Phenolic Contents and L-Phenylalanine Ammonia-Lyase Activity in Peach Fruit as Affected by Rootstocks

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Summary

Phenolic contents in ripe fruit of six peach cultivars (Prunus persica Batsch) grafted on P. persica (‘Juseito’ or ‘Nagano–yaseito’), P. tomentosa (Nanking cherry) or P. japonica (Japanese bush cherry) rootstocks were evaluated. In all cultivars, fruit from trees grafted on Nanking cherry contained more total phenolic compounds than did those on ‘Juseito’ or ‘Nagano–yaseito’ rootstocks. However, there were wide variations in total phenolic contents among the cultivars; being low in ‘Saotome’, ‘Yahata–hakubo’, and ‘Chikuma’ but high in ‘Sanyo–suimitsu’, ‘Setouchi–hakuto’, and ‘Golden Peach’, ‘Sanyo–suimitsu’/Japanese bush cherry combination had the highest total phenolic content, compared with the same cultivar grafted on Nanking cherry or ‘Juseito’. The level of higher molecular fraction of phenolic compounds was positively correlated with the total phenolic concentration. In addition, phenolic contents, L-phenylalanine ammonia-lyase (PAL) activity and L-phenylalanine content in fruit of ‘Sanyo–suimitsu’ trees, grafted on ‘Juseito’ and Nanking cherry, were investigated during fruit growth. Although the seasonal trends of total and higher molecular phenolic contents in ‘Sanyo–suimitsu’ grafted on ‘Juseito’ and Nanking cherry were similar, they were higher in Nanking cherry throughout fruit development, especially during Stage 2. PAL activity was high during Stage 1 and the first half of Stage 2, followed by a decrease to undetectable levels by harvest. During the early growth stages, higher PAL activity and lower L-phenylalanine content were found in fruit from trees on Nanking cherry than ‘Juseito’.

Key Words: astringency, PAL activity and phenylalanine, peach fruit, phenolic contents, rootstocks.

Introduction

In Japan, ‘Nagano–yaseito’ (P. persica, wild form) is the principal rootstock for peach. However, scions grafted on this rootstock are quite vigorous, and the trees sometimes attain heights in excess of 4.5 meters. After the report of Fisher (1971), there have been several attempts to increase the production efficiency of peach trees by lowering tree height and using dwarfing rootstocks, such as Nanking cherry (P. tomentosa), Japanese bush cherry (P. japonica), and European plum (P. domestica) (Bargioni et al., 1983; Mizutani et al., 1985; Shimamura et al., 1987). Some scion cultivars, grafted on these rootstocks, have yielded favorable results in tree height, fruit quality, yield, and harvesting time (Fisher, 1971; Kubota et al., 1990; Mizutani et al., 1985; Nakano and Shimamura, 1983). However, one of the problems of these rootstocks is the strong astringency of the fruit, a major limitation of these dwarfing rootstocks. The total phenolic compound is closely related to the degree of astringency (Kubota, 1995) but there is little information about the relationship between phenolic levels in ripe peach fruit harvested from trees grafted on different rootstocks.

Phenylalanine, an aromatic amino acid, is the most important precursor of phenolic compounds for phenylpropanoid metabolism in higher plants except for some Gramineae. Phenylalanine ammonia-lyase (PAL) catalyzes the deamination of L-phenylalanine and produces t-cinnamic acid in the first step of phenolic metabolism, so that PAL is referred to as the key or rate-limiting enzyme in the phenolic biosynthesis. The concentrations of phenolic compounds and PAL activity in peach fruit have been investigated (Aoki et al., 1971; Camm and Towers, 1973; Craft, 1961; Goldstein and Swain, 1963), but information about their relationship in peach fruit from trees grafted on different rootstocks is meager.

We studied levels of phenolic compounds and astringency in fruit of six peach cultivars grafted on different rootstocks. Furthermore, seasonal changes in phenolic contents, PAL activity, and L-phenylalanine contents in ‘Sanyo–suimitsu’ fruit were compared between trees grafted on ‘Juseito’ and Nanking cherry rootstocks.
Materials and Methods

1. Phenolic contents in mature fruit

Six peach cultivars (Table 1) grafted seedling rootstocks of ‘Juseito’ (P. persica) or ‘Nagano・yaseito’ (P. persica, wild form) or cuttings of Nanking cherry (P. tomentosa) and Japanese bush cherry (P. japonica). ‘Setouchi・hakuto’ was grown in a commercial orchard; the other cultivars at the Research Farm, Okayama University. Eight fruit, harvested in 1987 at commercial maturity from four trees in each rootstock for five cultivars, whereas only three fruits from one tree of ‘Setouchi・hakuto’ were analyzed for fresh weight, total soluble solids content, and phenolic compounds. Total and higher molecular phenolic contents in fruit were assayed according to the method of Folin–Denis (Kubota et al., 2000; Swain and Hillis, 1959). Moreover, the approximate degree of astringency in each fruit was classified organoleptically by two research staff members into four levels: none, weak, medium, and strong.

