Correlations between Soluble Sugar and Phenol Contents in Leaves and Pear Scab Resistance

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Abstract
Pear scab caused by Venturia nashiicola is one of the most important diseases in pears in China. Better understanding of the relationships between soluble sugar and phenol contents in the leaves and pear scab will assist in developing resistant cultivars for management of this disease. However, such relationships were poorly understood. In this study, we determined soluble sugar and total phenol contents in the leaves of 29 pear cultivars with varying levels of scab resistance in May, June, August and September of 2012 and related their levels to pear scab resistance. Results of this study demonstrated that: 1) the changes in soluble sugar and total phenol contents in the leaves of interspecific pears appeared to be relatively stable in May, June and August. In September, however, significant changes in their levels were observed. The levels of soluble sugar content in the leaves of Pyrus bretschneideri and P. communis were significantly lower. Total phenol content in the P. communis leaves reached the highest. 2) Differences in soluble sugar and total phenol contents in the leaves of pear cultivars with varying levels of scab resistance were insignificant in May, June, August, and September. Total phenol content in pear cultivars that were immune to scab was slightly higher than that in other resistant cultivars in May, June, August, and September. 3) Soluble sugar and total phenol contents in the leaves of different interspecific and scab-resistant cultivars had no significant correlations with scab incidence in May, June, August and September. 4) There was a negatively correlation (p=0.039, r=-0.386) between soluble sugar content in the leaves of different cultivars and scab incidence in August.

Keywords: Pear scab; Soluble sugar content; Total phenol content

Introduction
The genus Pyrus L. in the Rosaceae family is cultivated in the temperate regions of the world. China is one of the origins of this genus and has diverse cultivars and genotypes. Pear has become an important industry in China. However, pear diseases are one of the major factors limiting the pear production. Pear scab caused by Venturia nashiicola is one of the most important diseases in the southern and northern pear-producing areas of China as well as in the North America. Among more than 80 diseases reported in pear, pear scab is the number one disease causing significant damage to pear in China [1,2]. Pear scab can damage stems, petioles and apical shoots at the stages of flowering through maturity. The disease causes deformity and failure of uniform fruit enlargement. Stem infection by V. nashiicola also reduces yield and quality in the following year. In epidemic years, the disease is able to cause up to 90% of leaves infected and 50% to 70% fruit diseased [3].

Genetic resistance can be the most effective means to manage pear scab. Better understanding of the relationships between soluble sugar and phenol contents and pear scab resistance will assist in developing resistant cultivars. However, such relationships remain largely unknown. Phenolic compounds are among the most common compounds found in fruits and vegetables [4]. Production of phenolic compounds plays a role in plant genetic resistance against pathogen infection [5]. Production and accumulation of phenolic compounds occur in plant tissue where pathogens infect. Changes in the levels of phenolic compounds are frequently associated with susceptibility or resistance to plant diseases including apple scab (V. inaequalis) [5-9]. The levels of phenolic content in the apple fruit of scab-resistant cultivars have been shown to be significantly higher than in susceptible cultivars [6]. Sugar content in the fruits may also vary with fruit species and cultivars [10]. However, changes in phenol and sugar contents in the leaves of pear and their associated scab resistance are unknown. In this study, we focused on determining soluble sugar and total phenol contents in the leaves of 29 pear cultivars and their correlations with pear scab resistance. Understanding of the relationships between soluble sugar and phenol contents in the leaves, rather than in the fruit, and pear scab will shorten the selection process of developing resistant cultivars for management of pear scab.

Experiment design
This study was conducted as random experimental design in a 10-year-old pear tree orchard at the Pomology Institute, Shanxi Academy of Agricultural Sciences, Shanxi, China. This orchard consisted of 29 pear cultivars with different scab resistance within different interspecies (Table 1). Fifty fully-expanded leaves per tree were collected on the 15th of May, June, August and September in 2012. There were four trees (replicates) per treatment. Leaves collected from the trials were rated

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individually using the following scale: 0=no symptoms, 1=<5% of leaf area affected, 2=5 to 14% of leaf area affected, 3=15 to 24% of leaf area affected, 4=25 to 34% of leaf area affected, and 5=≥ 35% of leaf area affected. Based on disease ratings, disease incidence was calculated as the percentage of the leaves showing symptoms of scab. Disease index was also calculated as follow: Disease index=∑[number of leaves with affected, 4=25 to 34% of leaf area affected, and 5=≥ 35% of leaf area affected].

