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Yangling City, Shaanxi Province, China
October 12-14, 2017

Organized and edited by ISHS and NWAFU Kiwifruit experimental station

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Executive Director of ISHS
Peter Vanderborght

Secretariat of ISHS
PO Box 500
3001 Leuven 1
Belgium

Phone: +32.16.22 94 27
Fax: +32.16.22 94 50
E-mail: info@ishs.org
Internet: www.ishs.org

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Photograph on the front cover:
'Qihong' cultivar. Courtesy of 'NWAFU Kiwifruit experimental station'.

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Prof. Zhande Liu

Chief scientist of Shaanxi Kiwifruit industry system; Chief scientist of Northwest Agriculture&Forestry University Kiwifruit experimental station

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FOREWORD

Kiwifruit (*Actinidia*), also called Yang Tao or Mao Tao in Chinese language, is the temperate berry with delicious flavour mixed with sour and sweet. It is regarded as the fruit of high content and rich dietary fiber, plus 17 kinds of amino acids and 14 kinds of mineral elements. Among 27 kinds of common fruits, kiwifruit is the one of highest nutrient density with good functions for nutritional and healthful improvement. Early in the period of Pre-Qin Dynasty, kiwifruit was recorded in *The Book of Songs* and later in the following dynasties was introduced by medicinal annals describing its medical functions and health benefits. Kiwifruit, originated from China's mountains, now has been a fashionable fruit enjoyed by consumers worldwide.

Shaanxi, an original place of kiwifruit and also optimum and major area for kiwifruit growing in China, is of unique advantages and foundation for kiwifruit industry. Four most important advantages make Shaanxi province stand out in kiwifruit industry: i) ecological environment, due to locations in the north slope of Qinling Mountains and Qinling-Ba mountainous area where there is very good natural conditions for kiwifruit trees; ii) rich germplasm resources, due to Qinling mountains to be regarded as the gene bank containing plenty wild kiwifruit resources, out of which there have been 18 elite varieties selected and bred ripening in periods from early to late and with sarcocarp in green, yellow or red color; iii) science and technology, due to dependence on the support from Yangling State Agricultural High-tech Demonstration Zone and Northwest A&F University, which makes whole supply chain of kiwifruit industry covering breeding, cultivation, storage, packaging, transportation and marketing well integrated by technological and standards system; iv) marketing, due to famous brand of kiwifruit developed by leading companies and marketing teams through integrating the organic fruits strategy and protected designation of origin (PDO), which makes kiwifruit industry another highly competitive industry following apple in Shaanxi.

There is a popular saying that China's kiwifruit will follow the example of Shaanxi and Shaanxi's kiwifruit industry shall follow the example of Mei county where the major peak of Qinling Mountains is located. Mei county located in the core area of kiwifruit industry is already the main and best area of kiwifruit growing in Shaanxi. As early as in 1200 year ago in Tang dynasty, there was a poet called cén shēn who had a poem describing kiwifruit trees. Recent years, Mei county had a fast development in kiwifruit industry by making use of the unique advantages like appropriate locations, plenty variety resources, robust technical support and industrial circumstances. There are total kiwifruit cultivation area reaching 21,000 ha, accounting for one third of total kiwifruit growing area in Shaanxi province, one fifth of total kiwifruit growing area in China and one tenth of the world total. Total yield per year reached 460,000 t and output value reached RMB 3000 million yuan, which makes Mei county an iconic county of kiwifruit industry in Shaanxi or even in China.

To strengthen the cooperation with international institutions of kiwifruit for sharing the research achievements and exploring the cutting-edge technologies,

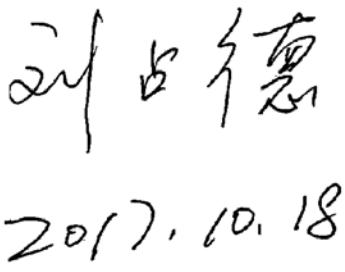
the World Kiwifruit Conference plus the 6th Shaanxi Kiwifruit Industrial Development Conference were organized as driving force for upgrading the kiwifruit industry in Shaanxi or even whole China.

The World Kiwifruit Conference held from October 13 to 15, 2017 with the theme as "let the world enjoy tasty kiwifruit" was sponsored by International Society for Horticultural Science and Chinese Society for Horticultural Science and jointly organized by Northwest A&F University, Shaanxi Fruit Industry Bureau, People's Government of Baoji City and People's Government of Mei county. There were 299 participants from world well-known kiwifruit research institutes of 16 countries or regions, such as New Zealand, Italy, Spain, Belgium, Korea and Japan. Domestically, there were 248 participants from 27 provinces or autonomous regions, 86 organizations, 15 universities, 36 research institutes and experts from major counties of kiwifruit growing. Among them, there were 75 participants who were master or Ph.D. candidates under age of 30. Apart from presentations, the conference received over 130 posters and papers which were reviewed by the expert panel and ten posters were granted the outstanding award.

The conference was comprised of three sessions: conference opening ceremony, field study in kiwifruit plantations and kiwifruit industry workshop. After opening ceremony held on October 13, the participants visited the kiwifruit plantations located in Tianjia village and Ningqu village of Mei county, kiwifruit experiment station of Northwest A&F University and other plantations in Zhouzhi county and Wugong county. Afterwards, the workshop on kiwifruit industry development was held in Yangling from October 14 to 15, 2017.

To commemorate the achievements generated from this conference, 25 papers selected from over 130 ones submitted were reviewed by editorial board of journal of International Society for Horticultural Science (ISHS) and shall be published by ISHS as one special issue *Proceedings of World Kiwifruit Conference*, covering the topics ranging from breeding and genetics, cultivation and management, pest and disease control, robotic use for harvest, storage, nutrition to in-depth processing. The publication of the proceedings was to promote the exchange and share of the conference achievements and jointly improve the development of kiwifruit industry both in China and all over the world.

Prof. Zhande Liu



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Experiment station-based sci-tech innovation, demonstration and extension for Shaanxi kiwifruit industry

Zhande Liu[a], Guangzhe Liu, Zhiyao Ma, Zhixiong Gao and Haohao He

College of Horticulture, Northwest A&F University, Yangling, 712100, Shaanxi, China.

PROFILE OF KIWIFRUIT PRODUCTION IN SHAANXI

Shaanxi Province is the major province of kiwifruit cultivation in China with 64,000 ha of kiwifruit orchards accounting for 42.7% of the total 1,500,000 ha of kiwifruit orchards currently (2017) in China (Figure 1). 39,000 ha of the kiwifruit orchards in Shaanxi have come into bearing,, with the yield reaching 1.3 million t.

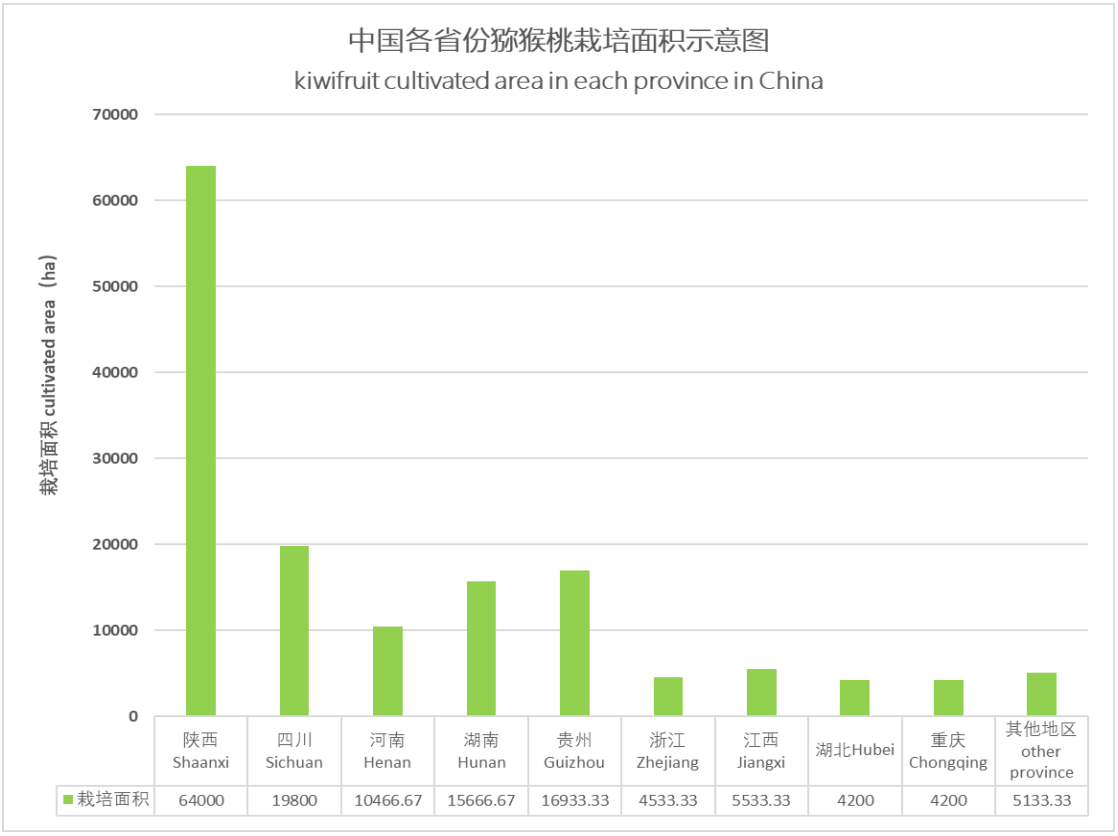

Figure 1. Area of kiwifruit orchards in different provinces of China.

