猕猴桃研究进展(VI)

Advances in *Actinidia* Research (VI)

黄宏文 主编

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内 容 简 介

本书收录了国内猕猴桃会议代表提交的论文，以及近两年国外猕猴桃研究的新动态论文，内容涉及从产业、栽培管理技术、病虫害防治、生物技术采后贮藏加工、遗传育种到资源利用的7个主要领域。所录论文是国内外近年来从事猕猴桃研究、管理、开发利用人员的成果或工作积累，以及针对一些产业发展问题和新技术应用提供建议。

本书是供广大从事猕猴桃科研、教学、推广与生产、市场销售等领域人员参考的重要资料，适合科研人员、教师、大中专学生、职业院校及从事果树行业管理的行政部门人员、基层科技人员，以及猕猴桃爱好者阅读。

图书在版编目(CIP)数据

猕猴桃研究进展(Advances in Actinidia Research). 第6辑/黄宏文主编. —北京：科学出版社，2011

ISBN 978-7-03-032872-4

Ⅰ. 猕… Ⅱ. 黄… Ⅲ. 猕猴桃－国际学术会议－文集 Ⅳ. S663.4-53

中国版本图书馆CIP数据核字(2011)第243267号

责任编辑：张颖兵/责任校对：梅 莹

责任印制：彭 超/封面设计：苏 波

科 学 出 版 社 出版

北京东黄城根北街16号

邮政编码：100717

http://www.sciencep.com

武汉市首壹印务有限公司印刷

科学出版社发行 各地新华书店经销

*

开本：787×1092 1/16

2011年11月第 一 版 印张：27

2011年11月第一次印刷 字数：681 000

定价：100.00元

(如有印装质量问题，我社负责调换)

前　言

（PREFACE）

猕猴桃（*Actinidia chinensis* Planch.）是20世纪初以来驯化栽培成功的水果，至今仅有100余年的历史。猕猴桃以其独特的风味，富含维生素C、饮食纤维、多种矿物营养，以及清肠健胃功效而得到广泛青睐，成为重要的水果种类之一。猕猴桃的驯化栽培被认为是近代由野生到人工商品化栽培最成功的植物驯化范例。

我国自1978年开展全国猕猴桃属植物野生资源普查以来，经过30余年的发展，充分利用自己的资源优势大步追赶世界先进水平，现已成为世界栽培面积第一、产量第二的猕猴桃生产大国。至2010年，我国栽培面积达7.0万hm^2，占世界栽培总面积的45%；产量达49.0万t，占世界产量的28%。我国猕猴桃产业的快速发展进程凝结了我国猕猴桃科技人员、果农及经销者多年的拼搏和不懈的追求！也正是为了这个追求，中国园艺学会猕猴桃分会于2004年开始，每2年召开1次全国学术与产业研讨会，已分别在湖南吉首、广东和平、陕西杨陵和四川蒲江举办了四届，每次会议之后均出版了《猕猴桃研究进展》系列学术论文集，本卷是继《猕猴桃研究进展（V）》之后的第VI卷，是在收集第四届猕猴桃研讨会会议论文的基础上，增加了世界主要生产国的知名专家在猕猴桃上的科研和产业等方面的研究论文，旨在为中外研究人员提供世界猕猴桃研究进展和市场、生产最新信息。

本书共分新品种选育、资源开发与利用、栽培及生理、生物技术、贮藏保鲜与加工、产业与市场6个部分，系统地提供了国内外猕猴桃科研与产业、市场发展趋势，也结合我国猕猴桃发展现状和存在问题，提出了我国猕猴桃科研重点、产业方向和市场策略。

由于能力和水平所限，书中疏漏之处在所难免，恳请大家批评指正。借此机会再次向为本书提供文献的作者和对分会和本书给予支持的领导和同仁们表示衷心的感谢，并希望继续得到你们更多的指导和支持！

