The Effect of Time, Cultivar, and Grafting Method on Graft Compatibility in Persimmon (*Diospyros*)©

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The formation of the graft union between scions of persimmon (*Diospyros kaki*) cultivars 'Fuyu' and 'Hiratanenashi' grafted on *D. lotus* and *D. kaki* rootstocks was studied. When studied over time, cultivar, and grafting method, visible differences in graft union formation were observed. Based on the results, late July to August was the best time to conduct budding operations and the best overall method to use was chip budding. 'Fuyu' budded on *D. kaki* showed the highest compatibility in contrast to other combinations tested. It would appear that the best time for veneer (shoot) grafting was April. 'Fuyu' when grafted on *D. kaki* showed the best compatibility. In the initial stage, both *D. kaki* and *D. lotus* developed callus and cambial cells in a similar manner, but the rate of growth was higher and faster with *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. In later stages, strength of the union was reduced and the tree weakened in 'Fuyu' grafted on *D. lotus* combinations.

INTRODUCTION

Asexual propagation in persimmon has proven more difficult when compared to other commercial fruit trees because of graft incompatibility. Comparative anatomical studies, over time with two *Diospyros kaki* cultivar scions ('Fuyu' and 'Hiratanenashi') and grafting different methods with *D. kaki* and *D. lotus* rootstocks, showed visible differences. Other research has shown in *Diospyros* that abnormal growth in the graft union can occur. Also Hodgson (1940) reported incompatibility problems between 'Fuyu' scions grafted onto *D. lotus* rootstocks. He observed that scions often grew more slowly, produced fruit earlier, and died within a few years. In this paper we focus on the effects of time, cultivar, and grafting methods on graft compatibility.

MATERIALS AND METHODS

Scions of 'Fuyu' and 'Hiratanenashi' were budded and grafted onto *D. kaki* and *D. lotus* in July, Aug., and Sept. of 2002 and March, April, and May of 2003, respectively, in order to observe graft union development. The grafting methods used in this experiment were chip budding and T-budding, and veneer and tongue grafting. The trees were planted in plastic containers and samples of 10 graft unions of each rootstock-scion combination were monitored and visual evaluation was done at 1, 3, 5, and 7 months after budding and grafting as well as anatomical observation with an Olympus BH-2 microscope.

RESULT AND DISCUSSION

The objective of this research was to study possible mechanisms that could be related to the adhesive phenomenon of *D. kaki* grafted with *D. lotus* and to provide

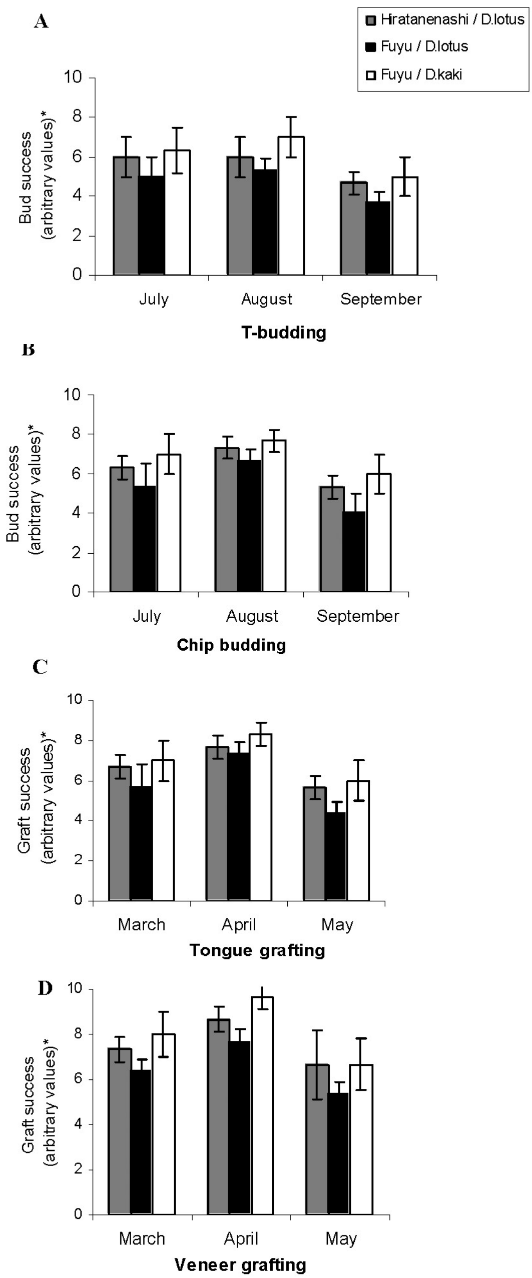

Figure 1. Graft compatibility T-(A) and chip (B) budding and tongue (C) and veneer (D) grafting using different scion / rootstock combinations. Qualitative assessment of the graft union: 10 = Excellent (perfect union), 7 = Good (graft union well formed but with callus tissue visible), 5 = Fair (union imperfectly formed with callus visible), 3 = Poor (deformed or swollen union and different diameters between stock and scion), 0 = Dead (dead scion due to incompatibility).