2. Changes in phenolic contents, PAL activity, and L-phenylalanine contents during fruit growth

To monitor changes in phenolic and L-phenylalanine contents and PAL activity during fruit growth, three, 5-year-old ‘Sanyo・suimitsu’ trees grafted on ‘Juseito’ and Nanking cherry rootstocks growing in the Research Farm, Okayama University were selected. Two fruits were sampled from each tree at weekly intervals from May 15 until the end of July, 1986.

The crude PAL was extracted from the mesocarp; its activity was determined spectrophotometrically (Kataoka et al., 1983), and was expressed as μ moles of t-cinnamic acid formed per g fresh weight (FW) per hour. The absorbption spectrum of the reaction product was identical with that of authentic t-cinnamic acid (Kubota, 1995). Determinations were triplicated with one set of two fruit for each rootstock.

Amino acids, including L-phenylalanine, were extracted from the fresh mesocarp with 70% (v/v) ethanol, according to Kato et al. (1984). The ethanolic extract was passed through an Amberlite CG-120 (H+) resin column, and the adsorbed components were eluted with 2N-NH₄OH. The eluates were dried under vacuum, dissolved in a sodium citrate buffer (pH 2.2), and filtered. Amino acids in the filtrate were determined in an autoanalyzer (JLC-6AH, JEOL, Tokyo) with a series of sodium citrate buffers. The L-phenylalanine content is expressed as μ moles per 100 g FW.

Results and Discussion

The total phenolic level in fruit harvested from trees grafted on Nanking cherry was higher than in those on ‘Juseito’ or ‘Nagano・yaseito’ for all peach cultivars examined (Fig. 1). This agrees with the previous results reported on other peach cultivars (Kubota, 1998). The degree of astringency of the fruit was related to the total phenolics (Table 1), as previously reported by Kubota (1995). These observations indicate that rootstocks affect total phenolic content and, therefore, the astringency of peach fruit as well as other fruit characteristics such as fruit size, sugar content, and yield per unit area (Kubota et al., 1990; Nakano and Shimamura, 1983; Shimamura et al., 1987). Similarly, Utashiro and Yamada (1996) observed that rootstock plays an important role in polyphenol content of the European pear fruits. They showed that ‘Le Lectier’ fruit produced on

Table 1. Harvest date, fruit weight, total soluble solids contents and astringency of ripe fruit from six peach cultivars grafted on different rootstocks.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Rootstocks</th>
<th>Harvest date</th>
<th>Fruit weight (g)</th>
<th>Total soluble solids (° Brix)</th>
<th>Astringency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saotome</td>
<td>Juseito</td>
<td>Jun.21</td>
<td>91.8 ± 4.5x</td>
<td>10.8 ± 0.4</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Nanking cherry</td>
<td>Jun.21</td>
<td>112.4 ± 6.9</td>
<td>13.0 ± 0.8</td>
<td>–</td>
</tr>
<tr>
<td>Yahata・hakubo</td>
<td>Juseito</td>
<td>Jul.15</td>
<td>199.3 ± 4.5</td>
<td>9.2 ± 0.3</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Nanking cherry</td>
<td>Jul.10</td>
<td>210.0 ± 8.2</td>
<td>10.8 ± 0.5</td>
<td>–</td>
</tr>
<tr>
<td>Chikuma</td>
<td>Juseito</td>
<td>Jul.20</td>
<td>165.4 ± 5.5</td>
<td>10.2 ± 0.8</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Nanking cherry</td>
<td>Jul.17</td>
<td>204.5 ± 5.1</td>
<td>13.0 ± 0.7</td>
<td>–</td>
</tr>
<tr>
<td>Sanyo・suimitsu</td>
<td>Juseito</td>
<td>Jul.27</td>
<td>196.7 ± 5.7</td>
<td>9.3 ± 0.5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Nanking cherry</td>
<td>Jul.20</td>
<td>211.6 ± 6.6</td>
<td>10.0 ± 0.2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Japanese bush cherry</td>
<td>Jul.20</td>
<td>229.3 ± 5.9</td>
<td>13.5 ± 0.6</td>
<td>++</td>
</tr>
<tr>
<td>Setouchi・hakuto</td>
<td>Nagano・yaseito</td>
<td>Aug.26</td>
<td>406.9 ± 19.9</td>
<td>12.5 ± 0.7</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Nanking cherry</td>
<td>Aug.26</td>
<td>349.3 ± 7.7</td>
<td>12.1 ± 0.7</td>
<td>+++</td>
</tr>
<tr>
<td>Golden Peach</td>
<td>Juseito</td>
<td>Sep.9</td>
<td>339.7 ± 3.6</td>
<td>11.6 ± 0.3</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Nanking cherry</td>
<td>Aug.26</td>
<td>333.8 ± 5.2</td>
<td>14.1 ± 0.5</td>
<td>+++</td>
</tr>
</tbody>
</table>