中国园艺学会猕猴桃分会

2011.6.10 于武汉

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（一）新品种选育

利用多因子分析鉴定二倍体中华猕猴桃品种果实品质相关挥发性物质种类

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摘　要　在对果实中的糖、酸类物质影响风味进行了很多关注的同时,往往对果实风味与香气同样有重要作用的挥发性物质却难以定义。那些对中华猕猴桃品种风味有重要贡献挥发性物质的鉴定,可以大大提高果实风味改良育种的效率与降低成本。本研究就是利用多因子分析来探讨挥发性化合物、品质鉴评和中华猕猴桃品种果实特性来确定影响风味的主要挥发性物质,以及优良亲本及选择方法。我们从一个育种群体中利用主成分分析(PCA)方法,依据果实特性和风味多样性相关性状为原则选择了24个基因型进行测试,发现了72个挥发性物质。事实证明,利用扩展的多因子分析可以从有限的材料中发掘最大量的信息,在主成分分析中,为避免其中单因子相关矩阵分析的缺陷,我们基于挥发性物质表型相关多因子进行聚类分析,成功将这些物质被聚类成4大家族。根据这些分支,进一步利用PCA和多因子线性回归分析,探讨了挥发性物质、感官评价和果实性状间的相关性。主成分分析提供了对影响消费者反应的挥发性物质进行综合权衡的依据,13种对中华猕猴桃品种果实品质有重要影响的挥发性物质被鉴定,'Hort16A'中有5种脂类对其果实风味和香气有重要决定性作用。与"酸味"、"果实成熟"、"Hort16A非典型性香气"和"非猕猴桃味(一般指'海沃德')"相关的挥发性物质也鉴定出来。同时,对具备不同挥发性物质的潜在亲本选择以及猕猴桃的风味改良选育的可行性方法也进行了总结。

关键词　猕猴桃　育种　风味　挥发性物质　多因子分析

Identifying Volatile Compounds Associated with Sensory and Fruit Attributes in Diploid *Actinidia chinensis*(kiwifruit) Using Mutivariate Analysis

1　Introduction

Kiwifruit belong to the genus *Actinidia*, which comprises more than 60 species native to large parts of China and some neighbouring countries. All known species are dioecious and grow as long-lived, perennial, woody vines. The familiar green-fleshed kiwifruit of the cultivar 'Hayward' belongs to the species *A. deliciosa*(A. Chev.) Liang et A. R. Ferguson and is grown commercially in many countries. Yellow-fleshed cultivars of *A. chinensis* Planch. are grown widely in China and increasingly in other kiwifruit producing countries. The 'Hayward' cultivar is characteristically described by consumers as having a fresh, sweet and acid flavour, while the commercial yellow-fleshed *A. chinensis* cultivar 'Hort16A' (marketed as ZESPRI® GOLD Kiwifruit) is described as having sweet, banana and blackcurrant-like flavours (Jaeger et al.,2003).

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Flavour is a key aspect of fruit quality and is generally defined as a combination of aroma and taste sensations. While the roles of sugars and acids in taste are well known and described, the volatile compounds that contribute to flavour and odour are more difficult to define. Many of the earlier studies on kiwifruit aroma focused on the composition of volatile compounds. Up to 90 volatiles were identified from fruit of 'Hayward' (Young et al., 1983; Young and Paterson, 1985; Takeoka et al., 1986; Paterson et al., 1991; Young and Paterson, 1995; Young et al., 1995; Perera et al., 1998). Between 40 and 80 volatiles were detected from fruit of *A. arguta* genotypes (Matich et al., 2003). Hex-*E*2-enal was identified as the major component in mature fruit of 'Hayward' but on further ripening, ethyl butanoate began to dominate the profile (Bartley and Schwede, 1989). Young et al. (1995) reported that the sweetness of ripe 'Hayward' kiwifruit was associated with high concentrations of esters, which account for up to 85 % of the total volatiles in kiwifruit (Crowhurst et al., 2008). In the yellow-fleshed cultivar 'Hort16A', acetaldehyde, hexanal, hex-*E*2-enal and ethyl butanoate were found to be important contributors to aroma (Friel et al., 2007).

