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

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Abstract

Pear scab caused by *Venturia nashicola* 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 bretschnrideri* 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 nashicola* 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. nashicola* 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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Cultivar	Species	Disease index	Disease incidence	Disease resistance rank
Red Bartlett	*P.communis* Linn.	0	0	immune
Lvbaoshi	*P. hybrid*	0	0	immune
Abate Fetel	*P. communis* Linn.	0	0	immune
Hongxiangsu	*P. hybrid*	3.6	23%	highly resistant
Jinzaosu	*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
Dangshansuli	*P. bretschnrideri* Rehd.	7.6	38%	moderately resistant
Whangkeum-bae	*P. pyrifolia* Burm Nakai.	6.4	34%	moderately resistant
Yuluxiang	*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
Zaomeisu	*P. hybrid*	12.3	40%	moderately susceptible
Qiyuesu	*P. hybrid*	11.7	38%	moderately susceptible
Zaosu	*P. hybrid*	14.2	45%	moderately susceptible
Kousui	*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
Bayuesu	*P. hybrid*	22.3	62%	highly susceptible
Xuehuali	*P. bretschnrideri* Rehd.	21.1	62%	highly susceptible
Fali	*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.

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 same rating scale×value of the corresponding scale]/[total number of leaves rated×greatest value of scale]×100. Cultivar scab resistance was ranked based on the following scale: immune=0 ≤ disease index <1, highly resistant=1 ≤ disease index <5, moderately resistant=5 ≤ disease index <10, moderately susceptible=10 ≤ disease index <20, and highly susceptible=disease index value ≥ 20. Collected leaves were stored in a freezer (-20°C) for 24 hours. The leaves were washed in water containing 5% laundry detergent and rinsed three times in distilled water, and blot dried with filter paper. The washed leaves were dried at 105°C for 30 min and then at 80°C for 24 h. The dried leaves were crushed, screened, and preserved in desiccators.

The contents of soluble sugar and total phenols in the treated leaves were determined separately. Soluble sugar content was determined using the Yemm and Willis' method [11]. Treated leaves (0.12 g) were placed in 10 mL of deionized water, boiled for 30 min and then centrifuged under 12 000×g for 10 min, repeated 3 times. The resulting extracts were dissolved in 50 mL deionized water and determined in the anthrone reagent composing of anthrone (0.15 g), oil of vitriol (84 cm^3) and H_2O (16 cm^3). The rate of absorbance (0.1 mL of the extracts and 3 mL of anthrone reagent) was recorded at 620 nm. Total soluble phenols in the treated leaves were extracted with 80% of methanol and quantified using the Folin–Ciocalteu method with gallic acid as a standard with detection at 750 nm [12]. Data were analyzed by analysis of variances (ANOVA) and treatment means were separately by the LSD test at P=0.05 with SPSS. Correlation analyses were also performed with SPSS.

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, August and September.

In general, the levels of total phenol content in leaves were not statistically different among different scab-resistant cultivars evaluated in May, June, August and September (Figure 2). However, the scab-

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

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

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

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.

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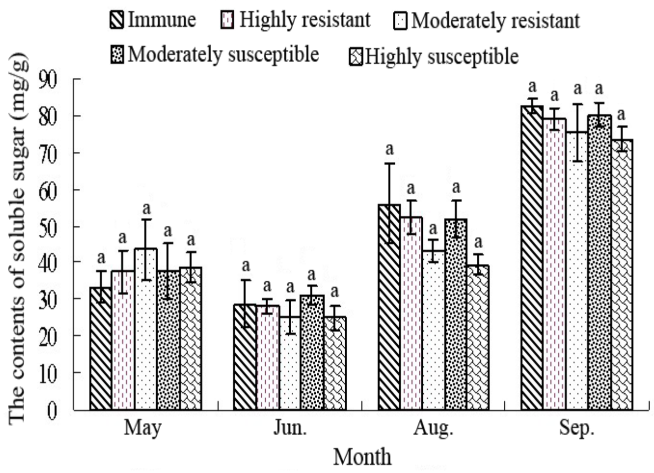

Figure 1: Changes in soluble sugar content in the leaves of different scab-resistant cultivars.

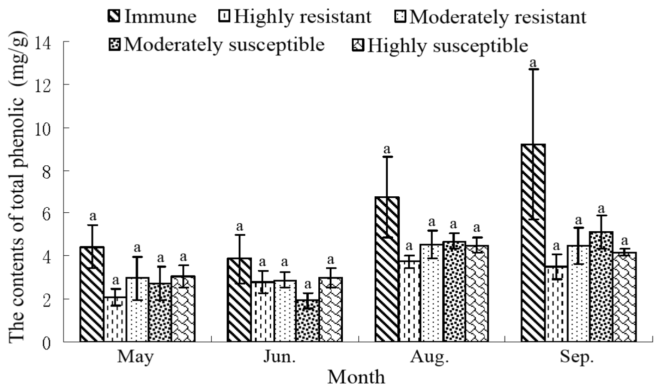

Figure 2: Changes in total phenolic content in the leaves of different scab-resistant cultivars.

		Disease index				Disease incidence			
		May	June	Aug.	Sep.	May	June	Aug.	Sep.
Soluble sugar content	r	-0.773	-0.838	-0.810	-0.787	-0.744	-0.773	-0.810	-0.787
	P	0.125	0.076	0.051	0.058	0.149	0.125	0.097	0.114
Total phenolic content	r	-0.262	0.005	-0.519	-0.606	-0.320	-0.163	-0.660	-0.710
	P	0.670	0.994	0.370	0.279	0.599	0.793	0.225	0.179

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 pear leaves of different interspecies and scab resistance

Table 4: The correlation between the contents of soluble sugar and total phenols of pear leaves and pear scab resistance in four different interspecies.

immune 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 different interspecies and *V. nashicola* and pear scab resistance.

		Disease index				Disease incidence			
		May	June	Aug.	Sep.	May	June	Aug.	Sep.
Soluble sugar content	r	0.298	-0.333	-0.762	-0.706	0.550	-0.401	-0.838	-0.823
	P	0.627	0.584	0.135	0.183	0.337	0.503	0.076	0.087
Total phenolic content	r	-0.272	-0.406	-0.393	-0.493	-0.495	-0.556	-0.617	-0.710
	P	0.658	0.497	0.513	0.399	0.397	0.330	0.268	0.179

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 scab-resistant 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 different scab-resistant cultivars and scab resistance

Table 5: The correlation between the contents of soluble sugar and total phenols of pear leaves and pear scab resistance among five scab-resistance ranks.

		Disease index				Disease incidence			
		May	June	Aug.	Sep.	May	June	Aug.	Sep.
Soluble sugar content	r	0.053	-0.133	-0.352	-0.251	0.094	-0.133	-0.386	-0.286
	P	0.784	0.501	0.061	0.189	0.626	0.493	0.039*	0.133
Total phenolic content	r	0.271	0.234	0.234	0.234	0.242	0.213	0.190	0.195
	P	0.155	0.222	0.205	0.223	0.205	0.268	0.324	0.311

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 contents of pear leaves and pear scab resistance in all 29 cultivars evaluated.

The results of correlation analysis indicate that the contents of soluble sugar and total phenols were not different among different scab-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) .

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. These results can be explained by the findings of Luo et al. [13] that the invasion of the fungus *V. nashicola* boosted the production of free 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.

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. bretschnrideri* 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. bretschnrideri* 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 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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