al., 1994). In these cases, an abnormal process of neocambium differentiation leads to a cambial involution and a lack of differentiation into new vascular elements, as pointed out for pear and quince grafts (Ermel et al., 1999) and apricot on *prunus* grafts (Errea et al., 1994). While it appears that the formation of functional vascular connections is essential for successful grafts in herbaceous plants, incompatible grafts in woody plants can grow for several years without any external indication of incompatibility, denoting the presence of functional vascular connections in incompatible grafts (Hartmann et al., 1997).

In this study, growth characteristics in ‘Fuyu’/*D. kaki* were superior than in ‘Fuyu’/*D. lotus* combination (Table 1). These results are in agreement with those in persimmon as affected by incompatible rootstocks (Yamada et al., 1997). Using *D. kaki* rootstock also increased the photosynthetic rate, stomatal conductance and transpiration rate relative to that with *D. lotus* rootstock (Table 2). In previous studies, photosynthetic rate of peach grafted onto *P. tomentosa* as incompatible rootstock tended to decrease relative to that

in compatible rootstock *P. persica* (Salvatierra et al., 1998). The inhibition of photosynthesis caused by decreased mesophyll capacity or by stomatal closure and distribution percentage, were related to the sink capacity as a whole organ, and the quantity of ^{13}C assimilated per unit dry matter was related to the sink activity in organ cells (Graan and Boyer, 1990).

The total DW as well as the DW of the parts above and below the graft union, trunk shoot, tap root, and roots ≥ 2 mm and < 2 mm were higher in *D. kaki* than in *D. lotus*. These results are consistent with the findings of Yano et al. (2002a), wherein the total DW in compatible combinations with *Prunus persica* as a rootstock was greater than that in *Prunus tomentosa* as an incompatible rootstock. The higher amount of DW in *D. kaki* indicated better assimilation of ^{13}C than in *D. lotus*. The absorption of ^{13}C was high in *D. kaki* and low in *D. lotus* combinations. ^{13}C distribution was greatest in trunk shoots, tap root, and leaves in both combinations, indicating that the applied carbon was transported mainly to the middle parts of scion regardless of the graft combination. Furthermore, the high accumulation of ^{13}C in the fine roots (< 2 mm size) of *D. kaki* implies that these roots are more active and have a higher demand for photosynthates. The better assimilation of ^{13}C in *D. kaki* would prove that it is a superior rootstock as compared to *D. lotus*. The poor performance of 'Fuyu'/*D. lotus* combination may be caused by malfunctioning of the graft union which adversely affected assimilate translocation. This can be supported by the higher amount of ^{13}C remained in parts above the graft union (68.5%) as compared to that in 'Fuyu'/*D. kaki* combination (53.4 %).

The amount of photosynthate export depends on the branch composition and shoot type. Translocation distance between branches and connection of the vascular bundles are simpler in younger limbs than in older branches (Teng et al., 2002) and our results confirm these statements as shown by the higher amount of ^{13}C in the middle part of scion, which is close to the ^{13}C -exposed branch. The sink-source relationship depends on the degree of light exposure, location of the leaves in the canopy, and the crop load. The new organs with high growth rates, which are referred to as growth centers, govern the relative activity, or sink capacity. These different growth centers may influence the distribution pattern of assimilates (Quinlan and Preston, 1971; Quinlan and Weaver, 1970). The unequal photosynthates partitioning easily occurs in incompatible combinations. As observed in this study, the partitioning of ^{13}C in most of the organs fluctuated more often in *D. lotus* than in *D. kaki* (Fig.36).

The tissues development, callus formation and vascular connections at the graft union zone were related to the translocation of photosynthates. As previously reported (Simkhada and Gemma, 2005), the necrosis and abnormal vascular differentiation in *D. lotus* revealed the lack of production of callus and vascular tissues by the new cambium, limiting the passage of nutrients and water between the rootstock and scion that may have led to the incompatibility phenomenon. Similarly we reported previously that in incompatible 'Fuyu'/*D. lotus* grafts, the failure of procambial development might be the result of the absence of an additional and more direct form of cellular communication between the graft partners (Simkhada and Gemma (2003). The anatomical studies of graft union in peach revealed the presence

of necrotic cell layers and defective vascular connection in the inter graft zone which were different between *P. tomentosa* and *P. persica* rootstocks (Salvatierra et al., 1998). Also, cyanide accumulation interrupts translocation by destroying phloem and xylem at the graft union and results in an incompatibility symptom (Andrews and Marquez, 1993). ‘Fuyu’ scion grafted onto *D. lotus* rootstocks often grow slowly, bear fruit early and die within few years (Hodgson, 1940) while astringent cultivars normally form good growth but excessive fruit dropping might be affected by certain rootstock.

The girdling experiment revealed that majority of ^{13}C -assimilates remained in the leaves and trunk shoot and the amount was higher in *D. kaki* than in *D. lotus*. In all the sampling periods, the ‘Fuyu’/*D. kaki* accumulated higher amount of ^{13}C than ‘Fuyu’/*D. lotus*. The partitioning of ^{13}C photosynthates in the leaves and branches decreased with time and the opposite trend happened in the trunk shoot in both combinations. The higher amount of carbon in *D. kaki* might be due to its better growth and physiological characteristics. Besides, the differences in the rootstock between the two plants also might have contributed to this difference in ^{13}C accumulation.

Carbohydrate distribution pattern in a plant is related to the relative competitive ability of the various sink regions within the whole plant. A high sink import rate is a function of its mobilizing ability (Daie, 1985). If the sink mobilizing ability or strength is a function of sink size and activity, then the high levels of ^{13}C labeled fraction found in *D. kaki* could indicate its high sink activity. In both combinations, girdling affected the downward movement and

consequently blocks the translocation of carbohydrates but the observed difference in the amount of ^{13}C accumulated in *D. kaki* and *D. lotus* can be attributed to the rootstock effect. Thus, carbohydrate allocation is affected by rootstock, so appropriate selection of cultivar is very important to ensure scion-rootstock compatibility.

5.5. Conclusions

Growth and physiological characteristics and carbon assimilation are superior in 'Fuyu'/*D. kaki* grafts as compared to 'Fuyu'/*D. lotus* grafts. This implies that 'Fuyu'/*D. kaki* combination is more appropriate as compared to 'Fuyu'/*D. lotus* combination. Therefore, selection of *D. kaki* as rootstock would enhance the translocation of photosynthates and thereby improve the productivity and longevity of persimmon trees.