| Cultivar          | Species               | Disease index | Disease incidence | Disease resistance rank |
|-------------------|-----------------------|---------------|-------------------|-------------------------|
| Red Bartlett      | P. communis Linn.     | 0             | 0                 | immune                  |
| Lvbaoshi          | P. hybrid             | 0             | 0                 | immune                  |
| Abate Fetei       | P. communis Linn.     | 0             | 0                 | immune                  |
| Hongxianshu       | P. hybrid             | 3.6           | 23%               | highly resistant         |
| Jinzao            | P. hybrid             | 3.3           | 21%               | highly resistant         |
| Early Red Du Comice | P. communis Linn.     | 4.6           | 27%               | highly resistant         |
| Jinmi             | P. hybrid             | 4.2           | 25%               | highly resistant         |
| Jinfeng           | P. bretschnrideri Rehd.| 2.3           | 18%               | highly resistant         |
| Meirensu          | P. hybrid             | 4.9           | 29%               | highly resistant         |
| Yusu              | P. hybrid             | 4.4           | 26%               | highly resistant         |
| Huangguan         | P. hybrid             | 4.0           | 24%               | highly resistant         |
| Jinsu             | P. hybrid             | 5.9           | 33%               | moderately resistant     |
| Bayuehong         | P. hybrid             | 6.7           | 35%               | moderately resistant     |
| Dangshansui       | P. bretschnrideri Rehd.| 7.6           | 38%               | moderately resistant     |
| Whangkium bai     | P. pyrifolia Burm Nakai | 6.4       | 34%               | moderately resistant     |
| Yuluixiang        | P. hybrid             | 10.3          | 38%               | moderately susceptible   |
| Housui            | P. pyrifolia Burm Nakai | 12.6         | 40%               | moderately susceptible   |
| Mantianhong        | P. hybrid             | 13.4          | 43%               | moderately susceptible   |
| Zaomeinhsu        | P. hybrid             | 12.3          | 40%               | moderately susceptible   |
| Qiyuesu           | P. hybrid             | 11.7          | 38%               | moderately susceptible   |
| Zaosu             | P. hybrid             | 14.2          | 45%               | moderately susceptible   |
| Kouisi            | P. pyrifolia Burm Nakai | 15.7         | 47%               | moderately susceptible   |
| Pingguoli         | P. bretschnrideri Rehd.| 23.2          | 63%               | highly susceptible       |
| Yali              | P. bretschnrideri Rehd.| 26.3          | 68%               | highly susceptible       |
| Shuofeng          | P. hybrid             | 24.2          | 64%               | highly susceptible       |
| Zhongxiang        | P. bretschnrideri Rehd.| 25.2          | 67%               | highly susceptible       |
| Bayueu            | P. hybrid             | 22.3          | 62%               | highly susceptible       |
| Xuehuai           | P. bretschnrideri Rehd.| 21.1          | 62%               | highly susceptible       |
| Fall              | P. communis Linn.     | 23.3          | 64%               | highly susceptible       |

Table 1: Scab disease index, disease incidence and scab resistance rank of 29 pear cultivars used in this study.

### Results

Differences in the contents of soluble sugar and total phenols in the leaves of different pear interspecies

The soluble sugar content in the leaves of interspecies remained relatively stable in May, June and August, and increased dramatically in September when the soluble sugar content in *P. pyrifolia* reached the highest (83.3 mg/g) (Table 2). On the other hand, soluble sugar content in *P. pyrifolia* was not significantly different from those in *P. communis*, *P. hybrid* and *P. bretschnrideri*.

The content of total phenols in the leaves of different interspecies were insignificant different in May, June and August. In September, the content of total phenols in *P. communis* reached the highest (8.7 mg/g) (Table 3), and were not significantly different from those in *P. bretschnrideri* and *P. hybrid*. There was no difference in the content of total phenols in *P. bretschnrideri*, *P. pyrifolia* and *P. hybrid*.

Differences in the contents of soluble sugar and total phenols in the leaves of different scab-resistant cultivars

The levels of soluble sugar contents of soluble sugar in leaves varied with different scab-resistant cultivars (Figure 1). However, there were statistically insignificant in soluble sugar content in different scab-resistant cultivars in May, June and August.

In general, the levels of total phenol content in leaves were not statistically different among different scab-resistant cultivars evaluated in May, June and August (Figure 2). However, the scab-resistant cultivars showed differences in the contents of soluble sugar and total phenols over a cropping season.