The main kiwifruit cultivars in Shaanxi are 'Xuxiang', 'Cuixiang', 'Hayward' and 'Qinmei' and the proportions of cultivation for each cultivar are respectively 30.21, 18.75, 17.71 and 14.58% (Figure 2). Cultivars of *Actinidia deliciosa* make up 88.54% while cultivars of *Actinidia chinensis* with yellow flesh or red core account for 6.46 and 5%.

The rapid development of kiwifruit industry in Shaanxi is due to its unique and advantageous climate. The Grand Qinling Area, covering the northern and southern slopes of the Qinling Mountains (the climatic division between of south and north China), the Wei River basin and the flat plain in central Shaanxi have become the major cultivation areas of

[a]E-mail: icki201710@163.com

kiwifruit in Shaanxi. These areas, characterized by their ample sunshine, four distinct seasons, warm and humid weather, and vast flat land with fertile soil, have good natural conditions for the development of the kiwifruit industry (Table 1).

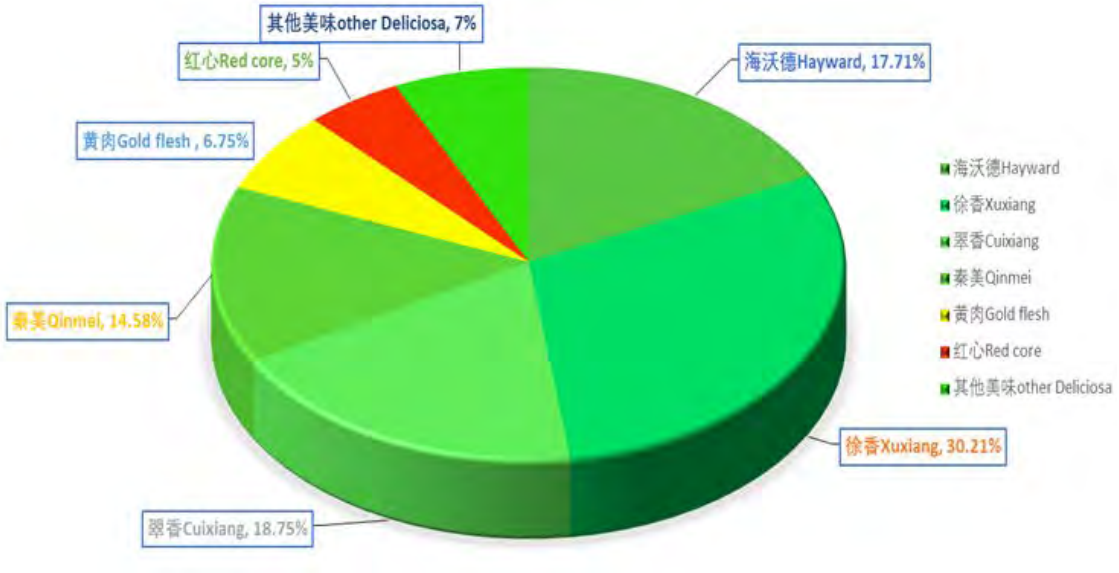

Figure 2. Percentage of different kiwifruit cultivars grown in Shaanxi Province.

Table 1. Climatic indices of the cultivation area in Shaanxi.

Annual sunshine duration (h)	1979.8
≥10°C accumulated temperature	4386.0
Frost-free days (d)	221.7
Annual precipitation (mm)	591.7
Relative humidity (%)	76
Altitude range (m asl)	400-600
Organic matter (%)	1.1
Depth of soil (m)	5-1200
Soil pH value	6.5-7.6

In recent years, kiwifruit yields have been rising gradually, bringing great benefits to local people. During 2015-2016, the unit yield mu^{-1} averaged 2.25 t (33.75 t ha^{-1}) with a price of about RMB 4.69 kg^{-1}, which generates an average income of RMB 39,000 per household for a total 210,000 grower households. The ratio of input to output is 1:4.6; an effective approach to help farmers get out of poverty. Figure 3 shows the different prices in different years and for various cultivars in Shaanxi, indicating the higher price for kiwifruit cultivars with a red core.

In the last five years, a new strategy called “cultivation expanding southward and moving eastward” was initiated in Shaanxi for kiwifruit. This made the cultivation of kiwifruit possible along the Qinling Mountains as the main axis including: one million mu (67,000 ha) on the northern slopes, 200,000 mu (13,000 ha) along the Hanjiang River, 100,000 mu (6,667 ha) along the Danjiang River, and 100,000 mu (6,667 ha) in the Bashan

mountainous area. The total cultivation area reaches 1.5 million mu (100,000 ha) with a yield of 1.8 million t and generates economic benefits of RMB 20 billion.

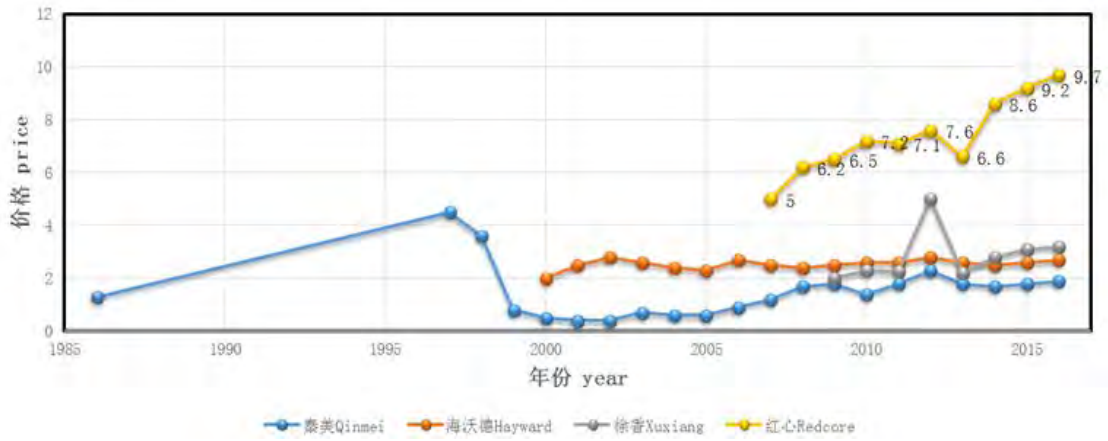

Figure 3. Variation in price of different kiwifruit cultivars 1986–2016.

There is a long history of cultivation of kiwifruit in China. According to a study by Prof. Xin Shu-zhi (1894-1977), the former president of Northwest Agricultural University and well-known scholar of agricultural history, *The Book of Songs* about 3000 years ago described the tree of kiwifruit, saying that in the moist place, there was a kiwifruit tree with graceful branches and twigs swinging in the wind and we are enjoying the beauty but the tree cannot feel our feelings. In ancient times, the kiwifruit tree had been an ornamental tree grown in the yard of a home. An ancient poem by a famous poet in the Tang Dynasty described a vivid scene: just after rain, the sky was clean and mountains could be clearly seen in the far distance. In a farmer's home yard, there is a well-grown kiwifruit tree with branches spreading like a cover, underneath a grandfather about 100 years old was entertaining his visiting friend (Figure 4).

Figure 4. Painting reflecting the Tang Dynasty poem.

Only in the last thirty years have kiwifruit been grown commercially in China. Shaanxi Province, with the largest cultivation area now in China, experienced three stages of development in kiwifruit production.

The period from 1980 to 1996 saw the initiation of development of kiwifruit cultivation, with only a single cultivar dominant and the cultivation area limited, together

with lower productivity and lower economic benefits. The period from 1996 to 2006 was a drifting period. The cultivation area was growing, more cultivars were being developed and yields plus economic benefits increased. The kiwifruit industry however, was still drifting owing to backward production techniques and management. The period from 2006 to 2017 is believed to be a fast growing stage. The cultivation area, yield and economic benefits, and the number of cultivars were enhanced significantly. A positive cycle of kiwifruit industry development was being realized (Figure 5).