In my idea, growth and physiological characteristics and carbon assimilation are superior in 'Fuyu'/*D. kaki* grafts indicate the better involvement of translocation of carbohydrates and nutrient materials. In contrast, the poor performance of 'Fuyu'/*D. lotus* combination may be caused by partial blockage at the graft union or reduction in movement of water or nutrient materials or both through the graft union ultimately, malfunctioning of the graft union which adversely affected assimilate translocation. In another possible reason in *D. kaki* is that superior ability of the vigorous rootstock to absorb and translocate mineral nutrients to the scion in comparison with the *D. lotus* rootstock.

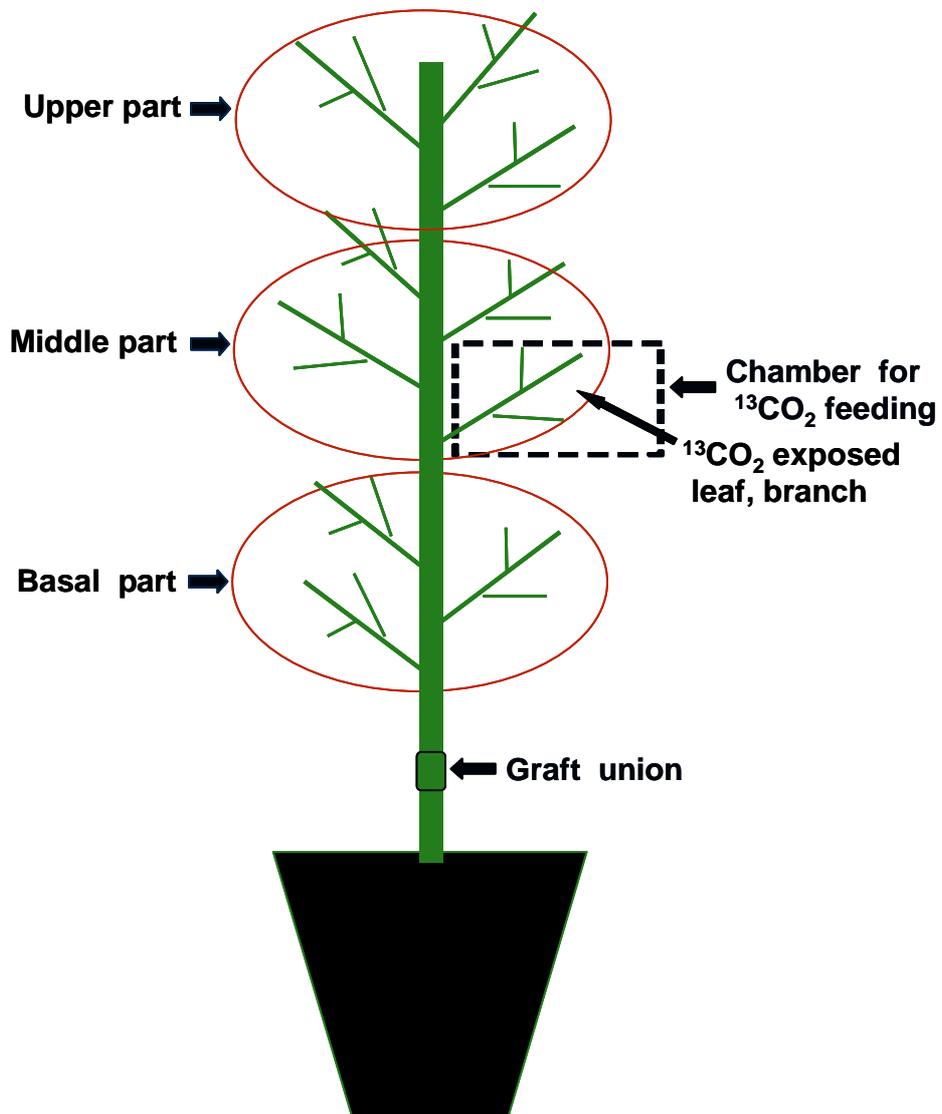


Fig. 35. A diagram of the treatment units showing the ¹³CO₂ feeding position of 2-year-old *D. kaki* cv. 'Fuyu' grafted onto *D. kaki* and *D. lotus* persimmon trees at mature leaf period.