Table 1. Average growth rate between *Diospyros kaki* and *D. lotus* in 2002/2003.

Cultivar	Trunk length (cm)	Trunk diameter (mm)	Ave. shoot length (cm)	Leaf ratio
'Fuyu'/*D. kaki*	126	24	19	more
'Fuyu'/*D. lotus*	97	19	11	less

new information which will help to elucidate the possible mechanism for the graft compatibility and incompatibility.

Results showed positive interactions of time, cultivars, and grafting methods, in relation to graft compatibility. Late July to August was the best time to perform budding, with chip budding as the preferred method over T-budding. Regardless of the scion cultivar used, combination with *D. kaki* yielded favorable results, with respect to cultivar (Fig. 1 A, B). April was the best time for grafting, with veneer grafting the preferred method. Combinations with *D. kaki* gave the best results with respect to cultivars (Fig. 1 C, D).

Comparisons of shoot, leaf, tree, and trunk growth rates (Table 1), as well as photosynthetic activity, stomatal conductance, and transpiration rate (data not shown) were higher in *D. kaki* than *D. lotus*.

The tissue development in heterograft combinations 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 seem 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. In later stages, the adhesive phenomenon is reduced and the tree weakens in 'Fuyu'/*D. lotus* combination (Fig. 2).

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 3 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 7 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. 3)

Once the stock and scion are in intimate contact, the cambial region through meristematic activity produce parenchymatic cells. These cells soon intermingle and interlock, producing the callus tissue that fills the space between the two components and connecting the scion and the rootstock (Hartmann 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 have 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 could 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