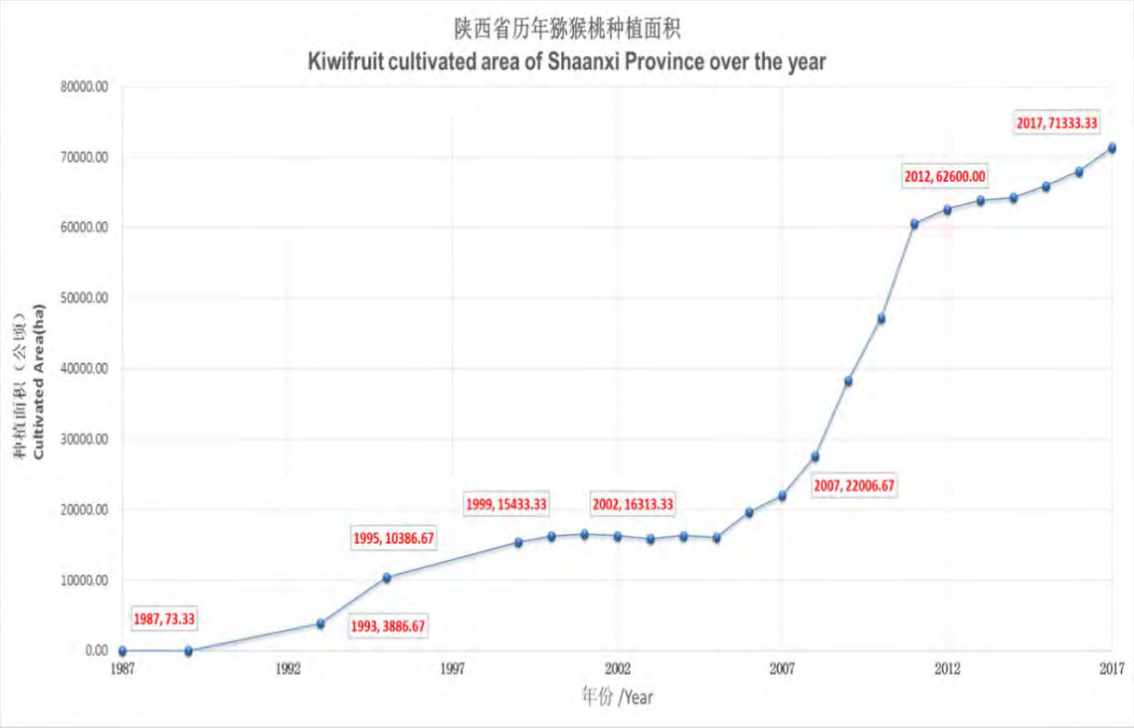

Figure 5. Area in kiwifruit orchards in Shaanxi from 1987 to 2017 indicating the stages of development.

THE ESTABLISHMENT OF KIWIFRUIT EXPERIMENT STATION OF NORTHWEST A&F UNIVERSITY (KES-NWAFU)

There is a complicated background to the establishment of KES-NWAFU. At that time, kiwifruit production in China was stagnant and breeding was still dependent on the traditional procedures: finding new germplasm, domesticating the germplasm and finally cultivating. Small-scale farming was popular among farmers who were less trained, and were lacking proper techniques and management skills. Cultivars such as 'Yate' and 'Qinmei' were not competitive in the market, generated low economic benefits to farmers which as a consequence made kiwifruit cultivation stagnant and there was a lack of incentives for growers. Under these circumstances, KES-NWAFU was established.

The station, located at the foot of the northern slope of the Qinling Mountains (Figure 6), just in the center of the cultivation area of kiwifruit, was established in 2006 covering 160 mu (10.7 ha), with 16 experts designated and over 10 postgraduates working as a group. It is one of the first eight experiment stations of NWAFU following the education principles: serving the direct demand of state and people through scientific approach, which has been passed down from generation to generation. With multi-disciplinary methods adopted, the station experts focus on the emerging problems in the whole production chain and integrate five functions together: field observation, experimental research, demonstration and extension, education, and internationalization, highlighting the combination of research,

education and extension in order to solve the key problems in kiwifruit industry development and contribute to the development of Shaanxi province.

Figure 6. The station, located at the foot of the northern slope of the Qinling Mountains.

KES-NWAFU, located within Mei County which has a long history of kiwifruit cultivation, has been therefore jointly implementing the project *Sci-tech Entering Specific Households* to serve the local kiwifruit growers through a model called "1+2+2+N": one expert plus two county technicians plus two village technicians and plus many farmers. Following that model, there are currently 16 university professors working with 32 county technicians followed by 32 village technicians and over 100 professional farmers (Figures 7 and 8). So far, there are 16 working groups in 16 demonstration villages together with 100 demonstration households for growing kiwifruit. The number of working days for those experts in villages is not less than 200. A great contribution to local kiwifruit industry development has been achieved.

Figure 7. Expert teaching postgraduates in the field.

Figure 8. Expert training farmers.

Over recent decades, the joint extension project *Sci-tech Entering Specific Households* has made great contributions to the local kiwifruit industry, making the cultivation area of kiwifruit during the period 2006-2016 increase from 83,000 mu (5,533 ha) to 300,000 mu (20,000 ha), with an annual growth rate of 27%, benefiting 66,800 households, covering 259,000 beneficiaries, and with an income from kiwifruit of RMB 8400 per capita and RMB 32,000 per household (Figures 9 and 10).

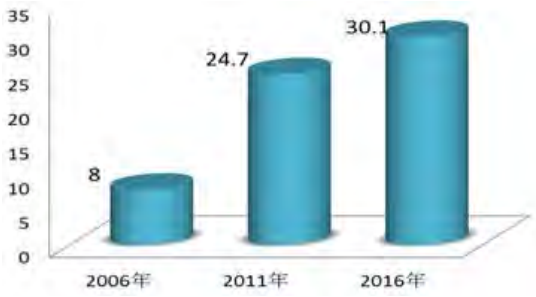

Figure 9. Area of kiwifruit plantings in Shaanxi 2006-2016 (ha×10,000).

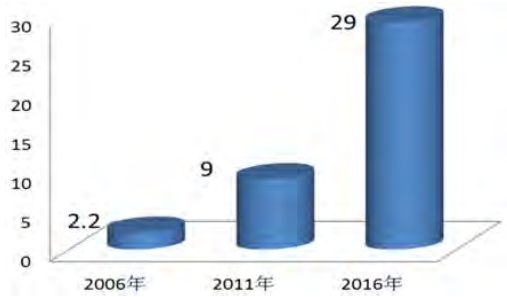

Figure 10. Economic benefits (RMB×100 million).

Located in Mei County, KES-NWAFU keeps an eye on the overall development of the kiwifruit industry in Shaanxi. There are currently six ecoregion-specific Kiwifruit Technological Extension Stations located respectively in Baoji, Xi'an, Linwei, Hanzhong, Ankang and Shangluo. A team consisting of thirteen experts and Shaanxi Kiwifruit Industrial System are formulated to serve the overall development of kiwifruit industry under the new strategy of *Expanding Eastward and Moving Southward*, with a recent expansion of kiwifruit orchards reaching 500,000 mu (33,333 ha).

Services by KES-NWAFU have been extended to other provinces owing to the effective work. One joint experiment station and six work stations have been established, respectively in Xixia, Henan Province; Jinggangshan, Jiangxi Province; Honghezhou, Yunnan Province; Chongmingdao, Shanghai; Qingdao, Shandong Province; Huzhou, Zhejiang Province; and Nanjing, Jiangsu Province, a network covering all of China to serve China's kiwifruit industry (Figure 11).

Figure 11. Kiwifruit experiment stations or work stations throughout China.

SCI-TECH INNOVATION, DEMONSTRATION AND EXTENSION

Quick propagation of young plants

One-year-old plants produced by rapid propagation methods can be used for orchard establishment and after three years they begin to bear fruit (Figure 12). Quick propagation techniques not only provide large quantities of quality young plants but also make orchard establishment much easier.

Figure 12. Quick propagation of seedlings for orchard establishment.

Assessment and selection of root stocks

Quality root stock is very important for orchard establishment of kiwifruit and has been the key research work of KES-NWAFU. After years of effort, robust and stress-resistant root stocks have been selected and passed through affinity test and assessment, which laid a solid foundation for propagation by grafting (Figure 13).