x ‘Juseito’ and ‘Nagano・yaseito’ are seedlings; others are from clonal rootstock cuttings.

Evaluation based on organoleptic tests by two researchers. –; none, +; weak, ++; medium, +++; strong.

n=3–8, means ± SE.
Old Home/Quince A interstock (*Pyrus communis*)/rootstock (*Cydonia oblonga*) had higher polyphenol content than that on *Pyrus betulaefolia*. In this study, the rootstock effects on phenolic contents in fruit varied significantly among peach cultivars. For example, ‘Saotome’, ‘Yahata–hakuho’ and ‘Chikuma’ had a small difference in the total phenolic contents in fruit between Nanking cherry and ‘Juseito’ rootstocks, whereas a large difference was noted in ‘Sanyo-suimitsu’, ‘Setouchi–hakuto’ and ‘Golden Peach’ fruit between Nanking cherry and ‘Juseito’ or ‘Nagano–yaseito’ rootstocks. Notably a five-fold variation occurred in ‘Golden Peach’ fruit. This difference is attributed to the degree of graft–incompatibility among the scion/rootstock combinations.

Yamasaki (1984) observed that fruit harvested from ‘Hakuto’ peach trees, grafted on Nanking cherry, was quite astringent, compared to those from trees on Japanese bush cherry. In this experiment, the total phenolic contents in mature fruit of ‘Sanyo–suimitsu’ peach trees were highest on trees grafted on ‘Japanese bush cherry’, followed by Nanking cherry, and ‘Juseito’ (Fig. 1). The reasons for different responses of ‘Hakuto’ and ‘Sanyo–suimitsu’ peach tree/Japanese bush cherry rootstock are not known so that further investigation is needed.

The level of total phenolic compounds correlated with that of higher molecular phenolics, irrespective of the cultivar and rootstock, as previously described (Kubota et al., 1992, 2000). Of all cultivars examined, the ratio of higher molecular phenolic contents to total phenolic contents was markedly higher in fruit harvested from ‘Setouchi–hakuto’ and ‘Golden Peach’ trees grafted on Nanking cherry. Accordingly, mature fruit in these cultivars grafted on Nanking cherry was more astringent than those on other peach cultivars because astringency is associated with phenolic compounds with a certain degree of polymerization, corresponding to molecular weights ranging between 500 and 3000 (Van Buren, 1970). Although a number of phenols have been found in peach fruit (Senter and Callahan, 1990), simple phenolic compounds, such as catechin and anthocyanin, are not astringent, whereas the condensed tannins are (Macheix et al., 1990; Sistrunk, 1985). The molecular weights of the higher molecular phenolics assayed by the Folin–Denis method have not been estimated (Nakabayashi et al., 1967).

In this investigation, peach cultivars grafted on Nanking cherry and Japanese bush cherry, which had high total phenolic content also had more total soluble solids compared with fruits from ‘Juseito’ except for ‘Setouchi–hakuto’ (Table 1, Fig. 1), which agrees with our previous findings (Kubota et al., 1992). Sugar content in peach fruit is higher in the trees grafted on dwarfing rootstocks, such as Nanking cherry than in those on vigorous stocks, such as ‘Juseito’ (Shimamura et al., 1987). Furthermore, Kubota et al. (1993) found that total soluble solids and phenolic compounds in peach fruit are increased by girdling which restricts the

![Graph](image-url)
basipetal translocation of carbohydrates, similar to trees grafted on dwarfing rootstocks (Kubota et al., 1990). Thus, the accumulation of sugars and phenolic compounds in fruit harvested from peach trees grafted on Nanking cherry and Japanese bush cherry rootstocks is attributed to graft-incompatibility.