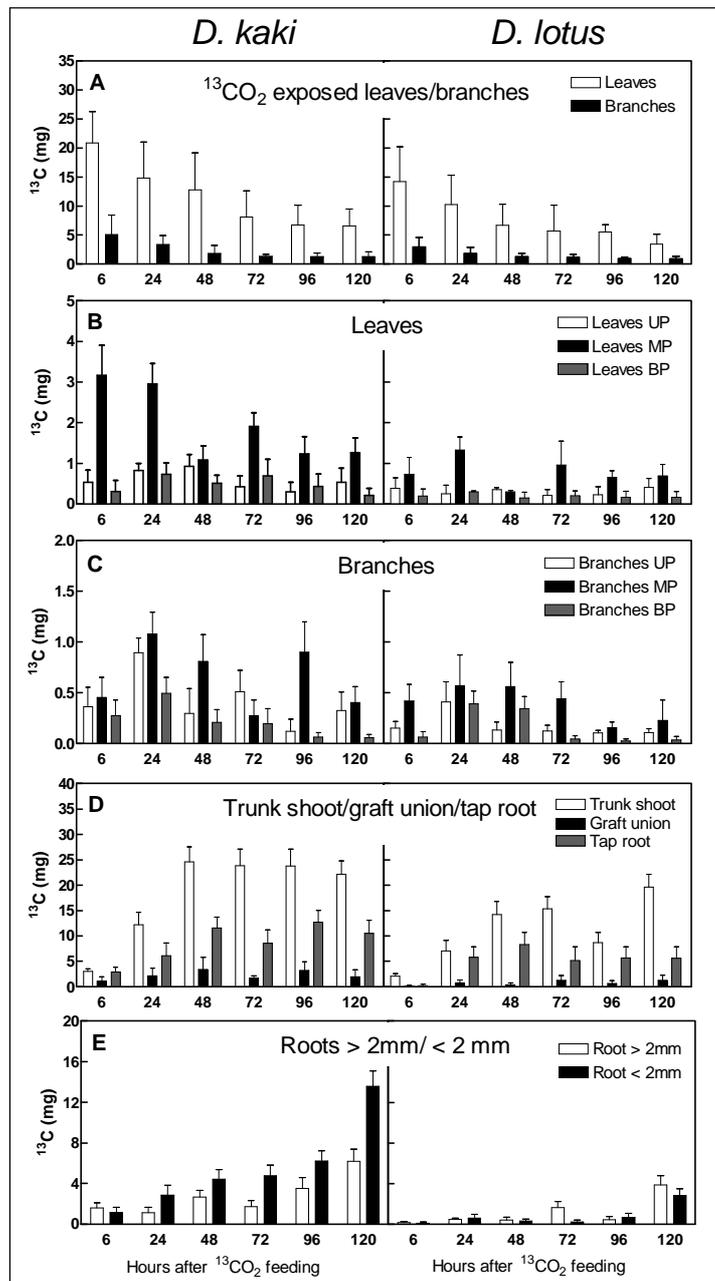


Fig.36. Partitioning patterns of ^{13}C assimilates: $^{13}\text{CO}_2$ exposed leaves and branches (A); leaves (B); branches (C); trunk shoot, graft union, tap root (D); and roots $\geq 2\text{mm}$ and $< 2\text{mm}$ (E) in each organ of 2-year-old *D. kaki* cv. 'Fuyu' grafted onto *D. kaki* and *D. lotus* rootstocks at mature leaf period after feeding. UP, MP, BP indicate upper, middle and basal parts. Vertical bars denote \pm SE (n=3).

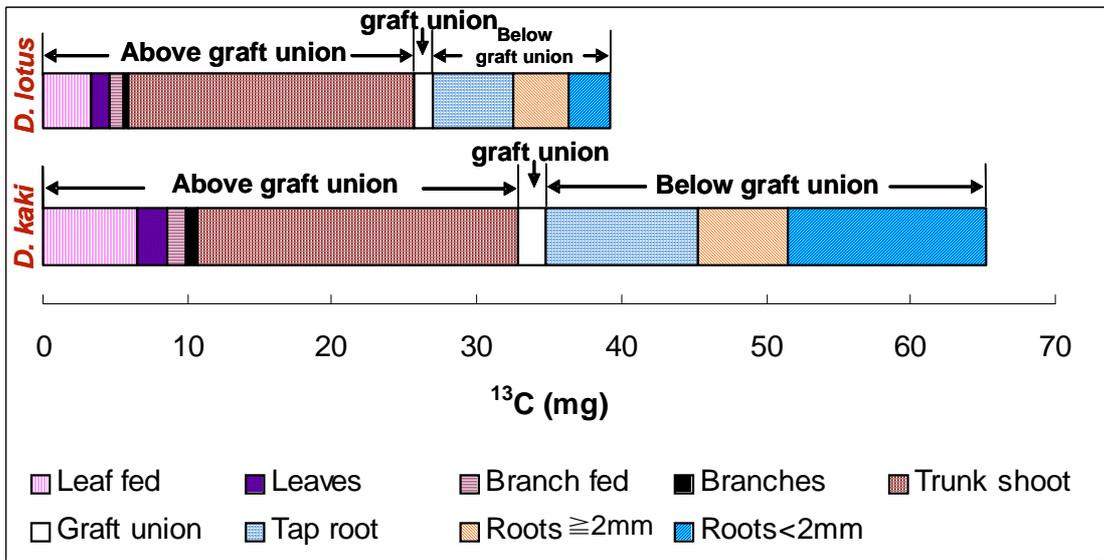


Fig. 37. Partitioning patterns of ^{13}C content at the parts above and below the graft union of 2-year-old *D. kaki* cv. 'Fuyu' grafted onto *D. kaki* and *D. lotus* rootstocks at mature leaf period at 120 hrs after feeding.

Table 14. Comparison of growth characteristics of 2-year-old *D. kaki* cultivar 'Fuyu' trees grafted onto *D. kaki* and *D. lotus* rootstocks at matured leaf period.

Graft Combinations	Leaf (number)	Branch (number)	Shoot growth (cm)	Tree height (cm)	Trunk diameter (cm)	Stunted shoot/total shoot (%)	Chlorophyll content (SPAD ^z)
'Fuyu'/ <i>D. kaki</i>	232.7	22	33.6	250.3	118.3	18.2	58.6
'Fuyu'/ <i>D. lotus</i>	113	11.7	12.7	186.3	93.4	42.8	25.9
Significance ^y	*	*	*	*	NS	*	*

^z Reading of a chlorophyll meter.

^y NS,*: non-significant or significantly different at $P < 0.05$, respectively, by student t-test (n=3).

Table 15. Comparison of photosynthetic rate, stomatal conductance and transpiration rate of 2-year-old persimmon trees *D. kaki* cv. 'Fuyu' grafted onto *D. kaki* and *D. lotus* rootstocks at matured leaf period ^z.

Graft combinations	Photosynthetic rate ($\mu\text{molCO}_2\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$)	Stomatal conductance ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$)	Transpiration rate ($\text{mmolH}_2\text{O}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$)
'Fuyu'/ <i>D. kaki</i>	13.62	0.14	2.08
'Fuyu'/ <i>D. lotus</i>	6.84	0.06	0.80
Significance ^y	**	**	**

^z Condition of light intensity was $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$.

^y **: Significantly different at $P < 0.01$, by student t-test (n=3).

Table. 16. Partitioning patterns of dry matter, ^{13}C content per unit dry matter, ^{13}C assimilates and ^{13}C partitioning in each organ of 2-year-old *D. kaki* cv. 'Fuyu' grafted onto *D. kaki* and *D. lotus* rootstocks at mature leaf period at 120 hrs after feeding.