Figure 13. Root stock selection and assessment through propagation as cuttings.

Micro-bud culture for propagation

A complete set of technologies on micro-bud culture for young plant production have been completed to ensure quality of young plants used in orchard establishment (Figure 14).

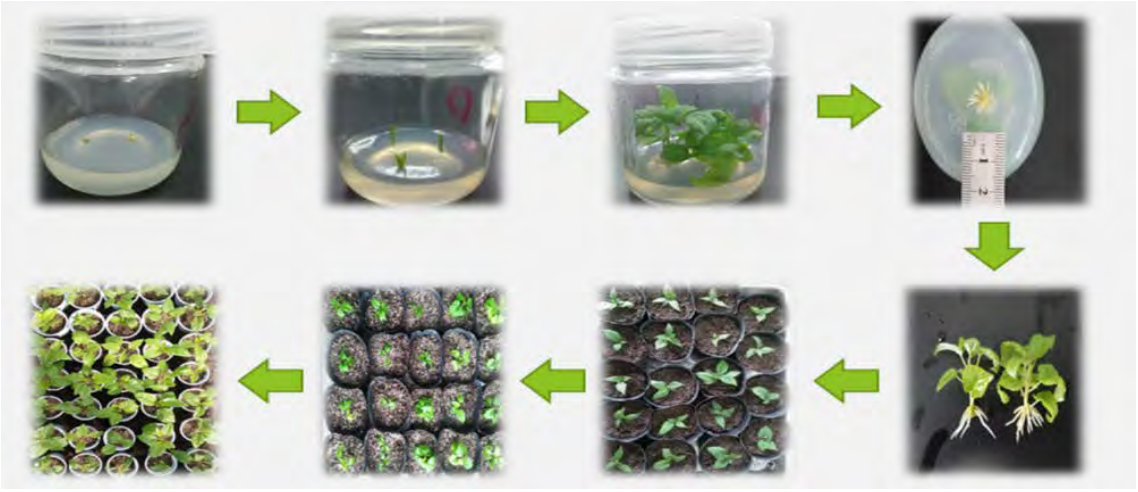

Figure 14. Micro-bud culture for vegetative propagation.

Pollination

Pollination technologies were refined to make sure of quality pollen and proper pollinating process for bearing quality fruits. Pollination tests with 'Xuxiang' showed that pollination of at least one third of the stigmata pollinated ensured good fruit size (Figure 15).

Clean production by organic fertilization

Based on sustainable principles and GAP requirements, the twigs and branches pruned from kiwifruit orchard are all chipped for compost with the help of certain microorganisms. The final production is organic fertilizer which would be reused in orchard for quality fruit production (Figure 16).

Figure 15. Correlation between number of pollinated stigmata and fruit size (number below the fruits indicates the number of stigmata pollinated).

Figure 16. Organic fertilizer generated from fermentation of orchard waste.

Environmentally friendly cultivation

Apart from the organic fertilization, the orchard of kiwifruit gives careful concerns to environment protection. Biological measures are applied to control pests. Growing clover in orchards improves the micro-habitat for reduction of pest and disease infection with kiwifruit vines and improves soil fertility. Furthermore, growing mushrooms between kiwifruit vnes is an effective way not only of protecting the soil but also of increasing economic output from orchards (Figure 17).

a b c

Figure 17. Environmentally friendly cultivation: a. biological control with predatory mites; b. growing clover; c. growing mushrooms between vines.

Trellis pergolas applied and proper training

The pergola system is the main support structure for kiwifruit vines. (Figure 18). To balance vegetative and reproductive growth, the stem height is 30 cm lower than normal and two branches of three are reserved hierarchic for consecutive fruiting from the current to the next two years.

Figure 18. Pergola system of support for kiwifruit vines.

Cultivar selection and breeding

Before establishment of the station establishment, 62 superior plants were collected from the kiwifruit germplasm resources in the Qinling-Bashan mountainous area, from which some cultivars such as 'Qinmei', 'Qincui', 'Yate', 'Jinxiang', 'Qiuxiang', etc. were selected and bred. Since establishment of the station, experts have collected an additional 27 species or cultivars, based on which 22 cross pollinations have been made producing 22,000 seedlings. Over the past ten years of selection and regional cultivation trials, four new cultivars have been developed: 'Nongda Mixiang', 'Nongda Yuxiang', 'Nongda Jinmi' and 'Qihong'.

Germplasm collection and evaluation

After the establishment of the station, almost every year the station has organized for experts and students together to investigate the wild germplasm of kiwifruit in the Qinling Mountains (Figure 19). 263 accessions of germplasm have been collected (17 with red core of fruits) and evaluated so far, and the elite materials will be used for hybridization to generate new cultivars.

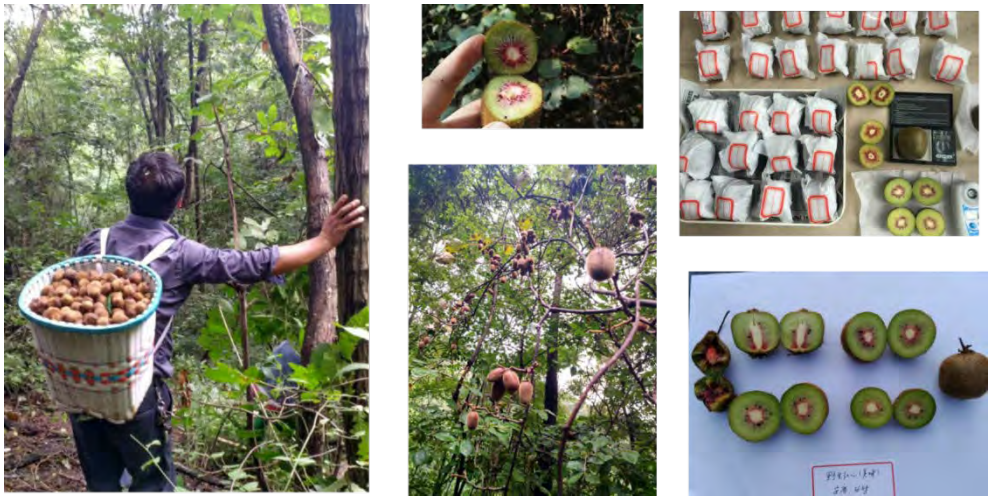

Figure 19. Searching for kiwifruit germplasm in the mountains and samples collected.

MAIN CONCERNS FOR CURRENT AND FUTURE KIWIFRUIT INDUSTRY

i) New requirements from kiwifruit industry will become the new direction for future breeding, such as focus on cultivars with multiple nutritional functions.
ii) Ecological cultivation model as well as organic plus high yield techniques will be the direction for standardized cultivation technologies development.
iii) PSA will still be the key during the pest and disease control. Green measures as well as ecologically friendly approach will become important.
iv) Modern equipment for orchard management together with modern intelligent technologies will play an important role for kiwifruit industry development.
v) More accurate technologies regarding supply chain quality control and fruit storage will be an important issue in future research and exploration.
vi) Further promotion of fruit quality, nutritional development and in-depth processing will be key orientations for kiwifruit industry development.

History, present, future of the kiwifruit industry

G. Costa

University of Bologna, Alma Mater Studiorum, Department of Agricultural Science, Bologna, Italy.

Abstract

Kiwifruit industry started in the last century; until 1970s many countries did not even know the existence of this fruit species while now it represents one of the most successful examples of fruit cultivation in many countries. The world plantings and production increased almost six-fold in only 40-50 years. Currently China, Italy, New Zealand, Chile and Greece account for more than 90% of the total world production and other countries, such as Turkey and Iran consistently increased their acreage.

The kiwifruit industry was based on a single green-fleshed cultivar ('Hayward') but now yellow- and some red-fleshed kiwifruit cultivars are being cultivated. The cultivars were practically disease-free until 2009 when the bacterial canker by Psa appears representing a real problem especially for the yellow- and red-fleshed cultivars of *A. chinensis* var. *chinensis* which are particularly susceptible. Breeding Psa tolerant or resistant cultivars is becoming "imperative". However the new cultivars recently introduced and the existence of Psa require modification of the growing systems and management techniques to ensure important yield and high quality fruit. Fruit quality is an issue since kiwifruit are now marketed internationally and consumers have become highly demanding in terms of fruit quality, safety, etc. In the next few years the "kiwifruit universe" (industry, research, etc.) will have to work together to face important challenges finding solutions for maintaining and increasing the high standard nowadays achieved.

A platform for communication of horticultural technology among researchers and the participants involved in the kiwifruit industry under aegis of the ISHS "Kiwifruit and its culture" working group to have a better understanding of kiwifruit production, to make significant progress against the Psa and to allow the introduction in the main growing areas of the best cultivars originating from the different breeding programs ongoing in the different countries is desirable.

