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The relationship of dwarfing and root anatomy in sweet cherry (*Prunus avium* L.) rootstocks

[Pertalian antara pengerdilan dengan anatomi akar pokok penanti ceri manis (*Prunus avium* L.)]

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Key words: dwarfing, vigour, rootstock, anatomy, sweet cherry (*Prunus avium* L.)

Abstrak

Kajian menggunakan bilik kabus dan bekas tanaman telah dijalankan di rumah hijau untuk mengkaji pertalian antara potensi pengerdilan dengan anatomi akar pokok penanti ceri manis jenis Mazzard (*Prunus avium* L.), Giessen (Gi) 148/1 (*Prunus cerasus* x *Prunus canescens*) dan Gi 148/8 (*Prunus cerasus* x *Prunus canescens*). Gi 148/1 yang mempunyai pertumbuhan cepat didapati sentiasa mempunyai luas saluran akar xilem yang tertinggi diikuti dengan Mazzard dan pokok penanti Gi 148/8 yang kerdil. Perbezaan nisbah kulit dengan kayu antara ketiga-tiga pokok penanti ceri manis adalah tidak ketara dan bernilai 0.67 hingga 0.77. Rawatan dengan 100 µM 2,3,5-asid triiodobenzoik (TIBA) sejenis perencat pengangkutan auksin menambahkan keluasan saluran xilem dan ketebalan lapisan kayu pada akar pokok penanti Giessen yang dicantum dengan varieti 'Bing' dan ini boleh mempercepat pertumbuhan pokok penanti Giessen. Tetapi rawatan dengan 100 bpj scopoletin, perencat oksidas asid indola-3-asetik (IAA) tidak memberi kesan yang ketara terhadap keluasan saluran xilem akar atau ketebalan lapisan kayu dalam kedua-dua kajian.

Abstract

Container and mist chamber experiments were conducted in a greenhouse to determine the relationship between the dwarfing potential of Mazzard (*Prunus avium* L.), Giessen (Gi) 148/1 (*Prunus cerasus* x *Prunus canescens*) and Gi 148/8 (*Prunus cerasus* x *Prunus canescens*), rootstocks and their roots anatomy. The vigorous Gi 148/1 consistently had the largest root xylem vessel areas followed by Mazzard and dwarfing Gi 148/8. The bark:wood ratios of Gi 148/8, Gi 148/1 and Mazzard roots were not significantly different and ranges from 0.67 to 0.77. Treatment with 100 µM 2,3,5-triiodobenzoic acid (TIBA), auxin transport inhibitor, increased xylem vessel area and wood thickness in roots of Giessen rootstocks grafted with 'Bing' and hence could enhance Giessen rootstocks vigour. But, treatment with 100 ppm of scopoletin, indole-3-acetic acid (IAA) oxidase inhibitor, had no significant effect on root xylem vessel area, bark or wood thickness in either experiment.

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Introduction

Anatomical studies of sweet cherry roots, stems and leaves are lacking; most anatomical studies of tree fruit have been of apple (*Malus domestica* Borck.). Anatomical structure of apple rootstocks has been shown to correlate well with the vigour they impart to scion cultivars. Extensive apple rootstock research has been conducted at the East Malling Research Station in England since the early 1900s. Beakbane and Thompson (1939) showed that dwarfing and vigorous apple rootstocks have bark/wood ratios of 1.0–2.3 and 0.61–0.98, respectively. In other words, vigorous rootstocks had relatively more wood consisting mostly of xylem vessels, fibres, parenchyma and rays. Vigorous rootstocks contained a higher concentration of IAA, or less auxin-degrading activity, than the dwarfing rootstocks. High IAA content favours xylem formation (Digby and Wareing 1966).

Rootstock vigour has been shown to be related to the vessel size of the root. In general, vigorous rootstocks possess larger diameter vessels (McKenzie 1961), although the relationship between the vessel size of rootstock and potential vigour of the scions was inconsistent. Beakbane (1941) reported that a very vigorous M.12 apple rootstock has significantly larger vessel diameter, but fewer vessels than a dwarfing M.9 rootstock. The vigorous M.2 rootstock showed a similar trend.