Organ	Dry matter		^{13}C content/ dry matter ($\text{mg}\cdot\text{g}^{-1}$)		^{13}C content/ organ (mg)		Signi- ficance ^z	^{13}C partitioning (%)	
	(g)								
	<i>D. kaki</i>	<i>D. lotus</i>	<i>D. kaki</i>	<i>D. lotus</i>	<i>D. kaki</i>	<i>D. lotus</i>		<i>D. kaki</i>	<i>D. lotus</i>
Leaf Fed	3.01 (1.6) ^y	4.13 (2.8)	2.19(6.4) ^x	0.83 (3.2)	6.59	3.42	NS	10.11	8.71
Leaf UP ^w	2.45 (1.3)	2.35 (1.6)	0.22(0.6)	0.17 (0.7)	0.53	0.41	NS	0.82	1.04
Leaf MP	3.06 (1.6)	3.33 (2.2)	0.41(1.2)	0.21 (0.8)	1.27	0.69	*	1.94	1.75
Leaf BP	2.36 (1.2)	1.69 (1.1)	0.09(0.3)	0.10 (0.4)	0.21	0.17	NS	0.33	0.42
Branch Fed	1.39 (0.7)	1.29 (0.9)	0.93(2.7)	0.71 (2.7)	1.29	0.92	NS	1.99	2.33
Branch UP	1.72 (0.9)	1.22 (0.8)	0.19(0.6)	0.09 (0.3)	0.33	0.11	**	0.50	0.27
Branch MP	1.87 (1.0)	1.50 (1.0)	0.22(0.6)	0.15 (0.6)	0.41	0.23	*	0.62	0.58
Branch BP	0.96 (0.5)	1.00 (1.7)	0.06(0.2)	0.04 (0.1)	0.06	0.04	NS	0.09	0.09
Trunk shoot	84.56 (44.3)	78.66 (52.4)	0.26(0.8)	0.25 (1.0)	22.14	19.63	NS	33.97	49.98
Graft union	7.07 (3.7)	8.50 (5.7)	0.28(0.8)	0.15 (0.6)	1.96	1.31	NS	3.01	3.34
Tap root	55.20 (28.9)	33.66 (22.4)	0.19(0.6)	0.17 (0.6)	10.55	5.65	**	16.19	14.38
Roots $\geq 2\text{mm}$	12.09 (6.3)	7.27 (4.8)	0.52(1.5)	0.53 (2.0)	6.23	3.88	**	9.56	9.87
Roots < 2mm	15.24 (8.0)	5.46 (3.6)	0.89(2.6)	0.52 (2.0)	13.60	2.83	***	20.87	7.22
Total /plant	191.00(100.0)	150.09(100.0)	0.34(1.0)	0.26 (1.0)	65.18	39.27	NS	100.00	100.00

^z NS, *, **, ***: non-significant or significantly different at $P < 0.10$ or $P < 0.05$ or $P < 0.01$, respectively, by student t-test.

^y Percentage of dry matter in each organ to total dry matter in whole tree.

^x Relative value of ^{13}C content per unit dry matter in each organ to that in whole tree (n=3).

^w UP, MP, BP, indicates upper, middle and basal parts.

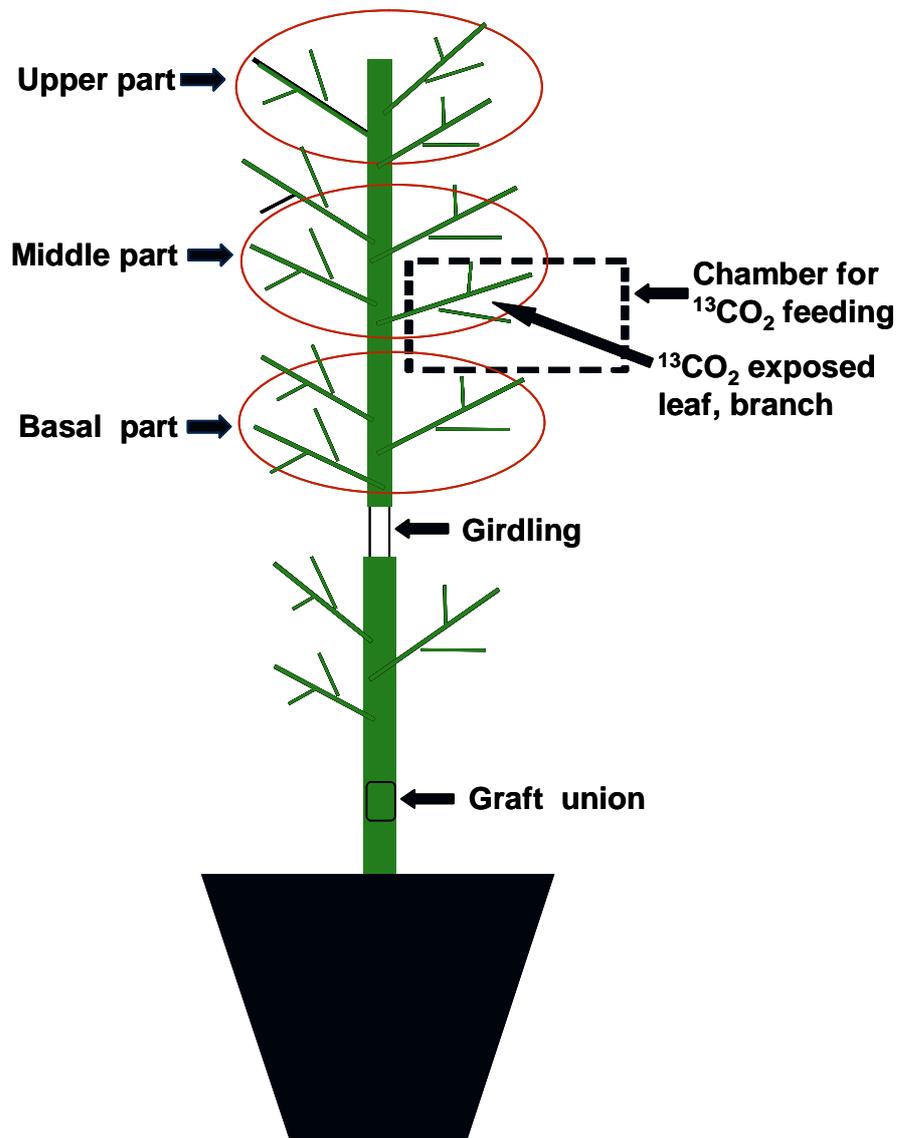


Fig. 38. A diagram of the treatment units showing the ¹³CO₂ feeding position of girdled 2-year-old *D. kaki* cv. 'Fuyu' grafted onto *D. kaki* and *D. lotus* persimmon trees at mature leaf period.

Table. 17. Partitioning patterns of dry matter, ^{13}C content per unit dry matter, ^{13}C assimilates and ^{13}C partitioning in each organ of girdled 2-year-old *D. kaki* cv. 'Fuyu' grafted onto *D. kaki* and *D. lotus* rootstocks at mature leaf period at 120 hrs after feeding.

