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Comparative assessment of sugar and malic acid composition in cultivated and wild apples

Baiquan Ma [a,d], Jie Chen [b,c,d], Hongyu Zheng [a,d], Ting Fang [a,d], Collins Ogutu [a,d], Shaohua Li [a], Yuepeng Han [a,*], Benhong Wu [b,c,*]

[a] Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden of the Chinese Academy of Sciences, Wuhan 430074, PR China
[b] Beijing Key Laboratory of Grape Sciences and Enology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, PR China
[c] CAS Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, PR China
[d] Graduate University of Chinese Academy of Sciences, 19A Yuquanlu, Beijing 100049, PR China

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ABSTRACT

Soluble sugar and malic acid contents in mature fruits of 364 apple accessions were quantified using high-performance liquid chromatography (HPLC). Fructose and sucrose represented the major components of soluble sugars in cultivated fruits, whilst fructose and glucose were the major items of sugars in wild fruits. Wild fruits were significantly more acidic than cultivated fruits, whilst the average concentration of total sugars and sweetness index were quite similar between cultivated and wild fruits. Thus, our study suggests that fruit acidity rather than sweetness is likely to have undergone selection during apple domestication. Additionally, malic acid content was positively correlated with glucose content and negatively correlated with sucrose content. This suggests that selection of fruit acidity must have an effect on the proportion of sugar components in apple fruits. Our study provides information that could be helpful for future apple breeding.

1. Introduction

Soluble sugars and organic acids are important components of fruit taste, and together with aromas, they have a strong impact on the overall organoleptic quality of fruits (Borsani et al., 2009). In fruits, soluble sugars are mainly composed of sucrose, fructose, and glucose, whilst malic, citric, and tartaric acids are the primary organic acids (Mahmood, Anwar, Abbas, Boyce, & Saari, 2012). Fructose, glucose, and sucrose differ significantly in sweetness (Doty, 1976). Similarly, malic, citric, and tartaric acids are not equally acidic. Therefore, fruit taste depends on the content and type of soluble sugars and organic acids (Bordonaba & Terry, 2010). Moreover, organic acids also play an important role in fruit coloration by serving to stabilize anthocyanins, and extend the shelf life of fresh fruits and their processed products. It is thus clear that soluble sugars and organic acids are important indicators of fruit taste quality. The soluble sugar and organic acid composition has become a major focus of fruit tree breeding programs worldwide (Basha, Vasanthaiah, Kambiranda, Easwaran, & Queeley, 2012).

Apple (*Malus* × *domestica* Borkh.) is one of the most important fruit crops in temperate regions. To date, many apple cultivars have been developed for various uses such as cooking, fresh eating, and cider production. HPLC assessment of sugar and organic acid composition has been conducted in many different fruits such as berries (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012), stone fruits (Bae et al., 2014), and apple (Wu et al., 2007). However, HPLC assessment of organic acid and sugar composition has been so far conducted for a limited number of varieties, and the results indicate that the sugar and organic acid accumulation in apple fruits has two distinct characteristics. One is that apple fruits are rich in fructose, which accounts for 44–75% of the total sugars (Wu et al., 2007). Another is that malic acid is the dominant acid in apple fruits, accounting for up to 90% of the total organic acids (Wu et al., 2007; Zhang, Li, & Cheng, 2010). A more detailed knowledge of sugar and organic acid composition in apple cultivars is still needed because it will not only benefit the selection of apple genotypes suitable for different kinds of utilisation, but it is also crucial for diabetics or people who react sensitively to fruit acids. There is evidence to suggest that the cultivated apple was initially domesticated from *Malus sieversii*, a wild apple native to the Tian Shan Mountains of central Asia

* Corresponding authors at: Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden of the Chinese Academy of Sciences, Wuhan 430074, PR China (Y. Han); Beijing Key Laboratory of Grape Sciences and Enology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, PR China (B. Wu).
E-mail addresses: yphan@wbgcas.cn (Y. Han), bhwu@ibcas.ac.cn (B. Wu).

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(Dzhangaliev, 2003). Subsequently, hybridization and introgression between cultivars and wild relatives have played an important role in the evolution of domesticated apples and may continue to be a factor in increasing the genetic diversity of domesticated apples (Cornille, Giraud, Smulders, Roldán-Ruiz, & Gladieux, 2014; Harris, Robinson, & Juniper, 2002). Wild *Malus* taxa are thus considered useful sources of genetic variation for apple breeding. The use of these natural resources in breeding for fruit quality improvement requires a good understanding of their fruit quality characteristics. However, little information is available concerning the evaluation of fruit quality of wild *Malus* species.

China is the leading apple producer in the world, generating more than 34 million tons annually, which represents approximately half of the world apple production. In China, apples are grown for fresh consumption and juice processing. Recently, fruit quality attributes such as taste, flavour and texture have gained increasing attention in Chinese apple industry, and fruit quality improvement is becoming an important part of the apple-breeding programme in China. Assessing fruit quality traits of apple germplasm including wild *Malus* species is an essential prerequisite to a proper utilisation of apple germplasm in breeding programmes. Thus, we recently initiated a programme to measure fruit quality traits in apple germplasm. In this study, we report on the assessment of soluble sugar and malic acid contents in mature fruits of 364 apple accessions, including 321 cultivars worldwide and 43 wild relatives. Sugars and organic acids are important components of fruit taste (Wu et al., 2007), and fruit taste is expected to be one of the phenotypes selected by human during the domestication process (Cornille et al., 2014; Parker et al., 2010). Therefore, we also compared the sugar and acid content between cultivated and wild apples, and the artificial selection on fruit acidity and sweetness during the process of apple domestication was discussed. Our study not only provides an organoleptic fruit database of apple germplasm which is helpful for future apple breeding, but is also useful for understanding the anthropogenic selection during the domestication process of apple and other fruit trees.

