Microscopic Observation on Graft Union of Dwarfing Rootstock for Japanese Persimmon (*Diospyros kaki* Thunb.)©

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INTRODUCTION

There are several reports relating to the growth of Japanese persimmon (*Diospyros kaki* Thunb.) grafted on dwarfing rootstocks (Yakushiji et al., 2008). However, the reason that the dwarfing rootstocks reduce the vegetative growth of scions is still unknown. Several factors, such as graft incompatibility and water conductivity between scion and rootstock (Izu et al., 2008), have been suggested as causing fruit trees to be dwarfed, and the same mechanisms may occur in the dwarfed trees of Japanese persimmon. Thus, we microscopically observed the graft union soon after grafting to verify whether there were any abnormalities of the regenerated tissues and also re-examined more than 1 year after the grafting.

MATERIALS AND METHODS

One-year-old 'Fuyu' seedlings, MKR1 (which is applying for cultivar registration Tetsumura et al., 2010), and FDR-1 (which dwarfed 'Fuyu' scion at Fukuoka Agricultural Research Center) were used as rootstocks. 'Fuyu' scions were grafted onto the rootstocks on 15 Mar. 2010. Every week graft unions were collected until 10 weeks after grafting. The samples were frozen by liquid nitrogen and then were embedded in carboxymethyl cellulose (CMC) gel (Fig. 1A). Sections 10 μm thick were produced by a cryostat (Leica CM1850, Leica Microsystems Co.), stained with hematoxylin-eosin, and observed microscopically. In addition, 2-year-old 'Fuyu' seedlings, MKR1, and FDR-1 were used for grafting 'Fuyu' scions on 14 Mar. 2011. One year and a half after the grafting, the graft union was observed microscopically.

RESULTS AND DISCUSSION

One week after the grafting, cell division at the cambium layer occurred in each rootstock. Vessels began to form in the regenerated calluses between 'Fuyu' and 'Fuyu' seedlings 6 weeks after the grafting and 8 weeks after the grafting between 'Fuyu' and MKR1 or FDR-1 (Fig. 1B and C). Vessels regenerated most quickly in the callus on 'Fuyu' seedling rootstocks, which had the largest number of vessel elements 10 weeks after the grafting (data not shown). Although no morphological abnormalities were observed at the graft union 10 weeks after grafting, we considered that the speed of vessel element restoration affected vegetative growth of the scions. One and a half years after grafting, overgrowth of FDR-1 under 'Fuyu' and gall formation at the graft union between 'Fuyu' and MKR1 were observed, whereas no specific feature at the graft union between 'Fuyu' and 'Fuyu' seedlings were observed (Table1). Morphological abnormalities at the graft unions were not microscopically observed in the cambium layer of 'Fuyu' seedlings and FDR-1, but the cambium layer of MKR1 waved severely (Fig. 2). We also investigated the effects of MKR1 and FDR-1 interstocks on the growth of 'Fuyu' scions grafted on 'Fuyu' seedlings (Ishimura et al., 2011). However, no morphological abnormalities at the graft unions were observed between stock and interstock nor between interstock and scion. Moreover, the interstocks dwarfed the scion less than those used as rootstocks. Therefore, we considered that the root system of the rootstocks effectively dwarfed the trees and affected the formation of union galls and the overgrowth of the rootstocks. Hasegawa et al. (2004, 2006)

reported that the strapping treatment on 2-year-old branch of Japanese persimmon 'Nishimurawase' increased the number of flower primordium and retarded the shoot growth, and concluded that the strapping treatment, which inhibited transport of nutrient and water in xylem, restricted growth of the branch, and percentage of dry matter increased to promote flower primordium formation. The trees grafted on MKR1 showed the same growth features. Therefore, the same phenomenon might occur at the graft union of MKR1. While the trees grafted on FDR-1 showed overgrowth of the rootstock, no morphological abnormalities were found in the cambium layer. But vessel of FDR-1 developed better than those of 'Fuyu' seedling and MKR1, and its diameter was larger. In addition, it was difficult to slice the graft union of FDR-1 by the cryostat. Thus, the cell structure of FDR-1 may make the trees dwarfed.

CONCLUSION

A lot of callus developed at the graft union between MKR1 and 'Fuyu', but vessels regenerated slowly. The cambium layer waved severely at one and a half year after the grafting. It was thought that this wave was one of the reasons for dwarfing; however, since the graft union between FDR-1 and 'Fuyu' did not show the waving cambium layer it was suggested that a different factor exists. We will try to elucidate the cause of dwarfing effects of MKR1 and FDR-1 by sap flow measurement and plant hormone treatment.

Table 1. Effects of rootstock on overgrowth of rootstock and gall formation at graft union of 'Fuyu' scion.

Rootstock	Overgrowth[z]	Gall formation[y]
'Fuyu' seedling	1.01 b[x]	1.30 b
MKR1	1.02 b	1.47 a
FDR-1	1.21 a	1.38 ab

[z] Diameter of rootstock/diameter of scion.

[y] Diameter of graft union/[(diameter of rootstock / diameter of scion) / 2)].

[x] Means in the same column followed by the same letter are not significantly different according to Tukey at $P<0.05$.