### Table 2: Changes in soluble sugar content (Mean ± S.E. in mg/g DW) in pear leaves of four different interspecies over a cropping season.

| Species               | Soluble sugar content (mg/g) |
|-----------------------|-----------------------------|
|                       | May | June | August | September | Value |
| *P. bretschnrideri* Rehd. (n=6) | 37.4 ± 3.7a | 26.8 ± 3.9a | 43.0 ± 3.3a | 68.1 ± 4.4b | 66.1 ± 4.8 |
| *P. pyrifolia* Burm Nakai (n=3) | 23.4 ± 4.4a | 32.0 ± 3.5a | 52.6 ± 12.2a | 83.3 ± 4.4a | 66.1 ± 4.8 |
| *P. communis* Linn. (n=4) | 39.1 ± 9.5a | 30.0 ± 6.2a | 50.5 ± 12.4a | 76.3 ± 5.5ab | 66.1 ± 4.8 |
| *P. hybrid* (n=16) | 41.2 ± 4.1a | 26.9 ± 2.0a | 48.7 ± 2.8a | 80.8 ± 1.8a | 66.1 ± 4.8 |

Values in column followed by the same letter are not significantly different at P=0.05

### Table 3: Changes in total phenolic content (Mean ± S.E. in mg/g DW) in pear leaves of four different interspecies over a cropping season.

| Species               | Total phenolic content (mg/g) |
|-----------------------|------------------------------|
|                       | May | June | August | September | Value |
| *P. bretschnrideri* Rehd. (n=6) | 3.0 ± 0.8a | 3.1 ± 0.6a | 4.4 ± 0.4a | 4.0 ± 0.1b | 66.1 ± 4.8 |
| *P. pyrifolia* Burm Nakai (n=3) | 4.0 ± 1.4a | 2.3 ± 0.6a | 4.7 ± 0.5a | 6.0 ± 0.8ab | 66.1 ± 4.8 |
| *P. communis* Linn. (n=4) | 4.0 ± 4.0a | 3.7 ± 0.8a | 5.9 ± 1.6a | 8.7 ± 2.6a | 66.1 ± 4.8 |
| *P. hybrid* (n=16) | 2.3 ± 2.3a | 2.5 ± 0.3a | 4.3 ± 0.3a | 3.9 ± 0.4b | 66.1 ± 4.8 |

Values in column followed by the same letter are not significantly different at P=0.05
Changes in total phenolic content in the leaves of different scab-resistant cultivars had the greatest levels of total phenols in each of the four months.

Correlation between the contents of soluble sugar and total phenols in leaves and pear scab

The contents of soluble sugar and total phenols were not significantly different in May, June, August and September (P>0.05) (Table 4), indicating that there was no correlation between the contents of soluble sugar and total phenols in the leaves of immune pear cultivars, resulting in restrained expansion of the invasive hyphal. Furthermore, the current research also shows that the content of total phenols in the leaves of different interspecies of P. communis was highest in September when pear leaves were at maturity.

Note: *r values represent the correlation coefficients between the contents of soluble sugar or total phenols of the pear leaves and scab resistance in different interspecies, positive values represent positive correlations, whereas negative values represent negative correlations; *P<0.05 indicates significant correlations between the contents of soluble sugar or total phenols in the leaves of different interspecies and scab resistance.*

| Month | Content | P | r | May | June | Aug. | Sep. | May | June | Aug. | Sep. |
|-------|---------|---|---|-----|------|------|------|-----|------|------|------|
| May   | -0.773  | 0.125| 0.058 | 0.149 | 0.125 | 0.097 | 0.114 |
| June  | -0.810  | 0.051| 0.058 | 0.149 | 0.125 | 0.097 | 0.114 |
| Aug.  | -0.744  | 0.058 | 0.058 | 0.149 | 0.125 | 0.097 | 0.114 |
| Sep.  | -0.773  | 0.125| 0.058 | 0.149 | 0.125 | 0.097 | 0.114 |

| Month | Content | P | r | May | June | Aug. | Sep. | May | June | Aug. | Sep. |
|-------|---------|---|---|-----|------|------|------|-----|------|------|------|
| May   | 0.094   | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| June  | -0.386  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| Aug.  | -0.286  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| Sep.  | -0.330  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |

Note: *r values represent the correlation coefficients between the contents of soluble sugar or total phenols of the pear leaves and scab resistance in different interspecies, positive values represent positive correlations, whereas negative values represent negative correlations; *P<0.05 indicates significant correlations between the contents of soluble sugar or total phenols in the leaves of different interspecies and scab resistance.*

Discussion

The current study investigated the contents of soluble sugar and total phenols in the leaves of 29 pear cultivars in relation to pear scab resistance. Results of this study show that the contents of total phenols in the immune cultivars of pear were higher than those in other disease-resistant cultivars in May, June, August and September (P<0.05) (Table 5).