Although all peach trees tested were similarly irrigated during fruit ripening, soil moisture stress might also be related to the increase in phenolics in fruit from trees on dwarfing rootstocks because of their shallow and narrow rooting zones (Shimamura et al., 1987). It has been reported that soil moisture tension above -P 2 during Stage 3 of fruit growth is closely related with the occurrence of astringent peach fruit (Kubota and Kudo, 1992).

Total and higher molecular phenols of ‘Sanyo-suimitsu’ fruits from trees grafted on Nanking cherry and ‘Juseito’ decreased toward the end of Stage 1; they increased rapidly until the middle of Stage 2 and then decreased sharply to their lowest level during Stage 3 (Fig. 2). These trends are similar to those of other peach cultivars (Craft, 1961; Goldstein and Swain, 1963; Kubota et al., 2000). The total and higher molecular phenolic contents were higher in fruit on Nanking cherry than in those on ‘Juseito’ throughout the fruit growth. The former varied between two years from 57.4 to 104.6 mg per 100 g FW, whereas such difference was not observed in the latter. The large difference is attributed to some environmental factors, such as light intensity, soil moisture content, and temperature during fruit growth (Hesse, 1975; Kubota and Kudo, 1992).

In both rootstocks, PAL activity was high during Stage 1 and the first half of Stage 2, after which it declined (Fig. 3), being higher in fruit on Nanking cherry than on ‘Juseito’. Similar results were reported for other peach cultivars by Kubota and Iwase (1993), who also noted that the phenolic contents were higher in ‘Hakuto’ fruit from girdled scaffold branches than in those from the undamaged limbs, especially during Stage 2. Increased PAL activity was also observed in fruit from the girdled scaffolds during Stage 1, but not during Stages 2 and 3. Thus, the increase in the total phenolic content during Stage 2 resulted from increased PAL activity.

The concentration of L-phenylalanine, the substrate for PAL, may affect its activity (Da Cunha, 1987). Margna (1977) suggested that the biosynthetic rate of phenylpropanoid might not be controlled by PAL activity but by the L-phenylalanine concentration. The L-phenylalanine concentration was high in the early stages of fruit growth, but it decreased and subsequently remained low until maturation of both rootstocks. However, it was lower in fruit on Nanking cherry than it was on ‘Juseito’ throughout Stages 1 and 2 (Fig. 3) which may be explained by high PAL activity during Stage 1.

We conclude that: 1) depending on cultivars, the astringency of the mature peach fruit is affected by the rootstock. More total phenolics accumulate in fruit from trees grafted on dwarfing rootstocks than in those on vigorous rootstocks; 2) this accumulation occurs throughout the fruit development period, especially in Stage 2; 3) this increase in the phenolic compounds in Stage 2 is positively correlated with high PAL activity during Stage 1; and 4) such effects should be considered when selecting peach rootstocks.

**Literature Cited**


モモ果実のフェノール含量とL-フェニルアラニンアミノあるいは活性に及ぼす台木の影響

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摘要

モモ果実の渋味発生に及ぼす台木の影響を明らかにするため、共台（寿星桃または長野野生桃）、ユスラメ台およびニワメ台に接いたモモ6品種の成熟果実についてフェノール含量を比較した。果実の全フェノール含量は、供試した全ての品種において共台よりもユスラメ台樹で多かった。しかし、台木による差は品種によって異なり、‘さおとめ’, ‘八幡白鶴’および‘千曲’で小さく、‘山陽水蜜’, ‘瀬戸内白桃’および‘ゴールデンベーク’で大きく、特に‘ゴールデンビーチ’ではユスラメ台樹が共台樹の5倍以上であった。‘山陽水蜜’の場合、ニワメを台木とした果実の全フェノール含量はユスラメ台樹よりも多かった。全フェノール含量の多い品種や台木では高分子フェノール含量も多く、また全フェノールに占める高分子フェノールの割合は全フェノール含量の多い台木で高かった。共台とユスラメ台の‘山陽水蜜’樹を供試し、果実発育期間を通じてフェノール含量、L-フェニルアラニンアミノあるいは活性（PAL）活性およびフェニルアラニン含量を調査した。果実発育期間中の全フェノール含量は高分子フェノール含量の変化の様相は、両台樹ともほぼ同じであったが、ユスラメ台樹では果実発育期間を通じて共台樹よりも多く、特に第2期の増加が大きかった。PAL活性は、両台樹とも果実発育第1期に高く、特にユスラメ台樹で高かった。果実発育第2期以降のPAL活性は低く、台木による差もみられなかった。ユスラメ台樹では果実発育第1~2期のフェニルアラニン含量が共台樹よりも少なかった。