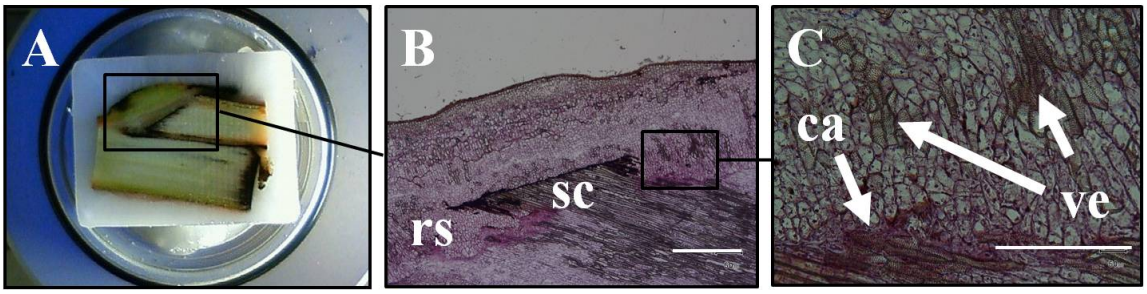

Fig. 1. (A): An embedded sample, (B, C): Longitudinal section of 'Fuyu' seedling (6 weeks after the grafting). rs: Rootstock, sc: Scion, ca: Cambium layer, ve: Vessel element Scale bar = 0.5 mm.

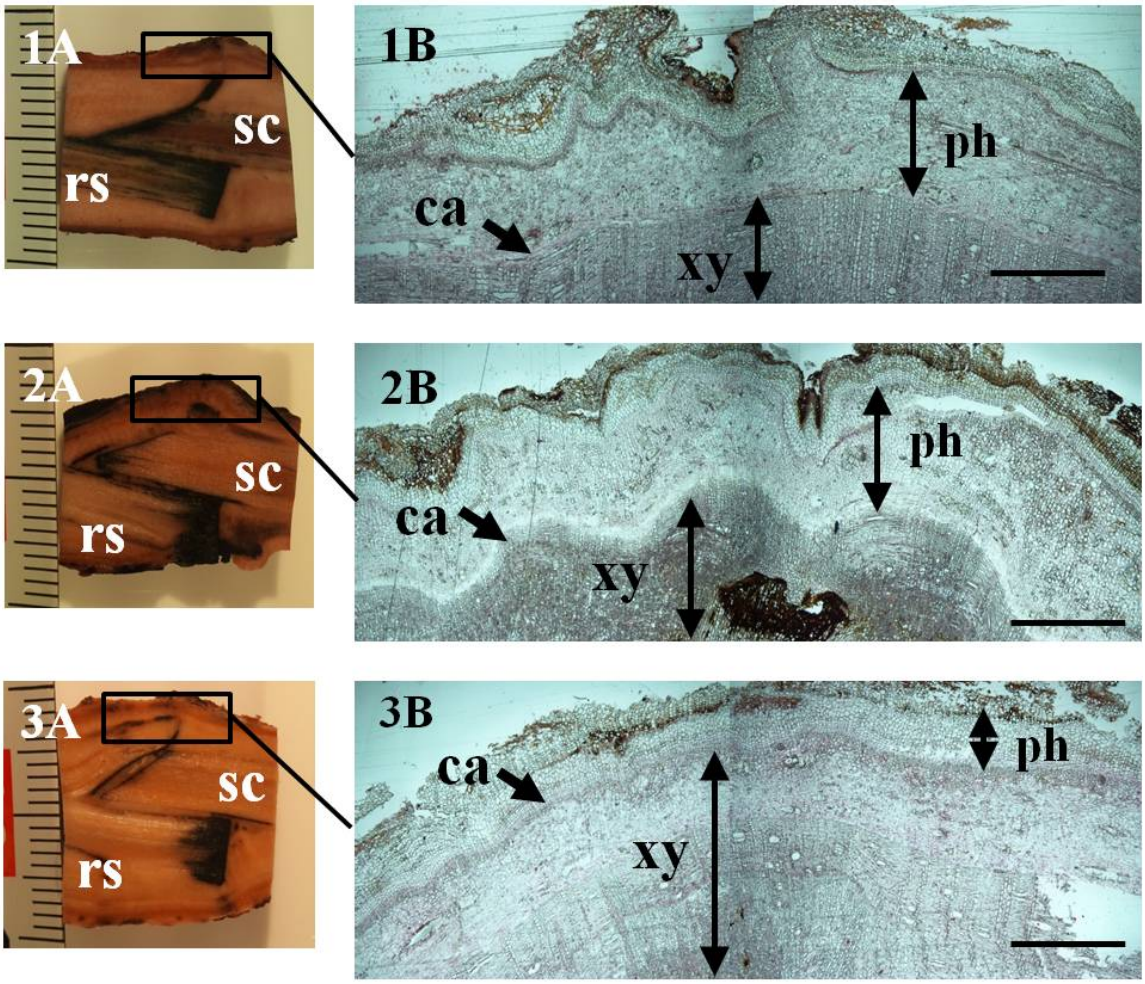

Fig. 2. Longitudinal sections of graft union between 'Fuyu' scions and rootstocks (1 year and a half after the grafting), 1 (A, B): 'Fuyu' seedling; 2 (A, B): MKR1; 3 (A, B): FDR-1. rs: Rootstock, sc: Scion, ca: Cambium layer, ph: phloem, xy: xylem. Scale bar=1.0 mm.

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