Keywords: plantings and yield, breeding, Psa, cultural management, harvest indices, fruit quality, international collaboration

INTRODUCTION

This brief review considers some of the main changes that have occurred in the species since its introduction to Italy as well as to other producing countries and how research could serve to overcome some of the main problems that the kiwifruit industry is currently facing. The kiwifruit was introduced to Italy in the 1970s and the University of Bologna focused research activity on several aspects. In the early 1970s, the methods of propagation were studied (Costa and Baraldi, 1983). When the first orchards were established the main cultural management techniques were studied in detail. More recently the research focus has been on breeding (cultivar 'Dorì') (Costa et al., 2014) and on "non-destructive evaluation of fruit quality" (NIRs, e-nose, kiwi-Meter instruments). After *Psa* appeared several research projects evaluated the relationship between the main cultural management techniques and Psa infection and on the use of protected cultivation with plastic tunnels to control the disease.

WORLD KIWIFRUIT INDUSTRY: PLANTINGS AND PRODUCTION

In the late 1980s, New Zealand, Italy, France, Japan and USA were producing more than 98% of the total world commercial production of kiwifruit. New Zealand was producing half of the world's total kiwifruit with 10,000 ha of kiwifruit orchards while currently the area in kiwifruit there has reached 13,000 ha producing 500,000 t year^{-1} accounting for only 10% of the world's total production. In Italy, in the late 1980s, the total area was about 6000 ha whereas today it has increased fourfold reaching about 25,000 ha producing up to 500,000 t year^{-1} (Testolin and Ferguson, 2009). However, the most significant change over the last decade has been the expansion of kiwifruit in China, which is now producing more than half the world's kiwifruit and is the largest producer of yellow- and red-fleshed kiwifruit. Other countries, such as Chile and Greece, have increased their areas in kiwifruit and production but even more important plantings were established in Turkey and Iran, about 20,000 and 13,000 ha respectively with important production (Table 1).

Table 1. Yield of the main kiwifruit producing countries (source CSO, Centro Servizi ortofrutticoli S.c.a.r.l., Ferrara).

Rank 1987-1990	Country	Production (t)	Rank 2013-2016	Country	Production (t)
1	China	n.a.[1]	1	China	2,433,333
2	Italy	215,433	2	Italy	484,072
3	New Zealand	225,750	3	New Zealand	404,112
4	Chile	18,600	4	Chile	193,353
5	France	43,220	5	Greece	160,933
6	Greece	11,725	6	France	60,935
7	Japan	48,650	7	Iran	58,333
8	Iran[2]	1-2000	8	Turkey	42,545
9	USA	31,923	9	Japan	31,075
10	Spain	2,675	10	USA	24,419
	Top five	564,976		Top five	3,675,803
	%	98.92		%	93
	Top ten	597,977		Top ten	3,893,110
	%	98.98		%	99
	World	606,551		World	3,950,461

[1]first data available 8,500 t in 2000; [2]19,000 t in 1999.

ORCHARD MANAGEMENT

Productivity and orchard management are strongly linked. Management techniques have significantly changed in the last 30 years. For instance, originally 300-400 vines ha^{-1} were planted while now planting densities have reached 900-1200 vines ha^{-1}. For instance in Italy, the T-bar and the pergola are the training system most widely used (Costa et al., 1990, 1993; Costa, 1999; Testolin and Ferguson, 2009) although other systems which allow higher planting densities up to 2000 vines ha^{-1} such as the "Tatura", spindle and Geneva Double Curtain (GDC) have been tested to enhance yields in the initial years (Testolin et al., 1987; Costa and Testolin, 1995). In New Zealand the pergola system is the most widely used training system but other systems such as "alternate year cropping system" that allow yields up to 60 t ha^{-1} have been recently proposed (Thorp and Barnett, 2011).

The increase in planting density affects productivity. In New Zealand in the 1980s 'Hayward' orchards reached yields of 25 t ha^{-1} (Sale and Lyford, 1990) while now the yield ha^{-1} has increased to about 38 t ha^{-1}. Selected yellow-fleshed cultivars such as 'Zesy002' (Gold3, the fruit of which are marketed as Zespri® SunGold kiwifruit) can produce up to 50

t ha^{-1}.

The situation in Italy is similar. When kiwifruit were introduced, the average yield ha^{-1} of 'Hayward' orchards ranged about 25-30 t, while nowadays well-managed orchards can bear up to 30-40 t ha^{-1}. The yellow-fleshed cultivars recently introduced, 'Soreli', 'Zesy002' and 'Dorì', can easily carry yields of 40-50 t ha^{-1}.

In China in 1980s, the 'Hayward' orchard yield was less than 10 t ha^{-1} but a few years later increased to 15-20 t ha^{-1}. Other Chinese cultivars such as 'Qinmei' (green-fleshed), 'Jintao' (yellow-fleshed), 'Jinyan' (*A. eriantha* and *A. chinensis* hybrid) and 'Donghong' (red-fleshed) can reach up to 45 t ha^{-1}.

Since the introduction of kiwifruit in several countries as well as in Italy research has focused on cultural management techniques, evaluating the response to pruning of the vine, the effects of different bud-loads left after winter pruning (Testolin and Costa, 1992), the effects of fruit thinning on yield and fruit quality (Costa et al., 1995) and in general the physiology of the vine as related to the application of these techniques to select the most appropriate ones to be performed to obtain high quality fruit. Studies on pruning intensity and bud-load were carried out to understand the effects of light and carbohydrate partitioning in the vine (Succi et al., 1990) evaluating the vine gas-exchange and the characteristics of the fruit produced as related to the amount of light intercepted (Costa et al., 2002). Several studies were also performed testing the effects of "bud dormancy-breaking agents" (Dormex®, Citokin®, Erger) to allow better bud-break and bud fertility in growing areas characterized by mild winters (Montefiori et al., 2003; Costa et al., 1995), and of plant bioregulators (Forchlorfenuron, Maxim®, Spray Dunger Global) to affect fruit morphogenesis, induce fruit thinning and manipulate fruit ripening (Biasi et al., 1992).

FRUIT QUALITY

Whatever the cultivar or the cultural management chosen, fruit quality is the main objective to be achieved. Growers want to obtain high yields and fruit size since the financial returns depend on these aspects. However, consumers became demanding in term of fruit quality and safety of the fruits they eat and other parameters such as sugar and dry matter content must be considered to obtain a quality that will meet consumer expectations. (Burdon et al., 2004). These aspects might also represent an additional premium to the growers considering that high quality fruits are easier to manage in pre- (uniform size and ripening, easier harvest, etc.) as well in post-harvest (easier cold-storage, less fruit losses, etc.). As a consequence, the ripening stage of the fruit at harvest must be carefully determined with standard (SSC, dry matter, flesh color) and innovative methods (NIRs, impact method; Kiwimeter, etc.) (Costa et al., 2015), considering that it will influence the cold storage management and the acceptance by consumers at consumption.

CHANGES IN CULTIVARS

Most of the studies were carried out on 'Hayward' since the kiwifruit industry was based on only this cultivar belonging to one *Actinidia* species for many years. In 2000 another cultivar 'Hort16A', the first yellow-fleshed kiwifruit in international trade, was released by New Zealand. The new cultivar with a flesh color different to "green" was enthusiastically accepted by the market and consumers stimulating breeding efforts in the search for new yellow- and red- fleshed cultivars. The cultivars grown were essentially disease-free, but this unique situation changed when bacterial canker of kiwifruit (caused by *Pseudomonas syringae* pv. *actinidiae* -Psa-), emerged and devastated orchards in the main producing countries. 'Hayward' was not so seriously affected by PSA, although its susceptibility depends on climatic conditions from year to year. Instead, orchards of 'Hort16A', which is highly susceptible to Psa, had to be removed in both Italy and New Zealand in a few years. Although the yellow- and red-fleshed cultivars have been demonstrated to be more susceptible to Psa than 'Hayward', in recent years several new cultivars have been introduced in the market. In the case of Italy, 'Jintao', 'Soreli', Gold3

('Zesy002') and 'Dorì' are the yellow-fleshed cultivars while 'Hongyang', 'Donghong', HFR18 and RK 2018 are the red-fleshed cultivars currently grown (Costa et al., 2018). It is difficult to formulate an exhaustive evaluation of these cultivars since most of them are still under evaluation.

Although Psa is the most serious problem for the kiwifruit industry, the area in orchards did not change dramatically. Some cultivars, such as 'Hort16A' were totally replaced by 'Zesy002' (Gold3) while production of other cultivars, such as 'Jintao', 'Soreli' and 'Dorì' increased slightly (Costa et al., 2018).