Correlation between the contents of soluble sugar and total phenols in the leaves of all 29 cultivars evaluated and pear scab

The contents of soluble sugar and total phenols in the leaves of all 29 cultivars evaluated and pear scab resistance were not correlated significantly with disease index or disease incidence in May, June, August and September (P>0.05) except one case where disease incidence was negatively correlated with soluble sugar content in August (P=0.039, r=-0.386) (Table 6) .

Table 4: The correlation between the contents of soluble sugar and total phenols in the leaves of immune pear cultivars.

| Month | Content | P | r | May | June | Aug. | Sep. | May | June | Aug. | Sep. |
|-------|---------|---|---|-----|------|------|------|-----|------|------|------|
| May   | 0.298   | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| June  | -0.333  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| Aug.  | -0.776  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| Sep.  | -0.706  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |

Note: *r values represent the correlation coefficients between the contents of soluble sugar or total phenols of the pear leaves and scab resistance in all 29 cultivars, positive values represent positive correlations, whereas negative values represent negative correlations; *P<0.05 indicates significant correlations between the contents of soluble sugar or total phenols in the pear leaves of all 29 cultivars and scab resistance.*

Table 5: The correlation between the contents of soluble sugar and total phenols in the leaves of all 29 cultivars evaluated.

| Month | Content | P | r | May | June | Aug. | Sep. | May | June | Aug. | Sep. |
|-------|---------|---|---|-----|------|------|------|-----|------|------|------|
| May   | 0.271   | 0.784| 0.051 | 0.061 | 0.189 | 0.626 | 0.493 | 0.039 |
| June  | 0.234   | 0.155| 0.222 | 0.205 | 0.233 | 0.242 | 0.213 | 0.190 |
| Aug.  | 0.234   | 0.155| 0.222 | 0.205 | 0.233 | 0.242 | 0.213 | 0.190 |
| Sep.  | 0.234   | 0.155| 0.222 | 0.205 | 0.233 | 0.242 | 0.213 | 0.190 |

Note: *r values represent the correlation coefficients between the contents of soluble sugar or total phenols of the pear leaves and scab resistance in all 29 cultivars, positive values represent positive correlations, whereas negative values represent negative correlations; *P<0.05 indicates significant correlations between the contents of soluble sugar or total phenols in the pear leaves of all 29 cultivars and scab resistance.*

Table 6: The correlation between the contents of soluble sugar and total phenols in the leaves of all 29 cultivars evaluated and pear scab.

| Month | Content | P | r | May | June | Aug. | Sep. | May | June | Aug. | Sep. |
|-------|---------|---|---|-----|------|------|------|-----|------|------|------|
| May   | 0.298   | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| June  | -0.333  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| Aug.  | -0.776  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
| Sep.  | -0.706  | 0.627| 0.135 | 0.183 | 0.337 | 0.503 | 0.076 | 0.087 |
This result is in agreement with the finding of Li et al. [14] that scab resistance in pear leaves increased with the growth of leaves.

In this study, the content of soluble sugar within leaves varied significantly among different interspecies of *P. bretschneideri* and *P. communis* in September, which is similar to Li et al. [14] findings. In the structure, physiological and bio-chemical study of pear sprout, Liu et al. [15] put forwards that the content of sugar possibly could play a significant role in disease resistance. Low soluble sugar content may retrain the growth and expansion of vegetative hyphae of *V. nashicola* between plant cells. However, the results of our study show that the content of soluble sugar in the leaves of different pear varieties had a negative correlation with disease incidence in August, which is in disagreement with Liu et al. [15] findings.

The sugar content in the pear leaves increased in September for the immune cultivars and all other resistant types with no significant difference among them. However, increased values in total phenols were observed on the immune cultivars in September but not on all other resistant types. These results suggest that determination of total phenol content in the leaves near maturity may provide a valuable tool to evaluate pear scab resistance, especially to identify the immune-type resistance. However, more investigation is needed to verify the findings from the current study.

Based on the contents of soluble sugar and total phenols in the leaves of 29 pear cultivars within different interspecies and scab resistance evaluated in this study, the contents of soluble sugar and total phenols in the leaves of different interspecies remain relatively stable in May, June and August, whereas the content of soluble sugar in *P. bretschneideri* and *P. communis* had a notable change in September, when the content of total phenols in the *P. communis* leaves was highest. Meanwhile, the contents of soluble sugar and total phenols in the leaves of different interspecies and disease-resistant cultivars remained relatively stable in May, June, August and September, but the immune cultivars had much higher levels than other different disease-resistant cultivars in that period. The contents of soluble sugar and total phenols in the pear leaves of different interspecies and disease-resistant cultivars had no correlation with the incurrence of pear scab resistance. The content of soluble sugar in different pear leaf varieties had a negative correlation with disease incidence in August.

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