However, because many volatile compounds are not flavour active in a particular product, one of the major difficulties in studying odour is the determination of those compounds that make a real contribution to the flavour of food (Mistry et al., 1997). Odour activity values (OAVs) are used to estimate the odour contribution of individual food volatiles, and are calculated as the ratio between the concentration of an individual substance in a sample and the threshold concentration of that substance (i. e., the minimal concentration that can be detected by the human nose) (Rothe and Thomas, 1963). Buttery (1993) found that of 400 volatiles identified in tomatoes, only 16 reached their OAVs. Hundreds of volatiles have also been identified in apple (Dixon and Hewett, 2000) and strawberry (Dirinck et al., 1981; Pérez et al., 1996), but of these, only a small proportion was classified as 'character impact' volatiles. Of 60 volatiles identified from 'Hayward' kiwifruit using GC/MS-O (gas chromatography/mass spectrometryolfactometry), only 30 were odour-active (Frank et al., 2007). Hex-*E*2-enal ('marzipan, sweet') had the greatest odour impact, followed by 1-penten-3-one ('plastic, herbal, glue, kiwifruit-like') and hexanal ('green, fresh crushed leaves'). In 'Hayward' essence and puree, hex-*E*2-enal was perceived by GC-O as imparting a fruity, strawberry and cherry flavour (Jordán et al., 2002).

Olfactometry is thus a valuable tool as it allows a complex volatile mixture to be separated temporally into individual compounds, but it does not take account of the complexity of human sensory perception (Frank et al., 2007). Sensory evaluation methods offer an organized way to collect information on the sensory attributes of food samples as perceived by the human senses. To take into account the multivariate nature of the data, multivariate chemometric data analysis techniques ought to be applied (Daszykowski et al., 2007). Thus, a complementary approach to studying flavour is to use multivariate statistics to identify associations between sensory and chemical data that may indicate potentially important flavour volatiles that contribute most to the sensory experience. This approach recognizes that while individual flavour components are responsible for taste and odour, the total sensation determined from sensory analysis results from the integration of all the individual flavour stimuli in a mixture (Rouseff and Leahy, 1995; Frank et al., 2007). This type of approach has been used in studies of tomato, where relationships between sensory attributes and volatiles have been identified using multiple linear regression (MLR) (Baldwin et al., 1998; Tandon et al., 2003) and PCA (Krumbein et al., 2004).

Variation has been reported in the quantity of volatiles in thirteen apple cultivars (Young et al., 2004)

and six apricot cultivars(Guillot et al.,2006). López et al. (1998) found that odour components varied in class and quantity in two apple cultivars and were associated with sensory properties characteristic of each cultivar. During fruit ripening, a number of different biosynthetic pathways are involved in volatile synthesis (Dixon and Hewett,2000; González-Agüero et al.,2009; Zhang et al.,2009). These have not been fully described, but appear to be common to different fruits(Dixon and Hewett,2000). To date, only a few genes that directly influence fruit flavour biogenesis have been reported, including alcohol dehydrogenase(*ADH2*) in tomato(Speirs et al.,1998), terpene synthases in strawberry(Aharoni et al.,2004), antisense ACC (1-aminocyclopropane carboxylic acid) oxidase and alcohol acyltransferase (AAT) in apple(Schaffer et al.,2007; Souleyre et al.,2005; Li et al.,2006) and lipoxygenase (LOX) in kiwifruit(Zhang et al., 2009).

In an ever more competitive and global market, flavour quality is increasingly important to consumers. Therefore, knowledge of kiwifruit flavour is of utmost importance in developing new cultivars. While studies of the composition of volatile compounds have detected up to 90 volatiles in kiwifruit cultivars and genotypes, there are only a few published studies on 'flavour impact' volatiles related to sensory attributes in 'Hayward' (Frank et al.,2007; Jordán et al.,2002). Moreover, because of the large number of volatiles, the assessment of the volatiles contributing to flavour within breeding populations is especially challenging. Therefore, the main goal of this study was to evaluate multivariate analyses of associations between volatiles, sensory and fruit attributes to identify potential key impact volatiles that make a substantial contribution to the flavour and odour of *A. chinensis* fruit, using 24 genotypes representing the diversity of taste and fruit characteristics in a breeding population. The study also aimed to identify economic and efficient breeding methods for improving flavour volatiles and superior parents for use in our breeding programme.