Because of the emergence of Psa, research has focused on breeding for tolerant or resistant cultivars, on the relationship among management techniques and susceptibility to PSA and how protected cultivation modifying the microclimate under tunnels might reduce the incidence of disease (Costa and Ferguson, 2015). This latter technique is still being studied although consistently increasing. It has to be remembered that the new cultivars recently introduced and the existence of Psa require modification of the growing systems and of the management techniques to ensure yields and high quality fruit. Moreover under tunnels several cultural techniques must also be adapted and modified from those used in the open field conditions. *Pruning* must take into account the reduction in light reduction and a consequent possible reduced bud-fertility. Rain might encounter difficulties in reaching the roots of the vines and *irrigation* must be modified under-canopy to ensure an adequate degree of humidity of the soil. *Fertilization* can be limited and N in particular reduced. Under plastic tunnels *pollination* with bees could be problematic and "artificial pollination" is more frequently more used doing two or more passages with a portable or self-propelled pollen-distributing machine. The results of the "cultivation under tunnel" so far obtained are encouraging although there are real concerns because of the high cost of installation. It has, however, to be taken into consideration that the best results have so far been obtained with innovative plastic films (heat protective; light diffusion, etc.) and that must be maintained open throughout the year (Buriani et al., 2015; Costa et al., 2018).

FINAL CONSIDERATIONS

Market

The biggest change in world kiwifruit production over the past 30 years has been the rise of China from an insignificant producer of kiwifruit to the world's biggest player. The Chinese kiwifruit production is currently destined for the domestic market. However, if China starts to export kiwifruit a major effect on world markets should be expected. However, China so far is importing large quantities of kiwifruit (in 2015 up to 90,000 t, from New Zealand and Chile) (Belrose Inc., 2016).

Also Turkey and Iran, which are in strategic geographic positions for markets with great potential such as Russia and Arab countries, increased their kiwifruit plantings and more production is expected.

Breeding programs and new cultivars

In the near future new cultivars will be certainly introduced in the market: i.e., yellow- and red-fleshed kiwifruit that have been recently introduced in a small scale in different markets are meeting high consumer acceptance. Also the replacement of existing cultivars with new selections tolerant of or resistant to Psa is highly likely and when it will happen the cultivar standard is going to be strongly modified. The commitment for the breeders is not easy but the breeding programs throughout the world have included resistance to Psa as a priority. Being "realistic" it is therefore possible to expect positive results in this regard. As presented in the recent International Conference on Kiwifruit Industry, organized by Prof. Zhande Liu of the Northwest A&F University in Yangling, it has also been pointed out that China has large germplasm collections, trained scientists dedicated to breeding programmes and the Conference participants had the opportunity to see the

results of a massive breeding program that started some years ago and that is currently ongoing.

Management of protected cultivars and branding

These aspects are increasingly affecting the kiwifruit industry. Kiwifruit are no longer marketed just as "kiwifruit" but categorized by brand, flesh colour, country of origin or method of cultivation (Ferguson, 2015). Consortia and clubs are emerging in the main producing country: the Zespri® brand from New Zealand or the Summerkiwi™ and Jingold™ from Italy are promoting and making available to a limited number of growers the cultivars they own influencing the kiwifruit industry. Zespri Group Ltd is promoting 'Zesy002', Summerfruit™ is promoting the green-fleshed 'Meris', the yellow-fleshed 'Dorì' and the red-fleshed 'Honyang'; the Consortium Kiwigold® is promoting the green-fleshed 'BO-Erika' and the yellow-fleshed 'Jintao' and 'Jinyan' and the red-fleshed 'Donhong'. Biogold, a South Africa-based company, is promoting the yellow-fleshed cultivar 'Soreli' worldwide. Most of these cultivars have been recently introduced and are still under evaluation. These consortia and clubs take individual decisions; have specific market strategies, and searching for new cultivars for commercialization influencing grower decisions. Joint coordination could give them the possibility to be present in the different markets with higher fruit quantities that would cover a 12-month market.

INTERNATIONAL COLLABORATION AMONG THE MAIN COUNTRIES

Two main aspects originated from the Yangling conference:

- New cultivars, different environments, new management techniques will affect "fruit quality". It has been fully recognized that cultural techniques and post-harvest management are the basis for high quality fruit production and should not be neglected. Kiwifruit are now marketed throughout the whole world. Returns to kiwifruit growers are now affected not only by the total yield and the size of the fruit but also by other parameters such as sugar and dry matter content to fulfil consumer expectations. (Burdon et al., 2004). There is therefore very strong encouragement for growers to find a balance between total yield and fruit quality to maximize their returns.

- Another important aspect concerns the consideration of the role and the advantages that an international collaboration among researchers of the main producing areas might deliver to the kiwifruit industry. At the Yangling International Conference it was hoped to set a platform for communication of horticultural technology among researchers, Consortia/Clubs and growers around the world under ISHS aegis to have a better understanding of kiwifruit production, make significant progress against Psa and introduce in the main growing areas the best cultivars originating from the different breeding programs ongoing in the different countries. It would be desirable for the introduction of a new cultivar or technique to occur after research to validate and test them by a group of international colleagues coordinated and regulated under the aegis of ISHS. "Kiwifruit and its culture" is a working group belonging to the Section Wine and Berry fruits of ISHS. We have the instruments, in several ISHS Symposium requests of collaboration have been put on the table, at the last International Conference on Kiwifruit Industry organized by the North-West A&F University in Yangling this collaboration was once again desired and demanded. It is time for action, not words.

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Kiwifruit cultivars

A.R. Ferguson[a]

The New Zealand Institute for Plant & Food Research Ltd., Private Bag 92 169, Auckland Mail Centre, Auckland, New Zealand.

Abstract

'Hayward', which has green-fleshed fruit, is the most widely planted kiwifruit cultivar. In most countries growing kiwifruit, plantings of alternative cultivars are small and not yet commercially important. China and New Zealand are the two exceptions. In China, although 'Hayward' is the most extensively cultivated, there are also many other important alternative cultivars with green, yellow or red fruit flesh. Most of these are selections direct from the wild. Only one widespread cultivar, 'Jinyan', is the result of a planned breeding programme. In New Zealand, exports of kiwifruit are from two main cultivars, 'Hayward' (fruit marketed as Zespri® Green Kiwifruit) and the yellow-fleshed 'Zesy002' (fruit marketed as Zespri® SunGold Kiwifruit). 'Zesy002' has replaced 'Hort16A' which was particularly susceptible to *Pseudomonas syringae* pv. *actinidiae*.

Keywords: cultivars, *Actinidia chinensis*, fruit flesh colour, breeding

INTRODUCTION

Nearly all the kiwifruit (*mihoutao*) cultivars of commerce are large-fruited selections of *Actinidia chinensis* Planch. These have fruit flesh which is green, yellow (gold) or more rarely, partly or wholly red. Kiwifruit with green fruit flesh inside hairy skins (*A. chinensis* var. *deliciosa* [A. Chev.] A. Chev. syn. *A. deliciosa* [A. Chev.] C.F. Liang et A.R Ferguson) were the first to be cultivated commercially and they are still the most important category in the international trade in kiwifruit. Then, more recently, came yellow-fleshed kiwifruit with fruit skins which have fine, soft hairs (*A. chinensis* var. *chinensis*). Now, red-fleshed kiwifruit (also mainly *A. chinensis* var. *chinensis*) are being widely grown in China, but not yet extensively in other countries.

In addition, there are relatively minor plantings of other *Actinidia* species such as *A. arguta* or hybrids involving species such as *A. rufa* (Matsumoto et al., 2011). These are not discussed further here.

KIWIFRUIT CULTIVARS IN CHINA

Actinidia chinensis has been grown commercially for less than a century. It is native to China and many hundreds of thousands of plants exist in the wild. It is therefore not surprising that most of the kiwifruit cultivars grown in China were selected directly from the wild. Nearly all the green-fleshed kiwifruit grown in other countries are also only one or two generations removed from their wild progenitors. Only a small number of kiwifruit cultivars have resulted from planned breeding programmes and of these cultivars it is mainly those with yellow fruit flesh that are the most extensively planted.

This reliance on selection of cultivars from the wild or from descendants of plants from the wild is demonstrated by the kiwifruit orchards of Shaanxi Province which produce about half of China's total kiwifruit crop. In Shaanxi, the most important fruiting kiwifruit cultivars are 'Xuxiang', 'Hayward', 'Qinmei' and 'Cuixiang', all *A. chinensis* var. *deliciosa*, and these together make up about 90% of Shaanxi plantings (Lei Yushan, pers. comm.).