The stem anatomy of low vigour scions was shown to be different from that of the high vigour scions. Jaumien and Faust (1984) studied the anatomical structure of terminal shoots of 'Delicious' x 'Golden Delicious' F_2 hybrids that has varying degrees of vigour. The stem anatomy of these trees showed similar trends to the root anatomy of rootstocks of varying vigour, that is larger, but fewer vessels for high vigour trees and smaller, but more vessels for low vigour trees. In eight hardwood species studied by Baas et al. (1984), vessel frequency is higher and vessel diameter smaller in dwarf trees compared with vigorous trees. In some diffuse and semi-ring-porous species dwarf trees, however, there was little reduction in vessel diameter. In the ring-porous species, *Kalopanax pictus* (Thumb.) Nakai. and *Quercus acutissma* Carruthers., only the earlywood vessels were strongly reduced in diameter, and the latewood vessels were of equal diameter in dwarf and vigorous trees.

In addition to xylem, sieve tube elements of phloem have been studied in stems of sweet cherry clonal rootstocks. Misrili et al. (1996) found a direct relationship between vigour and the size of sieve tube elements in 12-year-old wood of Mahaleb rootstocks of varying vigour, but no correlation was found in 2-year-old trees.

The fruitfulness of the scion cultivar also has been shown to be related to root anatomy. Beakbane and Thompson (1947) found that apple rootstocks which induced dwarfing and fruitfulness in the scion also contain relatively fewer fibres but more rays in their roots. They also reported that cultivars with more than 60% root bark are more fruitful and dwarfing than vigorous unfruitful cultivars which has less than 50% root bark.

In these studies, root anatomy of three sweet cherry rootstocks was investigated. Bark and wood thickness and bark:wood ratio, and xylem vessel size were examined in roots of selected sweet cherry rootstocks. The effects on root anatomy of the indole-3-acetic acid (IAA) oxidase inhibitor, scopoletin and auxin transport inhibitor, 2,3,5-triiodobenzoic acid (TIBA) were also studied.

Materials and methods

Container experiment

Rootstocks of Mazzard, Gi 148/1 and Gi 148/8 were planted in containers on 16 May 1996. Peat, pumice and sand in 6:3:1 ratio were used as potting mix. The rootstocks were grown in a greenhouse in randomized complete block design consisting of four blocks with one plant of each genotype either treated with scopoletin, TIBA or untreated. These rootstocks were spaced 36 cm apart and grown at air temperatures between 20–31 °C, with 14 hours daylength.

TIBA (100 µM), an auxin transport inhibitor (Botia et al. 1992; Soumelidou et al. 1994), was applied in a lanolin paste around the trunk 5 cm above soil level. Scopoletin, an IAA oxidase inhibitor (Andreae 1952; Imbert and Wilson 1970), was applied at 100 ppm foliarly to run-off. Both treatments were repeated at 2, 4 and 6 days after initial treatment.

After 30 days, the potting medium was washed thoroughly from the roots. Mature brown roots, about 2 mm in diameter, were collected and fixed immediately in FAA solution for anatomical studies. Safranin and fast green were used to stain the sections according to the procedure of Berlyn and Miksche (1976).

An image analysis software system was used to quantify anatomical features. Bark and wood thickness, and xylem vessel lumen area were measured. A total of four replicates with three measurements per replicate were made for each parameter. An ANOVA General Linear Models Procedure of SAS was performed and a Least Significant Difference test was used to separate the mean differences at $\alpha = 5\%$, when ANOVA showed significant differences.

Mist chamber experiment

Dormant bareroot ‘Bing’ sweet cherry grafted on Gi 148/1 and Gi 148/8 rootstocks were gently washed off sawdust and planted in two identical mist chambers in a completely randomized design. TIBA (100 µM) was applied as a 3–5 mm wide band of lanolin paste around the graft union. Scopoletin (100 ppm) was sprayed foliarly to run-off. Both treatments were repeated 3 days and 6 days after initial treatment. The experiment was terminated 21 days after initial treatment. Mature root samples about 2 mm in diameter were collected at the end of the experiment from each plant and immediately fixed for anatomical studies as described previously. An image analysis software system was used to quantify bark and wood thickness and xylem vessel area. Statistical analysis was performed as in the previous experiment.