2 Materials and methods

2.1 Plant material

Twenty-four genotypes of diploid *A. chinensis* were sampled in April 2001 and were numbered from P1 to P24. Twenty-two were seedlings chosen from among 289 females in a population derived from a factorial mating design(3 females × 13 males), planted in 1994 at the Te Puke Research Centre, New Zealand. The other two genotypes were female parents of this population, Female B (P23) and Female C (P24), respectively. The third female parent, Female A, was missing from our study. The three female parents(A, B and C) were selected as testers based on results from previous studies(Cheng et al.,2004). Both Female A and Female B had moderate dry matter(DM) and soluble solids content(SSC). Female C was 'Hort16A', a yellow-fleshed cultivar with large fruit renowned for their good flavour(Jaeger et al.,2003). The 13 male parents were selected randomly from our germplasm collection. The 22 seedling genotypes were selected to be broadly representative of the population, based on the first two principal components(PCs) derived from 14 variables analysed in a previous study (Cheng et al.,2004). The variables included four sugars (fructose, glucose, sucrose and *myo*-inositol), three organic acids (malic, quinic and citric), fruit pH, titratable acidity, vitamin C and four fruit characters(fruit number per vine, fruit weight, DM and SSC at eating ripeness). The two PCs were associated with the traits "sweet taste factor" and "sour taste factor", and accounted for 50.7% of the total variance. The 22 genotypes selected showed extreme differences in combinations of the two PCs. This approach was expected to maximise the discrimination of phenotypes

among vines in this population.

From 50 to 60 fruit were harvested at random from each vine when the mean SSC of a three-fruit sample reached 10 %. SSC and DM were measured at harvest as described in Cheng et al. (2004). The remaining fruit were cool stored at 1.5 ℃ before sensory and chemical analyses were performed.

2.2 Fruit sampling and chemical analysis

After 1 month of storage, fruit were removed from the cool store and ripened at 20 ℃ for 3 ~ 5 days. Fifteen fruit (firmness 0.5 ~ 0.7 kgf) from each vine were selected for sensory analysis. Each end of each fruit was sampled for SSC, and a longitudinal slice (1/3) was removed for chemical analysis. One half of this slice was used for sugar (fructose, glucose, sucrose and *myo*-inositol) and acid (malic, quinic and citric acids) analysis and the other half was sampled for volatile analysis. The remaining part of the fruit was used for sensory analysis (see below). The fruit samples for sugar and acid analysis were frozen in liquid nitrogen immediately and stored at − 20 ℃ prior to analysis. The individual sugar and acid contents were measured by GLC (gas-liquid chromatographic) analysis as described by Cheng et al. (2004).

For volatile analysis, the methods of Matich et al. (2003) were used with minor modifications. Headspace volatiles released from 1 to 1.5 g of the pulped tissue were collected with a Chromosorb 105 absorbent trap (100 mg per trap) for 15 min at 23 ℃ at a flow rate of 25 ml min^{-1}. The sampled traps were stored at −15 ℃ and analysed using GC-MS (HP5890-VG70) with a DB-wax column (*J&W*, 30 m × 0.25 mm × 0.25 μm), within 2 weeks. Peaks were identified and quantified as described by Matich et al. (2003). All analyses were performed on triplicate tissue samples.

2.3 Sensory analysis

A panel of eight in-house assessors, trained to evaluate the flavour of kiwifruit, was selected (Marsh et al., 2006; Wang et al., 2010). Sensory analysis was performed in a sensory panel room at 20 ℃. Sensory attributes were recorded for sweet taste, sour taste, 'Hort16A'-like flavour and odour and ethyl butanoate flavour and odour. The attribute intensities were recorded on 150 mm unstructured line scales anchored at zero for absent and 150 for extreme, using CompusenseTM 5 sensory software. The attributes were calibrated using 'Hort16A' fruit for both 'Hort16A'-like flavour and odour (100), 20 g l^{-1} and 40 g l^{-1} sucrose solutions for sweet taste (40 and 100, respectively), and 1 g l^{-1} malic acid solution for sour taste (115) as in Marsh et al. (2006). Sensory samples of fruit were presented monadically to panellists stem-end up in coded plastic crème cups. Each sample was cut into three slices. The stem end and middle slice were used for odour and flavour assessment, respectively. The remaining third was used for texture assessment (data not shown). The eight assessors analyzed 24 genotypes of kiwifruit during six sessions. The final sensory data were averaged across the eight assessors.