[a]Email: Ross.Ferguson@plantandfood.co.nz

- 'Xuxiang' was selected by the Xuzhou Fruit Orchard, Jiangsu from seedlings of open-pollinated plants introduced in 1975 from the Beijing Botanic Gardens, Institute of Botany, Chinese Academy of Sciences (Huang, 2014). In 1957, the Institute was the first in China to start cultivating *A. chinensis* var. *deliciosa* using seed from Mt Zhongnan, Qinling Mountains, Shaanxi. 'Xuxiang' was released to growers in 1990.
- 'Hayward' is now the secondly most widely grown kiwifruit cultivar in Shaanxi. It is one or possibly more generations removed from plants raised from kiwifruit seed introduced in 1904 to New Zealand through Yichang, Hubei (Ferguson and Bollard, 1990). The seed were probably collected inland to the west of Yichang by E.H. Wilson, the plant explorer. Fruit from one of these original plants in New Zealand (or a descendant) were sent to Hayward Wright, a nurseryman of Auckland, New Zealand. He extracted seed from the fruit and from the very small number of seedlings raised he selected 'Hayward'. This was released to growers in New Zealand in the late 1930s but was not introduced to China until about 1980.
- 'Qinmei' was selected from the wild, at Hanyangpo, Jiuyu, Zhouzhi County, Shaanxi, in 1979 by the Shaanxi Provincial Fruit Research Institute, Yangling and the Zhouzhi *Actinidia* Research Station, Zhouzhi, Shaanxi (Ferguson et al., 2012). It was released to growers in 1986. At one stage, it was the most widely planted kiwifruit cultivar in Shaanxi and, indeed, the whole of China. The vine is robust, drought-tolerant, adaptable and very productive. However, returns to growers have decreased as consumers preferred the fruit of other kiwifruit cultivars.
- 'Cuixiang' ('Ximi No. 9') was also selected from the wild from the Qinling Mountains near Jiyu (now Louguan), Zhouzhi County, Shaanxi (Huang, 2014). It was released in 2008.

These four cultivars, all *A. chinensis* var. *deliciosa* and comprising the bulk of kiwifruit plantings in Shaanxi, differ little from plants in the wild. This is also true of kiwifruit orchards in most other parts of China (Li et al., 2014; Ferguson, 2015): most kiwifruit cultivars, whether *A. chinensis* var. *chinensis* or *A. chinensis* var. *deliciosa*, have been selected directly from the wild (e.g., 'Qinmei'), have been selected from seedlings from seed collected in from the wild (e.g., 'Hongyang') or have been selected from seedlings from the open-pollination of vines coming from seed collected in the wild (e.g., 'Xuxiang' or 'Hayward') or existing kiwifruit cultivars (e.g., 'Emihoutao No 1' formerly known as 'Jinkui'). 'Bruno', the other cultivar from New Zealand to be extensively cultivated in China, is, like 'Hayward', a seedling one or possibly more generations removed from plants raised from kiwifruit seed introduced in 1904 to New Zealand.

Although there are a number of controlled breeding programmes in China, only one product of such programmes has so far been widely planted. This is 'Jinyan', an F_1 seedling resulting from crossing female *A. eriantha* by male *A. chinensis* var. *chinensis*. It is the first interspecific *Actinidia* cross to have been widely planted (Zhong et al., 2012). There is enormous diversity within the genus *Actinidia*, and in the future, controlled crossing between *Actinidia* species is likely to result in a wide range of new and novel kiwifruit cultivars.

KIWIFRUIT CULTIVARS IN OTHER COUNTRIES

By far the most widespread kiwifruit cultivar outside China is the green-fleshed 'Hayward' (*A. chinensis* var. *deliciosa*). The New Zealand kiwifruit export industry became based on 'Hayward' because its large, good-flavoured fruit stored particularly well. As the exports of kiwifruit from New Zealand increased, it became apparent that 'Hayward' fruit reached the overseas markets after a long sea voyage in much better and more consistent condition than did fruit of the other kiwifruit cultivars then grown. From 1975 till 2000, only 'Hayward' kiwifruit were accepted for export from New Zealand. The successful development of kiwifruit as a commercial crop is largely due to this predominance of 'Hayward'. It allowed the development of the New Zealand kiwifruit industry primarily

supplying fruit for export to overseas markets. When kiwifruit cultivation started in other countries, growers in those countries also started growing 'Hayward' and today at least 95% of the kiwifruit grown outside China and New Zealand are of this one cultivar: it dominates international trade. Apart from the accompanying male pollenisers, kiwifruit orchards in most parts of the world are essentially a monoculture of 'Hayward'.

China and New Zealand are the exceptions. In China, although 'Hayward' is probably the most widely grown cultivar (Ferguson, 2015), many other kiwifruit cultivars also grown. New Zealand is also different because of breeding programmes to diversify the kiwifruit cultivars grown there. New Zealand pioneered kiwifruit cultivation and initially had a monopoly in world markets. Eventually, however, kiwifruit exporters from New Zealand faced increasing competition as cultivation of 'Hayward' began in other countries. One solution was the branding of kiwifruit produced in New Zealand to distinguish them from the kiwifruit coming from elsewhere. An even better solution was to develop new protectable kiwifruit cultivars that could be grown only in New Zealand or under licence.

The first commercially successful product of breeding programmes in New Zealand was the yellow-fleshed *A. chinensis* var. *chinensis* cultivar 'Hort16A', the fruit of which were marketed as Zespri™ Gold Kiwifruit. Commercial export of the new fruit started in 2000 and, for more than a decade, 'Hort16A' was the most widely grown, yellow-fleshed kiwifruit outside China. It was initially very successful and changed completely the international market in kiwifruit. Consumers liked the novel, sweeter fruit. Growers liked 'Hort16A' because the vines carried much heavier crops than 'Hayward' and the prices for the fruit were higher. Marketers liked it because the fruit were distinctive and easily distinguished. Furthermore, 'Hort16A' had the great advantage, from a marketing perspective, of being a protected cultivar that could be grown only under licence. By the 2011/2012 season, just over a quarter of all the kiwifruit exported from New Zealand were Zespri™ Gold Kiwifruit, 30 million tray equivalents. (A tray of 'Hort16A' fruit weighed about 3.2 kg) The export of the yellow-fleshed kiwifruit had become the most profitable part of the New Zealand kiwifruit industry and it was confidently predicted that production and sales would continue to expand rapidly. Instead the arrival of a highly virulent strain of bacterial canker of kiwifruit (caused by *Pseudomonas syringae* pv. *actinidiae* or Psa), first detected in New Zealand in November 2010, quickly meant that 'Hort16A' could not survive as a commercial cultivar. This was a severe financial blow to the industry a whole and to many individual growers.

It was fortunate that the Plant & Food Research kiwifruit breeding programmes had identified several promising alternative yellow-fleshed selections. These were originally chosen to complement 'Hort16A' because of their fruit maturing earlier or having greater storage life but one selection also proved to be much less susceptible to Psa. This selection, 'Zesy002', is commonly known as Gold3 and its fruit are marketed as Zespri® SunGold Kiwifruit. Nearly all the 'Hort16A' orchards in New Zealand have been grafted over to the new cultivar and production of the fruit has expanded rapidly from 1 million tray equivalents in 2012 to 48 million tray equivalents in 2016/2017. It seems that the greater tolerance of 'Zesy002' to Psa will allow sustained commercial production of yellow-fleshed kiwifruit in New Zealand and that the industry can look forward with renewed confidence.

These yellow-fleshed cultivars were bred and selected for New Zealand's growing conditions and marketing requirements. Both have been grown under licence in northern hemisphere countries to ensure year-round supplies of fruit meeting Zespri's marketing strategies. As most kiwifruit exported from New Zealand are handled by Zespri, the only cultivars likely to be successful in New Zealand are those that Zespri decides to market. Cultivars that are adopted have the advantage of Zespri's promotion and marketing but orchardists will have little incentive to experiment with alternative cultivars.

Yellow-fleshed kiwifruit cultivars are also grown in China but probably represent little more than 10–15% of the total production. In other countries, especially Chile, Italy and Japan, there are less extensive plantings of other yellow-fleshed kiwifruit cultivars. These include selections from the wild, e.g., 'Jintao' (marketed as Jingold™ by Consorzio

Kiwigold®) (Ferguson et al., 2012), and cultivars resulting from planned breeding programmes, e.g., 'Dorì', 'Soreli' and 'Sanuki Gold' (Ferguson et al., 2012).