Results and discussion

Differences in root anatomical features were related to rootstock vigour. The dwarfing Gi 148/8 rootstock (480 μm^2) had significantly smaller xylem vessel area compared to the more vigorous Gi 148/1 rootstock (720 μm^2; *Plate 1* and *Figure 1*). Mazzard roots did not have statistically different xylem vessel area than the Giessen rootstocks. Beakbane (1941) also found that the very vigorous M.12 apple rootstock has significantly larger but fewer vessels than the dwarfing M.9 rootstock. McKenzie (1961) found vessel size in apple roots are related to rootstock vigour, with vigorous rootstocks possessing larger vessels than dwarfing rootstocks.

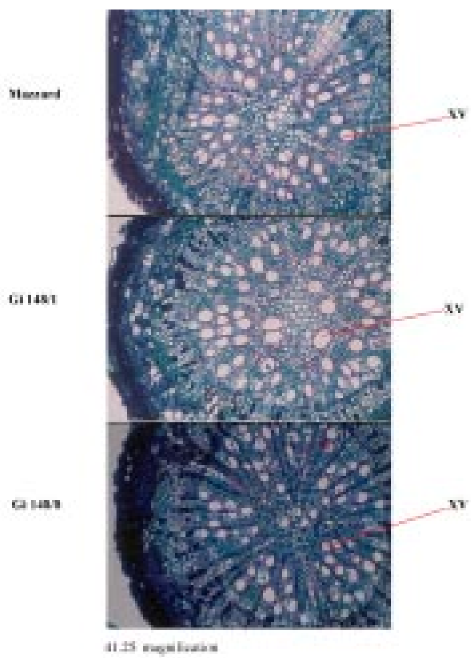

Plate 1. Root cross section of sweet cherry rootstocks stained with safranin-fast green. Note the relative size of the xylem vessel (XV)

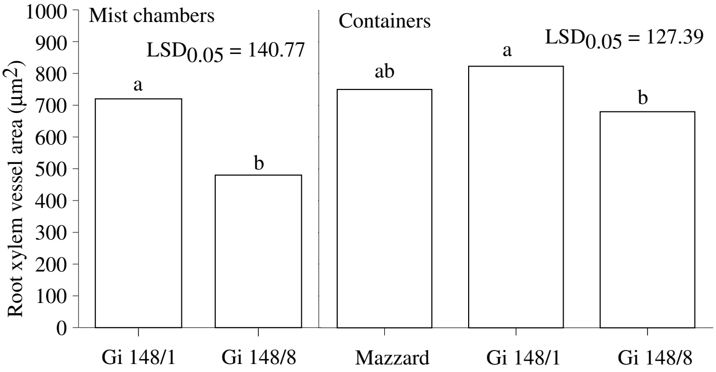

Figure 1. The root xylem vessel area of sweet cherry rootstocks of mist chamber and container experiments

Table 1. Thickness of bark, wood and bark:wood ratios of roots of sweet cherry rootstocks grown in containers and mist chambers

Rootstock	Bark (µm)	Wood (µm)	Bark:wood
Containers			
Mazzard	192.52	291.07	0.67
Gi 148/1	196.25	277.28	0.71
Gi 148/8	203.89	280.60	0.73
$LSD_{0.05}$	22.93	22.61	0.107
Mist chambers			
Gi 148/1	233.13	336.60	0.71
Gi 148/8	221.76	294.90	0.77
$LSD_{0.05}$	34.59	56.83	0.103

Unlike apple, in which dwarfing and vigorous rootstocks has bark:wood ratios of 1.0–2.3 and 0.61–0.98 respectively (Beakbane and Thompson 1939), the bark:wood ratios of these sweet cherry rootstocks were not significantly different and ranged from 0.67 to 0.77 *(Table 1)*. In other words, these ratios could not indicate sweet cherry rootstock vigour.

The auxin transport inhibitor, TIBA may block the polar transport of IAA from shoot to root in this experiment. Therefore, if TIBA is applied at the graft union it would be expected that auxin levels below the graft union would be reduced. IAA favours xylem formation, and high IAA levels induce larger xylem vessels and vice versa (Digby and Wareing 1966). But, when TIBA was applied to the graft union, wood thickness and xylem vessel area in the roots was significantly increased in both Giessen rootstocks (*Figure 2*).

TIBA inhibits IAA transport in *Lupin* hypocotyls and decapitates apple shoots (Botia et al. 1992; Soumelidou et al. 1994). The results with cherry were opposite to those of Digby and Wareing (1966), in that when they applied high levels of IAA, the stems produce more wide, "springwood" type vessels than narrow "summerwood" vessels. However, the results presented are in agreement with Aloni and his co-workers' hypothesis and findings for xylem vessel size control by auxin where larger xylem vessels size are induced by low auxin concentrations and smaller xylem vessels size is the result of high auxin concentrations (Aloni and Zimmermann 1983; Aloni and Indig 1985; Aloni 1987).