2. Materials and methods

2.1. Plant material

Three hundred and sixty-four apple accessions used in this study are grown at Xingcheng Institute of Pomology of the Chinese Academy of Agricultural Sciences, Xingcheng, Liaoning, China. Of the 364 accessions, 321 are cultivars, including 75 from China, 64 from United States, 33 from Japan, 22 from Russia, 12 from Canada, 11 from France, 10 from England, 5 from New Zealand, 3 from Belgium, 3 from Germany, 2 from North Korea, 1 from Australia, 1 from Estonia, 1 from Holland, 1 from Italy, 1 from The Czech Republic, and 76 with uncertain origin. The remaining 43 are wild relatives, including 6 *Malus prunifolia*, 5 *Malus baccata*, 5 *Malus robusta*, 4 *Malus hupehensis*, 4 *Malus toringoides*, 3 *Malus spectabilis*, 2 *M. sieversii*, 2 *Malus ioensis*, 1 *Malus asiatica*, 1 *Malus kansuensis*, 1 *Malus manshurica*, 1 *Malus micromalus*, 1 *Malus zumi*, 1 *Malus floribunda*, 1 *Malus sylvestris*, 1 *Malus angustifolia*, 1 *Malus sargentii*, 1 *Malus sikkihensis*, 1 *Malus sieboldii*, and 1 *Malus xiaojinensis*. Approximately 5 g of young leaves were collected in the spring season, and 200 mg was used for genomic DNA extraction.

Leaf samples were immediately frozen in liquid nitrogen, and then stored at −75 °C until use. Fruits were randomly harvested in 2010 when most were considered to be mature based on background colour and blush development followed by a confirmation of the seed colour changing to brown, together with the previous records of maturity date. Each accession had three replicates, consisting of 10 fruits. Fruit samples were mechanically peeled and cored. Pulps were cut into small sections, immediately frozen in liquid nitrogen, and then stored at −40 °C for sugar and acid measurement by high performance liquid chromatography (HPLC).

2.2. Measurement of sugars and malic acid

The samples from each replicate were ground into powder in liquid nitrogen using an A11 basic Analytical mill (IKA, Germany). One gram of power was extracted with 6 ml deionized water obtained from a Milli-Q Element water purification system (Millipore, Bed ford, MA, USA). After centrifugation at 5000×*g* for 15 min, the supernatants were decanted, passed through a SEP-C18 cartridge (Supelclean ENVI C18 SPE), and filtered through a 0.45 μm Sep-Pak filter.

The filtered supernatants were used to measure fructose, glucose, sucrose and malic acid using a Dionex P680 HPLC system (Dionex Corporation, CA, USA). Sugars were detected by a Shodex RI-101 refractive index detector with reference cell maintained at 40 °C. A Transgenomic CARB Sep Coregel 87C column (300 mm × 7.8 mm i.d., 10 μm particle size) with a guard column cartridge (Transgenomic CARB Sep Coregel 87C cartridge) was used. The column was maintained at 85 °C with a Dionex TCC-100 thermostated column compartment. Degassed, distilled, deionized water at a flow rate of 0.6 ml/min was used as the mobile phase. The injection volume was 10 μL. Malic acid was detected using a Dionex PDA-100 detector. The Inertsil ODS-3 column (250 mm × 4.6 mm i.d., 5 μm particle size) with a guard column cartridge (Sunchrom C18 cartridge) was used. The column was maintained at 40 °C. Samples were eluted with 0.02 mol/L KH_2PO_4 solution with pH 2.4. The flow rate was 0.8 ml/min. Eluted compounds were detected by UV absorbance at 210 nm. The Chromeleon chromatography data system was used to integrate peak areas according to external standard solution calibrations (reagents from Sigma Chemical Co., Castle Hill, NSW, Australia). Sugar and acid concentrations were expressed in mg/g fresh weight (FW).

2.3. Estimation of apple taste parameter

Total sugar content was indicated by the amount of three major sugars found in apple fruits, i.e., glucose, sucrose, and fructose. Sweetness index for each apple accession was estimated according to a modified method as previously described by Keutgen and Pawelzik (2007). Briefly, the contribution of each major sugar found in apple fruits was calculated, considering that fructose and glucose are 1.7 and 0.75 times sweeter than sucrose, respectively. As a result, sweetness index = 0.75 [Glucose] + 1.0 [Sucrose] + 1.7 [fructose].

2.4. Data analysis

All statistical analyses were carried out using SPSS statistics 17.0 (SPSS Inc., Chicago, Illinois). Correlations between experimental variables were calculated using Spearman's rank correlations. Significant difference values were estimated using two-tailed tests. Unless otherwise stated, significant differences were $P < 0.05$. The variation in the sugar and organic acid content was calculated using Origin 8.0 software (OriginLab Corp.). The dendrogram showing genetic relationships between wild and cultivated apples was constructed using DARwin software 4.0 (http://darwin.cirad.fr/darwin/Home.php).

2.5. Genotyping of apple germplasm

A total of 17 SSR markers distributed across different linkage groups of the apple genome were selected to screen apple germplasm (Table 1). The PCR reaction was performed using the