2.4 Statistical and multivariate analysis

In this study, there were 72 volatile compounds (attributes), but only 24 genotypes (observations). Thus, the dimensions of the data matrix were 24 × 72. For PCA, the correlation matrix for the data was singular and some scoring coefficients were zero (Daszykowski et al., 2007). To overcome this problem, the volatile data were regrouped, using hierarchical clustering of variables as implemented by PROC VARCLUS (SAS Institute Inc., 2003), based on a phenotypic correlation matrix between volatiles estimated by PROC CORR. The VARCLUS procedure divided volatile variables into hierarchical clusters. Four clusters were arbitrarily chosen, to make the number of volatile attributes within the cluster equal to or less than the number of observations (24) (Anderberg, 1973). The four clusters had 19, 13, 20 and 19 volatiles,

respectively(Table 1). In Cluster 1, because four ketones (2-methylpentan-3-one, 4-methyl-2-hexanone, 4-methylpentan-2-one and 5-methylhexan-2-one) and butyl acetate were found only in P2, some scoring coefficients of the PCA were zero because of the singular correlation matrix. Thus, only butyl acetate was included in Cluster 1 for PCA (Table 1). A PCA was carried out on the volatiles in each of the four clusters, using the PRINCOMP procedure of SAS(SAS Institute Inc., 2003). In accordance with Morrison (1990), the analysis was performed on standardised data. The un-rotated structure of each analysis was used to interpret the meaning of the components because the emphasis was on ordination rather than structure explanation. To minimize the number of principal components used for each character ('parsimony') (Morrison, 1990), we set the cut-off at 70 % of the total variance. This meant it was necessary to consider only 14 principal components(PCs) for all four clusters(Table 2).

Multiple linear regressions(MLR) of DM, sugars, acids and sensory attributes were conducted as a function of the 14 PCs, to explore associations between volatile compounds, sensory and fruit attributes and to identify potential key 'flavour impact' volatiles of fruit of *A. chinensis*. MLR was carried out using PROC REG with the STB(standardized β) option of SAS(SAS Institute Inc., 2003). A standardisation for the regression coefficient(STB) is the process whereby raw data are transformed into new variables with a mean of 0 and a standard deviation of 1. DM and SSC were very highly correlated and showed very similar correlations with other taste and fruit characters(Cheng et al. 2004). Therefore, this study reports only the relationship between DM and volatiles. The combined analysis of PCA and MLR enabled 13 potential key 'flavour impact' volatiles in *A. chinensis* fruit to be identified. Another PCA was carried out with only the 13 volatiles, to enable differentiation of genotypes. The parsimony criterion subsequently led to the first two PCs being used. MLRs of sensory attributes were conducted as a function of the two PCs of key volatiles, to specify the relationship between key volatiles and sensory attributes and to identify superior parents for flavour breeding.

Table 1 Occurrence and relative abundance of volatile compounds found in 24 genotypes of *Actinidia chinensis*

Compound	Occurrence (genotypes)	Mean /(ng g^{-1} FW)	Minimum /(ng g^{-1} FW)	Maximum /(ng g^{-1} FW)	'Hort16A' /(ng g^{-1} FW)	Cluster no.
Aldehyde						
Acetaldehyde	15	6.37	0	42.27	5.28	4
Propanal	24	3.97	0.83	7.83	3.60	1
Butanal	22	0.99	0	2.60	0	2
2-methylbutanal	3	0.21	0	2.54	0	1
Hexanal	24	128.95	3.94	616.98	28.24	4
Hex-*E*3-enal	22	4.76	0	23.93	0	4
Hex-*Z*3-enal	21	19.84	0	433.16	0	3
Heptanal	23	2.93	0	11.42	1.82	1
Hex-*Z*2-enal	22	11.74	0	39.01	4.45	4
Hex-*E*2-enal	24	686.48	17.15	2 484.81	123.50	2
Octanal	24	4.87	0.98	22.86	2.06	3
Nonanal	15	10.86	0	34.30	0	2
Decanal	20	17.00	0	78.05	0	3
Benzaldehyde	24	2.26	0.40	19.22	1.01	1