Organ	Dry matter		^{13}C content/ dry matter ($\text{mg}\cdot\text{g}^{-1}$)		^{13}C content/ organ (mg)		Signi- ficance ^z	^{13}C partitioning (%)	
	(g)								
	<i>D. kaki</i>	<i>D. lotus</i>	<i>D. kaki</i>	<i>D. lotus</i>	<i>D. kaki</i>	<i>D. lotus</i>		<i>D. kaki</i>	<i>D. lotus</i>
Leaf Fed	3.51 (9.1) ^y	3.1 (10.2)	3.87(6.0) ^x	3.51 (6.3)	13.61	10.90	NS	54.97	64.58
Leaf UP ^w	2.62 (6.8)	3.26 (10.7)	0.23(0.4)	0.12 (0.2)	0.59	0.38	*	2.39	2.23
Leaf MP	5.17 (13.4)	3.74 (12.3)	0.22(0.3)	0.17 (0.3)	1.12	0.62	**	4.51	3.65
Leaf BP	5.94 (15.4)	3.37 (11.1)	0.07(0.1)	0.12 (0.2)	0.42	0.41	NS	1.71	2.43
Branch Fed	2.26 (5.9)	1.78 (5.9)	0.84(1.3)	0.64 (1.2)	1.90	1.15	NS	7.69	6.79
Branch UP	3.51 (9.1)	2.01 (6.6)	0.14(0.2)	0.13 (0.2)	0.49	0.27	NS	1.98	1.59
Branch MP	1.67 (4.3)	2.02 (6.7)	0.65(1.0)	0.22 (0.4)	1.08	0.45	**	4.34	2.68
Branch BP	2.16 (5.6)	1.37 (4.5)	0.31(0.5)	0.15 (0.3)	0.68	0.20	**	2.73	1.19
Trunk shoot	11.81 (30.6)	9.69 (31.9)	0.41(0.6)	0.26 (0.5)	4.87	2.51	*	19.67	14.86
Total /plant	38.65(100.0)	30.35 (100.0)	0.64(1.0)	0.56 (1.0)	24.77	16.88	NS	100.00	100.00

^z NS,*, **: non-significant or significantly different at $P<0.10$ or $P<0.05$, respectively by student t-test.

^y Percentage of dry matter in each organ to total dry matter in whole tree.

^x Relative value of ^{13}C content per unit dry matter in each organ to that in whole tree (n=3).

^wUP, MP, BP, indicates upper, middle and basal parts.

Chapter 6

General Discussion

Persimmon (*Diospyros kaki* Thunb.) is a deciduous fruit tree commercially cultivated worldwide. Although often regarded as a strictly temperate species, persimmon appears to be readily adapted to a wide range of climatic conditions (warm temperate to sub-tropical) resulting to its cultivation in unexploited areas of Asia, Europe, and Latin America. Recently, production has spread to other regions and consequently, persimmon became a familiar fruit in non-traditional countries like Nepal, India, Pakistan, Bhutan and Myanmar.

The world production is about more than 2.3 million metric tons but this is bound to increase in the coming years because cultivation is rapidly expanding worldwide. However, the persimmon industry has been suffering from inadequate vegetatively propagated plant material, lack of suitable cultivars, and poor standardization in training, pruning and other orchard management practices (Mehata et al., 2003). Especially, relatively low success rate of grafting has hindered the mass propagation of persimmon with more desirable traits. Overcoming these problems could strengthen the persimmon industry and diversify the cultivation in other areas.

Propagation in persimmon has been proved the difficulty as compared to other commercial fruit trees due to the problem of low success potential of grafting. Comparative studies using different astringent and non-astringent

cultivar and their grafting methods, and anatomical studies of graft union with the combination between *D. kaki* and *D. lotus* showed visible differences. Differences in species within the genera between scion and rootstock sometimes cause abnormal growth within the graft union.

In the present study in chapter 3, as regards total percentage of success with different grafting methods, tongue grafting was found to be the preferred method over side-grafting. It was observed that *in-situ* grafting is the preferred method over grafting in transplanted rootstocks. With respect to scion cultivar used, the combination with 'Dhaura' with 'Kirtipur', 'Hiratanenashi', 'Fuyu', and 'Jiro' yielded favorable results. Top-worked plant had higher percentage success than others. Scions of 'Fuyu' and 'Jiro' top-worked with 'Dhaura', 'Zenjimar' and 'Kirtipur' were better in comparison with other rootstocks used.

The research conducted in the University of Tsukuba in relation to tissue development on the inter-graft zone showed differences in development of the union region between scion and rootstock in the case of 'Fuyu' on *D. kaki* had higher sap plug, more live cells with cellular activity and uniform growth and vigor. In contrast, 'Fuyu' grafted on *D. lotus* showed lesser sap plug inside the wounded graft which resulted in lowering the graft adhesion. Suriapananon and Suriyapananon (1997) reported that even if 'Fuyu' on 'Kluairusi' stock showed callus proliferation and extension of new cambium from the stock side, it failed to produce a successful union. The critical structural event in the formation of a successful graft is the unification of the vascular elements of both components (Yeoman, et al., 1978) while vascular continuity between the

components provided the most efficient system for the transport of water and nutrients.

The cultivar stability of most fruit tree species must be ensured by grafting selected rootstock and scion genotypes. The grafted partners often belong to the same species or genus, but the use of genetically divergent genotypes is also common. In these cases, the stock and scion do not always constitute a successful graft and the result is low success potential of grafting. This graft incompatibility or low success potential of grafting observed in some scion-rootstock combinations have been classified into two categories (Herrero, 1951; Mosse, 1962); the so-called translocated and localized incompatibility. The translocated incompatibility is associated to symptoms that resemble leaf aging and alterations in the redicular system. The localized incompatibility is associated to malformation at the graft union that results in mechanical weakness and subsequent breakdown of the union. A number of morphological and physiological alterations are associated to the incompatibility process preventing the development of a successful union between scion and rootstock.

In the present study in chapter 4, the results show that with respect to timing of grafting and budding operations, late July to August was the ideal time to perform budding with chip budding as the recommended method. Fuyu budded onto *D. kaki* showed the highest compatibility in contrast to other combinations tested. Likewise, April was the ideal time to perform grafting using veneer (shoot) grafting, which was a more superior compared to tongue grafting. Fuyu grafted on *D. kaki* showed highest compatibility than others. In the initial stages, both *D. kaki* and *D. lotus* developed callus

and cambial cells in a similar manner but the ratio of growth was higher and faster in *D. kaki* than *D. lotus* combinations. The callus cells showed an irregular appearance and the cell walls seem to be thinner than the callus cells on the homograft union. At the later stages, adhesive phenomenon was reduced and the tree weakened in 'Fuyu' grafted with *D. lotus* combinations.