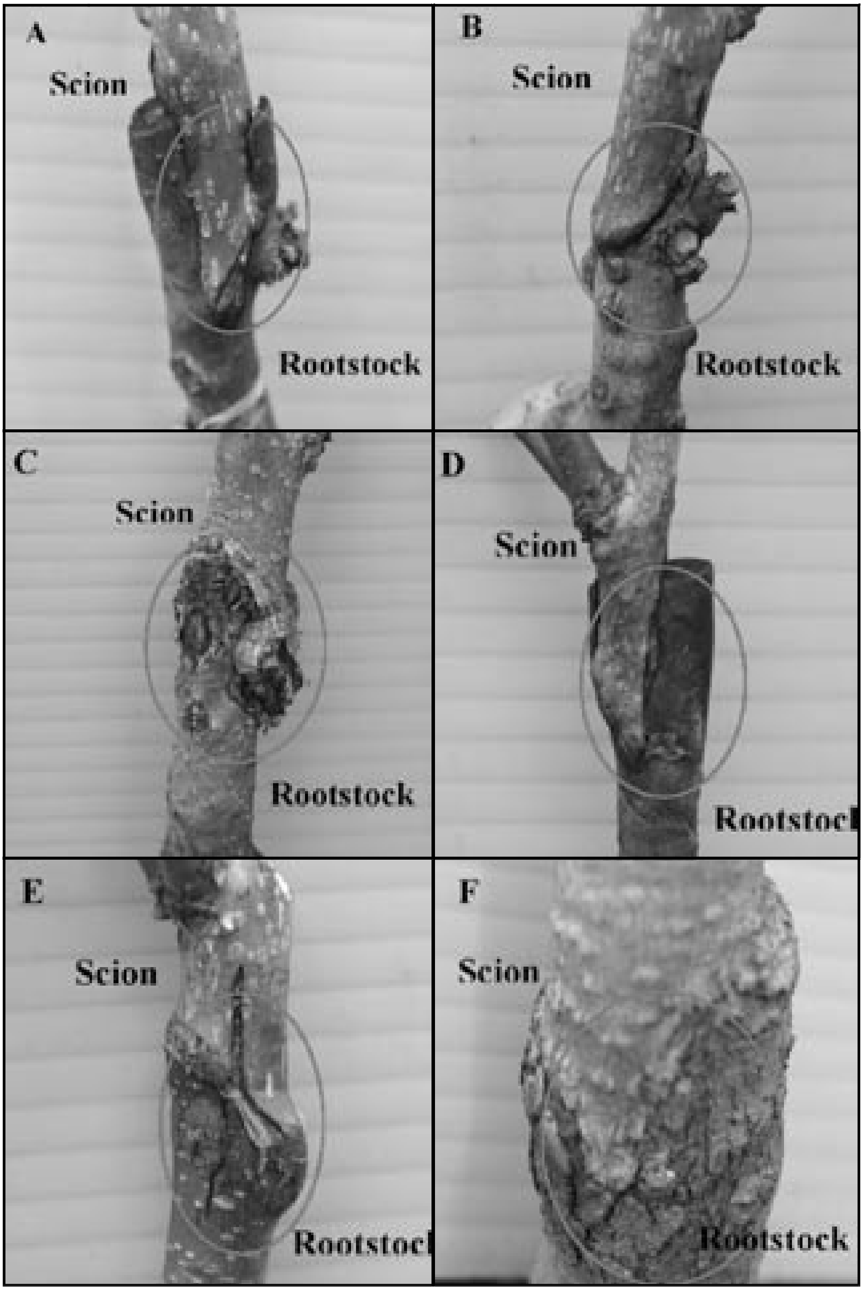

Figure 2. Photographs showing the graft union development with different scion and root-stock combinations: 'Fuyu' / *Diospyros lotus* (A.B.C), 'Fuyu' / *D. kaki* (D.E.F).Veneer grafting method (A.D) and tongue grafting method (B.E) after 5 month. Veneer grafting after 2.5 years (C. F).

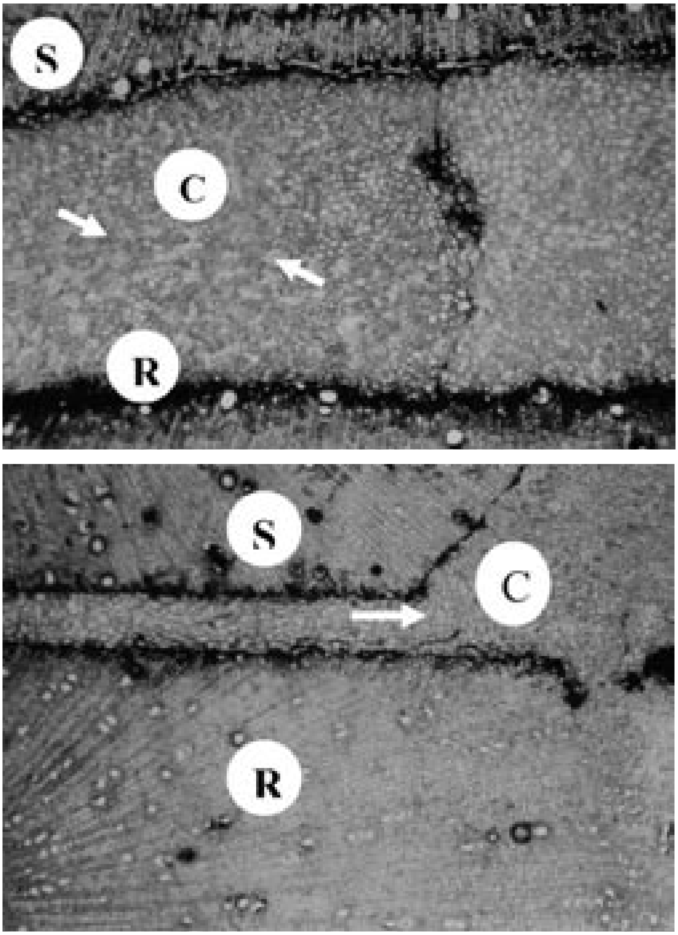

Figure 3. Transverse section of a 4-month old graft union of 'Fuyu' with *Diospyros kaki* (A) and 7-month old 'Hiratanenashi ' with *D. lotus* (B). Callus (C) cells (head of arrow) are observed between rootstock (R) and scion (S).

special kind of protein, called lections produces a mutual rejection of the opposing graft cells leading to the formation of an incompatible graft (Yeoman and Brown, 1976). In case of persimmon further study is needed.

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The Effect of Abscisic Acid on Growth Promotion©

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The involvement of gibberellins (GA) in plant growth promotion, stem elongation by stimulating cell division and elongation, and stimulation of bolting and flowering response under long days are widely known functions. Another hormone, abscisic acid (ABA), originally known as a hormone responsible for stimulation of stomata closure because of water stress (environmental response) and induction and maintenance of seed dormancy, is considered a growth inhibitor. Recently, research has shown a reverse effect of the natural type of ABA [(S)-(+)-Abscisic acid], which has shown an early differentiation of bud and flower promotion depending on the timing of application. Until recently, the synthetic racemic mixture of ABA (RABA) was used in research studies examining its physiological response and/or effects. This has lead to potential incorrect information obtained regarding ABA function (Kamuro, 1994). Because the natural type of ABA (SABA) was expensive to produce and apply to evaluate its functions was difficult. However, with the development of an inexpensive technique to synthesize SABA, it can now be artificially produced on a large scale at a moderate price.

The effects of exogenous ABA differ with species. In this work a mixture of SABA and GA was applied at a low concentrations to long-day, short-day, and day-neutral plants. Flower bud differentiation and flowering in long-day plants was promoted by application of these hormones. However, in short-day plants flowering was not promoted, but vigorous plant growth was induced. This suggests that SABA and GA interact and have synergetic effects on growth and flowering in plants. We expect that these results are applicable to increases in plant propagation and production.