Scopoletin, may be an enzyme inhibitor at high concentrations or a promoter at low concentrations depending on the plant species (Andreae 1952; Imbert and Wilson 1970). In these studies, scopoletin was applied foliarly at 100 ppm, where it promoted IAA oxidase activity in the container experiment but not in the mist chamber experiment (Chong and Preston 2001). The probable reason is that 'Bing' leaves may have a different capacity for absorption and translocation of scopoletin. Higher IAA oxidase activities usually result

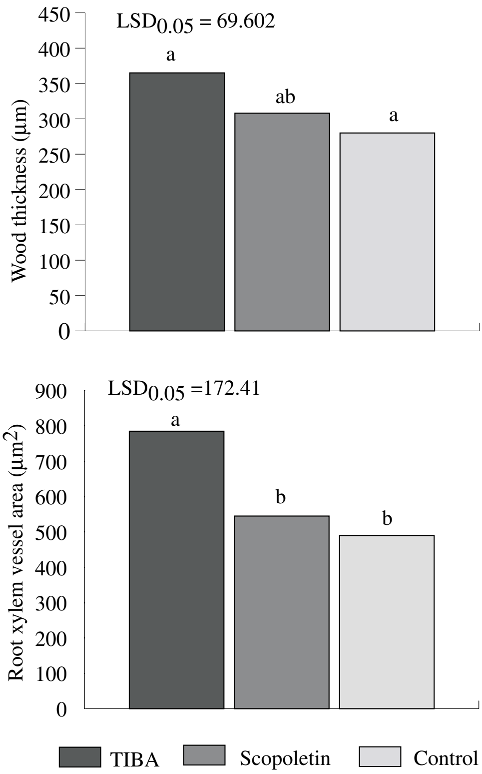

Figure 2. The treatment effects of TIBA and scopoletin on wood thickness and root xylem vessel area of two sweet cherry clonal rootstocks of mist chamber experiment

in lower IAA levels (Miller 1965; van Overbeek 1935), which would be expected to reduce xylem vessel growth and development (Digby and Wareing 1966). There were no significant differences in root xylem vessel area between scopoletin-treated and untreated cherry trees in either experiment (*Figure 2*). This suggests that there are probably other factors controlling xylem vessel growth and development besides auxin.

Digby and Wareing (1966) postulated that the relative levels of IAA and gibberellic acid (GA_3) are important in determining whether either xylem or phloem is produced. This view is supported by the work of Badr et al. (1970) who found that a certain proportion of auxin to gibberellins is required to stimulate cambial activity and subsequent xylem development. The actions of gibberellins on growth can be explained by its inhibitory influence on auxin degradation through its promotion of an unidentified endogeneous inhibitor of IAA oxidase activity (Pilet 1957; Galston 1959; McCune and Galston 1959; Housley and Deverall 1961; Kuraishi and Muir 1962)

Conclusion

There were some significant anatomical differences in roots among Mazzard, Gi 148/1 and Gi 148/8 sweet cherry rootstocks of varying vigour. Just like for apple and some hardwood species, root and stem xylem vessel are good indicators of sweet cherry rootstock vigour. These criteria could be used for evaluating and screening sweet cherry or tropical fruit trees rootstocks in selecting potential dwarfing genotypes. Bark:wood ratio is a good measure of vigour in apple, but was not in these sweet cherry rootstocks.

TIBA, an auxin transport inhibitor, increased xylem vessel area and wood thickness in roots of Giessen rootstocks grafted with ‘Bing’ but not in roots of ungrafted Giessen rootstocks. The reason for such response was not clear. Scopoletin had no significant effects on root xylem, bark or wood thickness of these three sweet cherry rootstocks studied. Probably 100 ppm concentration used was not the optimum concentration for sweet cherry rootstocks.

Another important area of future research in sweet cherry rootstocks is the relationship between tree vigour and area of the sieve tube elements. Phloem transport rather than xylem transport may be a more important limiting factor in dwarfing rootstocks. Since both xylem and phloem transport are important in tree growth and development, the ratios between area and number of xylem vessel and phloem sieve tube elements merit further investigation.

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