The tissue development in 'Fuyu' grafted onto *D. lotus* combination showed a visible differences between the components in shape of cells and some cell contents and defective union as compared to 'Fuyu' grafted onto *D. kaki* perhaps due to callus unresponsiveness to the stimulus for differentiation. These results agree with the comparative anatomical studies of Suriyapananont and Suriyapananont (1997) on 'Fuyu' as scion on Date plum (*D. lotus*) rootstock. Other main differences at the early stages were the presence of parenchymatous tissues without lignin coexisting with vascular tissues and necrotic cells in 'Fuyu'/*D. lotus* combination. In 'Fuyu' budded or grafted onto *D. lotus* there is a gradual reduction in the adhesive phenomenon, afterwards abnormalities appear at the graft union and the tree eventually weakens. The present results are in agreement with the results of Bellini (1982) who reported some incompatibility problems between Date Plum and pollination constant, non-astringent cultivars such as 'Fuyu.' 'Fuyu' scion grafted onto Date Plum (*D. lotus*) rootstock often grow slowly, bear fruit early and die within a few year (Hodgson,1940). This phenomenon may be due to the presence of phenolic compounds in the graft union zone. Many phenolic compounds are natural constituents of plants, which can be observed near the graft union. Prunin- a characteristics phenol of prunus found at the phloem above the union of cherry trees, was associated with incompatibility of the trees (Treutter and Feucht, 1988). However, the

phenolic compounds, depending on their chemical structure, have different roles in the physiological process. Some of them activate IAA oxidase whereas others inhibit this enzyme (Lee et al., 1982). Thus, phenols occurring at the graft union might have positive or negative effects on the tissue development at the graft union. When grafting is performed, the pattern of release, as a response to stress, or phenol accumulation at the graft union may change. This different pattern, depending on the substance, could have physiological effects that finally would produce abnormalities at the graft union.

The present findings are in agreement with the report of Morton (1987), where in India, cleft-grafting on stem were 88.9 % successful; while cleft-grafting on crown as tongue-grafting on stem were 73.4 % successful when the grafted plants were left for 2 weeks at about 25°C and a relative humidity of 75 % for 2 weeks before planting. In another experiment in the Kulu valley, India, scions were grafted onto 2-year old *D. lotus* seedlings, which are mounted with earth to cover the graft until it begins to sprout. At the Fruit Research Station, Kandaghat, 2-year old *D. lotus* seedlings were used as rootstock for veneer and tongue grafts from cv. Hachia between late June and the third week of August. Success rates ranged from 80 to 100 %.

A successful graft union is characterized by a proliferation of parenchyma tissues (callus) and differentiation of vascular tissues. The sequence of structural events in compatible grafts is the rupture of cells at the graft interface, and collapse and formation of a necrotic layer, which disappears during subsequent events. This is followed by living cells from both stock and scion extending into the necrotic zone. A callus bridge of

interdigitating parenchyma cells is formed by division of cells rupturing and invading the necrotic layer. During these events the tensile strength of the graft increases due to physical cohesion between stock and scion. The new vascular component is differentiated from parenchyma cells. Finally, secondary xylem and phloem are produced by the reconstituted cambium, providing a vascular connection between rootstock and scion. Since these events also occur during wounding, they may not be unique to grafting (Esau, 1965; Moore, 1984).

Anatomy of the graft union between 'Fuyu'/*D. lotus* indicated that success of grafting persimmon is directly related to the living cells, cellular activity and proper alignment of cambial layer between stock and scion. Living cells were most numerous in first-flush and younger stems but diminished rapidly with age. Proper alignment of cambial layers between stock and scion is conducive to rapid formation of the graft union. Rapid formation of callus may be expected to prevent excessive accumulation of resinous material, which is thought to interfere with the success or 'take' of grafted trees (Suriyapanon, et al., 1997).

Rapid proliferation of callus may be found where tissues, especially the cambium, of both components are close enough to cause a rapid connection of callus and cambial bridge. Cellular activity of both components is necessary for the completion of the cambial bridge (Suriyapananont, et al., 1997). The patterns of growth observed were also in agreement with previous reports, in which the cultivars seemingly affected the rootstocks (Larsen and Higgings 1990). The shoot length and growth ratio was lower in 'Fuyu'/*D. lotus* combinations. The trees on 'Fuyu'/*D. kaki*

combinations showed a fast shoot growth in June and July, as compared to *D. lotus* combinations. The present findings were consistent to the findings of Salvatierra et al. (1998) in peaches.

According to Hartmann and Kester (1975), auxin flux stimulus for vascular tissue development would be through the callus cells from the cambium. As the new xylem was not developed in normal orientation, and xylem development produced swirling xylem elements and vessels of differences sizes in the graft zone, these symptoms would be related to auxin levels (Soumelidou et al., 1994). The polar transport of hormones is impeded by harmful metabolites such as cyanides or phenols (Rubery, 1989). The less proportion of wood in the stock might be caused by a lower level of auxin in the rootstock tissues, or by a difficulty to pass to the scion to the stocks. Xylem differentiation only takes place at higher auxin levels (Aloni, 1987). He suggested that the non-polar transport of auxin in the phloem promoted vascular differentiation in instances of wounding and served as an additional mechanism that regulated vascularization in wounded regions. The size of vessels is determined by the rate of cell differentiation, therefore decreasing auxin concentration from leaves to roots results in an increase in vessel size (Aloni, 1989).

In addition, the relationships between scion and stock are affected by growth regulators, and it has been postulated that graft incompatibility may also exist. For example, an important substance involved in the development of compatible unions is auxin, which is released from vascular strands of the rootstock and the scion and induces the differentiation of vascular tissues, functioning as morphogenic substances (Moore, 1984; Aloni, 1987;

Mattsson et al., 2003). Its translocation from the root system has been studied in apples and has been related to graft incompatibility, since a supra and basipetal movements of auxin can organize the morphogenic pattern of the entire plant body (Zajaczowski et al., 1983), even accelerating the formation of a successful graft (Shimomura and Fujihara, 1977). Additionally, other compounds, like polyphenols also play a prominent role in graft union formation by influencing lignification processes and by their protein-precipitating feature (Haslam, 1979). It has been proposed that stress situations can lead to both the accumulation of flavanols and their degradation by oxidases (Van Sumere et al., 1985), inhibition of the lignin pathway (Buchloh, 1960). Some papers report observations on the characterization of monomeric and oligomeric flavan-3-ols in apricot cultivars and rootstocks (Errea et al., 1994a) and their accumulation in apricot combinations with a different degree of compatibility (Errea et al., 1992, 2000). Synthesis of flavanones can determine incompatibility in *Prunus*, such as prunasin (Moing et al., 1987; Moing and Carde, 1988), and can be stimulated by ABA and GA (Treutter and Feucht, 1988). The modulation of prunin levels is affected by growth regulators. All these macromolecules (phloem proteins, RNA, hormones) that are present in the sap phloem might appear to be important during vascular differentiation in compatibility process (Pina and Errea, 2005). In incompatible grafts differentiation is not complete (Errea et al., 1994c). The presence of phenols was generally associated with small cells in incompatible combinations, which did not form successful unions (Errea et al., 2001). Gebhardt and Feucht (1982) reported a lack of compatibility with a pronounced accumulation of polyphenols above the graft union. Relatively small quantities of polyphenols could be enough to produce locally limited

disfunction in the interphase between two cells (Erra, 1998). The theory that an accumulation of catechin above the graft union can be used as a biochemical marker of graft incompatibility (DeCooman et al., 1996; Musacchi et al., 2000) have been examined in the research. It is known that accumulation of polyphenols is also related with different types of stress (Dirr et al., 1994). Environmental conditions, site effects and orchard management modify the phenol content (Mayr et al., 1994, 1997) as well as physiological stage of the tissues (Michalek et al., 1999).

Auxin (IAA) is involved in the development of compatible unions and induces the differentiation of vascular tissues. The activity of IAA oxidase (Aloni, 1987) and transport of IAA transport (Stenlid, 1976) can be altered by naturally occurring substances such as phenols. *p*-Coumaric acid is an IAA antagonists or auxin-oxidase cofactors (Lockard and Schneider, 1981), but phloroglucinol is an IAA oxidase inhibitor (DeKlerk et al., 1999). Truetter and Feucht (1988) found an accumulation of pruning above the incompatible union of *Prunus avium* grafted to *Prunus cerasus*. From *p*-Coumaric acid pruning can be synthesized under stress conditions. Prunin enhances oxidative decarboxylation of IAA (Truetter and Feucht, 1988).

On the other hand, the involvement of certain enzymes in the cellular behavior during the first steps of graft formation have been studied in different species, although the specific role and effects on incompatibility is still not clear. However, Quessada and Macheix (1984) found a specific peroxidase in apricot trees, which can be specifically implicated in lignifications. The presence of peroxides that catalyze the oxidation or degradation of phenolic compounds as substrate, producing browning

reactions in the phloem of *Prunus* trees (Schmid and Feucht, 1981). Schmid and Feucht (1985) had studied proteins, peroxidases in the phloem and acid phosphatases in *Prunus avium/Prunus cerasus* grafting confirming that it is possible to define a good union not only by morphological characters but also by biochemical methods. Based on biochemical assays, Gulen et al. (2002) concluded that the absence or presence of peroxidase isozymes profile in the pear-quince graft combination is an experimental approach to predict incompatibility reaction. Some researchers found that the different pattern of isoenzymes in the partner could affect the graft healing and subsequently the graft union formation. Santamour et al. (1986) reported that isozyme analysis of scions and rootstocks could be used to predict incompatibility before grafting in different cultivars of Chinese chestnut (*Castanea mollissima* Blume). When peroxidase isozyme phenotypes of stock and scion matched, grafted resulted in a compatible union and the restoration of vascular continuity. In contrast, if isozyme phenotypes of graft partners were different, callus formation was impaired at the graft union (Santamour, 1988b). Similar results were obtained for intraspecific grafts of red oak (*Quercus rubra* L.) (Santamour, 1988c). Santamour (1988) found that the grafting between partners that had similar enzyme constitution was successful and vascular continuity was restored through the union. More recently, investigations suggest that increased peroxidase and catalase activities might be involved in graft development in tomato plants (Fernandez-Garcia et al., 2004).

With regard to grafting process in fruit trees more studies should be needed to obtain a deeper knowledge about the mechanisms that take

place during the graft incompatibility reactions, which will allow us to make an early rootstock selection, before observing the any external incompatibility symptoms.

In the present study in chapter 5, the growth characteristics in 'Fuyu'/*D. kaki* were superior than in 'Fuyu'/*D. lotus* combination (Table 12). These results are in agreement with those in persimmon as affected by incompatible rootstocks (Yamada et al., 1997). Using *D. kaki* rootstock also increased the photosynthetic rate, stomatal conductance and transpiration rate relative to that with *D. lotus* rootstock (Table 13). Stomatal density, which is one of the factors that influence photosynthesis, was higher in *D. kaki* than in *D. lotus*. Morinaga and Ikeda (1990) found that the photosynthesis of scion leaves grafted on various citrus rootstocks varied in comparison with those on common trifoliolate orange. Since photosynthesis had a significant positive correlation with stomata density, this was considered to be one of the most important factors affecting photosynthetic capacity. Breakbane and Majumder (1975), as cited by Morinaga and Ikeda (1990) showed the influence of rootstock on the density of stomata in scion leaves, and growth was closely related to the density of stomata. Therefore, the density of stomata might be an index related to photosynthesis of scion. Barden and Ferree (1979) found that rootstock of apple trees did not influence photosynthesis of scion leaves. However, citrus rootstocks did influence photosynthesis of scion (Morinaga and Ikeda, 1990). It was assumed that an increase or decrease in photosynthesis was related to morphological factors such as stomata density, or to biochemical factors such as RuBPCase activity.

In previous studies, photosynthetic rate of peach grafted onto *P. tomentosa* as incompatible rootstock tended to decrease relative to that in compatible rootstock *P. persica* (Salvatierra et al., 1998). The inhibition of photosynthesis caused by decreased mesophyll capacity or by stomatal closure and distribution percentage, were related to the sink capacity as a whole organ, and the quantity of ^{13}C assimilated per unit dry matter was related to the sink activity in organ cells (Graan and Boyer, 1990).

The total DW as well as the DW of the parts above and below the graft union, trunk shoot, tap root, and roots ≥ 2 mm and < 2 mm were higher in *D. kaki* than in *D. lotus*. These results are consistent with the findings of Yano et al. (2002), wherein the total DW in compatible combinations with *Prunus persica* as a rootstock was greater than that in *Prunus tomentosa* as an incompatible rootstock. The higher amount of DW in *D. kaki* indicated better assimilation of ^{13}C than in *D. lotus*.

The absorption of ^{13}C was high in *D. kaki* and low in *D. lotus* combinations. ^{13}C distribution was greatest in trunk shoots, tap root, and leaves in both combinations, indicating that the applied carbon was transported mainly to the middle parts of scion regardless of the graft combination. Furthermore, the high accumulation of ^{13}C in the fine roots (< 2 mm size) of *D. kaki* implies that these roots are more active and have a higher demand for photosynthates. The better assimilation of ^{13}C in *D. kaki* would prove that it is a superior rootstock as compared to *D. lotus*. The poor performance of 'Fuyu'/*D. lotus* combination may be caused by malfunctioning of the graft union which adversely affected assimilate translocation.

The amount of photosynthate export depends on the branch composition and shoot type. Translocation distance between branches and connection of the vascular bundles are simpler in younger limbs than in older branches (Teng et al., 2002) and our results confirm these statements as shown by the higher amount of ^{13}C in the middle part of scion, which is close to the ^{13}C -exposed branch. The sink-source relationship depends on the degree of light exposure, location of the leaves in the canopy, and the crop load. The new organs with high growth rates, which are referred to as growth centers, govern the relative activity, or sink capacity.

The tissues development, callus formation and vascular connections at the graft union zone were related to the translocation of photosynthates. As previously reported (Simkhada and Gemma, 2005), the necrosis and abnormal vascular differentiation in *D. lotus* revealed the lack of production of callus and vascular tissues by the new cambium, limiting the passage of nutrients and water between the rootstock and scion that may have led to the incompatibility phenomenon. Similarly we reported previously that in incompatible 'Fuyu'/*D. lotus* grafts, the failure of procambial development might be the result of the absence of an additional and more direct form of cellular communication between the graft partners (Simkhada and Gemma (2003). The anatomical studies of graft union in peach revealed the presence of necrotic cell layers and defective vascular connection in the inter graft zone which were different between *P. tomentosa* and *P. persica* rootstocks (Salvatierra et al., 1998).

The girdling experiment revealed that majority of ^{13}C -assimilates remained in the leaves and trunk shoot and the amount was higher in *D. kaki*

than in *D. lotus*. In all the sampling periods, the 'Fuyu'/*D. kaki* accumulated higher amount of ^{13}C than 'Fuyu'/*D. lotus*. The partitioning of ^{13}C photosynthates in the leaves and branches decreased with time and the opposite trend happened in the trunk shoot in both combinations. The higher amount of carbon in *D. kaki* might be due to its better growth and physiological characteristics. Besides, the differences in the rootstock between the two plants also might have contributed to this difference in ^{13}C accumulation.

Carbohydrate distribution pattern in a plant is related to the relative competitive ability of the various sink regions within the whole plant. A high sink import rate is a function of its mobilizing ability (Daie, 1985). If the sink mobilizing ability or strength is a function of sink size and activity, then the high levels of ^{13}C labeled fraction found in *D. kaki* could indicate its high sink activity. In both combinations, girdling affected the downward movement and consequently blocks the translocation of carbohydrates but the observed difference in the amount of ^{13}C accumulated in *D. kaki* and *D. lotus* can be attributed to the rootstock effect. Thus, carbohydrate allocation is affected by rootstock, so appropriate selection of cultivar is very important to ensure scion-rootstock compatibility.

Summary

Persimmon belongs to the genus *Diospyros*, consisting of nearly 190 species mainly in Asian countries, but with time being it is expanding to African, North and South American continents, too. They are fairly common trees in the warm regions of the world; only 4 species, *D. kaki*, *D. lotus*, *D. virginiana* and *D. oleifera* have been used for the production of fruit.

In Nepal, all the native varieties are of the astringent type and they have certain advantages: adaptability to a wide range of climatic conditions, excellent taste and fruit quality. While, the astringent varieties are not suitable to export in large amounts because post harvest self life is too short. But the farmers/fruit growers are preferred PCNA varieties characterized by early ripening, larger fruits, less cracking, high eating and long keeping quality with high productivity. However, in the government farms and private nurseries the problem of graft-incompatibility has hindered the mass propagation of persimmons with more desirable traits. The existing major budding and grafting methods show less than 60% success.

The objective of this research was to study possible mechanisms in scion and rootstock relation, and to provide new information which will enable the elucidation of the graft compatibility phenomenon. To achieve this, comparative formation of the graft union was studied on the basis of effect of time, cultivar and grafting methods, observation of graft union development at the union zone and ^{13}C tracer experiments in 'Fuyu' persimmon (*Diospyros kaki*) grafted onto different rootstocks in relation to physiological and morphological behaviors.

The result presented in this study show that (a) budding: late July to August was the ideal time, chip budding as the best method, 'Fuyu'/*D. kaki* showed the highest compatibility, (b) grafting: April was the ideal time, veneer grafting was the best method. 'Fuyu'/*D. kaki* showed highest compatibility than others. Initial response to grafting was the development of callus cells along the cut surfaces between the rootstocks and scion. Necrosis of the xylem elements near the cut surfaces were observed and after three to twelve months, new generating and connective tissues were seen in the transverse sections with necrotic cells in the callus at the base of the scion. The callus cells showed an irregular appearance and the cell walls seem to be thinner than the callus cells on the homograft union. After one to five years of grafting, the graft union zone of *D. lotus* showed more necrotic points than *D. kaki* and adhesive phenomenon was reduced which resulted in tree weakening in Fuyu / *D. lotus* combination.

Based on the results, all the growth and physiological parameters were significantly affected by the type of rootstock. The total DW was higher (191g) in *D. kaki* than in *D. lotus* (150g). Based on ¹³C-tracing experiment, the total amount of ¹³C was substantially higher (65.18mg) in *D. kaki* than in *D. lotus* (39.27mg). There were differences in the partitioning patterns of the ¹³C toward the organs with respect to the time after ¹³CO₂ feeding. The partitioning percentage on the parts above and below the graft union of *D. kaki* were 53.4 and 46.6, while in *D. lotus*, 68.5 and 31.5, respectively. The amount of ¹³C partitioned to leaves was significantly higher in middle parts. The translocation of ¹³C toward roots ≥ 2 mm and < 2 mm of *D. kaki* were significantly higher at each sampling period.

The girdling treatment was conducted to assess the direct effect of rootstock on ^{13}C assimilation. The ^{13}C per unit dry matter and ^{13}C in each organ were slightly higher in *D. kaki* than in *D. lotus*.

From these results above, the superior in 'Fuyu'/*D. kaki* grafts indicate the better involvement of translocation of carbohydrates and nutrient materials. The poor performance of 'Fuyu'/*D. lotus* combination in roots may be caused by partial blockage at the graft union or reduction in movement of water, nutrient through the graft union ultimately, malfunctioning of the graft union which adversely affected assimilate translocation. This implies that 'Fuyu'/*D. kaki* is more appropriate as compared to 'Fuyu'/*D. lotus* combination. Therefore, to overcome existing problems either to perform more research for selection of appropriate compatible rootstock within Nepalese local cultivars or it may introduce ideal Japanese persimmon rootstock to improve the productivity and longevity of persimmon industry in Nepal.

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