Although 'Hayward' appears to be adaptable and it is successfully grown in many different countries, it is not perfect: it is not particularly productive, its fruit mature late in the season and many consumers prefer a sweeter fruit (Ferguson et al., 1990). Breeding programmes have resulted in interesting selections of green-fleshed alternatives but none has yet been grown extensively. In China, there are many alternatives and, as production is expanding rapidly, possibly faster than demand, it will be interesting to see how consumers choose between the fruit of the different kiwifruit cultivars available.

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Breeding of red-fleshed kiwifruit cultivars with high quality and promotion

Mingzhang Li[1,3,a], Yong Yin[2], Qiguo Zhuang[1], Lihua Wang[1,3], Yue Xie[1,3], Qian Zhang[1,3] and Min Yuan[1,3]

[1]Sichuan Provincial Academy of Natural Resource Sciences, China; [2]Plant Protective Station, Sichuan Provincial Agricultural Department, China; [3]Kiwifruit Breeding and Utilization Key Laboratory of Sichuan Province, China.

Abstract

The *Actinidia* Germplasm is originally from China. It is one of the most favourite fruits by customers which is rich in Vitamin C. In the recent 20 years, the cultivated area of kiwifruit in China has increased 50 times, accounting for 70% of the world's production. It is an important featured fruit in China. Before the implementation of the project, the major kiwifruit cultivar was the green kiwifruit cultivar 'Hayward' from New Zealand, which accounts for about 98% of the global yields. Because of its sour taste, the consumer group is relatively small. In view of the fact that the world lacks red kiwifruit and the supporting cultivation and management techniques, the project team took 32 years to develop a new red cultivar with moderate sweetness, good quality and high market acceptance by utilizing the unique and rare red kiwifruit resources in China. The supporting research has integrated the technology system of industrialization. It is of great significance to promote the structural reformation on the supplying side of agriculture, to overcome poverty in poor areas and to build a healthy China.

RESULTS

Broadened a new field of red flesh kiwifruit breeding; took the lead in breeding a batch of high-quality red-fleshed kiwifruit cultivars authorized both at home and abroad; enriched the structure of kiwifruit cultivars; highlighted the characteristics of Chinese kiwifruit industry; promoted international competitiveness.

1. A new field of red-fleshed kiwifruit breeding has been created, and the first red-fleshed kiwifruit cultivar in the world, 'Hongyang', has been bred. The main economic characters of 'Hongyang' have unique advantages compared with those of green-fleshed kiwifruit (Table 1).

Table 1. Comparison of main economic characters of 'Hongyang', green- and yellow-fleshed kiwifruit.

Content	Red 'Hongyang'	Green 'Hayward'	Yellow SunGold
Core color	Red	Green	Yellow
Soluble solids (%)	19.6	13.3	15.1
Total sugar (%)	13.5	8.46	9.62
Total acids (%)	0.49	1.25	1.38
Vitamin C (mg 100 g^{-1})	135.77	106	137.7
Dry matter (%)	19.11	16.50	17.8
Growth and development period (days)	140	180	170

In 1982, the project team collected wild *Actinidia chinensis* seeds from Funiu Mountain, Xixia County, Henan Province. The wild seeds were planted and the seedlings cultivated for 15 years. And in 1997, a selection passed the examination and approval of the

[a]E-mail: 13981769856@163.com

Sichuan Provincial Variety Review Committee. It was named 'Hongyang' and got the authorization from both China and Argentina in 2005. 'Hongyang' is not only the most important red-fleshed kiwifruit cultivar in China, but also the parental material for red-fleshed kiwifruit breeding throughout the world.

Compared with 'Hayward', the total sugar content of 'Hongyang' is 5.04 % units higher (60% higher), the soluble solids content is 6.30% units higher (47% higher), the dry matter content is 2.61% units higher (16% higher) and the vitamin C content is 135.77 mg 100 g^{-1} fresh weight. Compared with the yellow-fleshed cultivar 'Zesy002' (known as G3 and fruit sold as SunGold Kiwifruit) from New Zealand, the total sugar content of 'Hongyang' is 3.88% units higher (40% higher) the soluble solids content is 4.50% units higher (30% higher) and the dry matter content is 1.31 % units higher (7% higher). 'Hongyang' has the characteristics of early maturity, bright flesh color, high sugar content, rich aroma, fine taste and high quality (Figure 1).

Figure 1. Cultivars 'Hongyang', 'Hayward' and 'Zesy002' (SunGold).

2. Breeding of new red kiwifruit cultivars with different harvest times.

From 1995 to 2014, a male parent with the red gene was used to do controlled hybridization with 'Hongyang' as the female parent. Four cultivars were selected: two medium-maturing cultivars ('Hongshi No. 1', 'Hongshi No. 2') and two late-maturing cultivars ('Honghua' and 'Hongmei'). The reasonable matching of early, mid and late harvest time cultivars of red kiwifruit was realized.

3. Breeding of new red kiwifruit cultivar – 'Hongshi No. 2'.

In 2000, a new paternal parent (SF0612M) with the red gene was used to do controlled directional crosses with 'Hongyang' as the female parent. After 14 years, a new red kiwifruit cultivar was developed. The cultivar gained the new cultivar rights in China in 2016, and gained the cultivar rights in the EU in 2017.

'Hongshi No. 2' is superior to 'Hongyang' in terms of the main economic characters, its yield is 30% more and its disease resistance is stronger Table 2). It has gradually become an alternative cultivar to 'Hongyang'.

Table 2. Comparison of main economic characteristics of 'Hongshi No. 2' and 'Hongyang'.

Cultivar characteristics	'Hongshi No. 2'	'Hongyang'
Single fruit weight	75-100 g	50-60 g
Redness	More red, 40%, stable	Less red, 20%, big variation
Core	Not hollow	Hollow
Taste	Sweet and sour moderate	Sweet
Resistance to Psa	Medium-resistant	Sensitive

The genetic trend of the main economic characters of *Actinidia chinensis* was revealed, and the breeding technology system of red-fleshed kiwifruit was established by the cultivation of male parent kiwifruit with red gene as the key technique.

1. Genetic trend of the main economic characters of the cross progeny of *Actinidia chinensis* var. *rufopulpa* was revealed.

From 2003 to 2010, a total of 375 hybrid seedlings were cultivated by crossing the female parent 'Hongyang' and the male parent (SF0611-M, SF0612-M, SF0613-M, SF0616-M, SF0616-M, SF0618-M). The genetic tendency of economic characters of F_1 hybrids were studied. The results can be found in Table 3.

Table 3. Analysis of red pigment grade of F_1 hybrids of 'Hongyang' (HY).

Hybridized combination	Female parent (level)	Grade ratio of red pigment in F1 hybrid (%)						Similar maternal type (%)	Supermaternal ratio (%)
		0	1	2	3	4	5		
HY×SF0611-M	4	72.22	5.56	0.00	11.11	11.11	0.00	11.11	0.00
HY×SF0612-M	4	28.57	14.28	21.42	0.00	7.14	28.57	7.14	28.57
HY×SF0613-M	4	66.67	16.67	0.00	0.00	0.00	16.67	0.00	16.67
HY×SF0616-M	4	60.00	20.00	20.00	0.00	0.00	0.00	0.00	0.00
HY×SF0618-M	4	62.50	12.50	0.00	12.50	0.00	12.50	0.00	12.50

The average heritability of red in fruit of F_1 hybirds was 48%, the heritability of 3-5 grade red was 19.92%, the ratio of male and female plant was 1:1.3-2.3, fruit size ranged from 30-95 g, the soluble solids content of fruit ranged from 16-20%, and the dry matter content of fruit was higher than that of 'Hongyang' by 0.05-1.03 % units.

2. Based on the breeding of male parent with red gene, the breeding technology system of red kiwifruit was established.

Based on the fact that kiwifruit is dioecious and the male does not bear fruit, it is impossible to judge whether male plants have the red gene or not. The key to breeding a red cultivar is to select a male parent with the red gene. In the first stage, the male seedlings of 'Hongyang' were selected, and in the second stage, the male plants were hybridized with yellow-fleshed female plants, respectively. After the hybrid seedlings were obtained, the red pigment degree of the hybrid combinations was evaluated. When the proportion of plants of red pigment grade between 3-5 level was more than 60%, the male plant could be identified as having the red gene (Table 4).

Table 4. Analysis of red pigment grade of the hybrid progenies of yellow-fleshed cultivars and selected males (partial combinations).

Hybridized combination	Flesh color of female parent	Degree ratio of red pigment in the hybrid progeny (%)						3-5 Level
		0	1	2	3	4	5	Total
Yellow×SFM0809	Yellow	10.55	5.56	10.53	28.65	25.78	18.93	73.36
Yellow×SFM1998	Yellow	18.24	5.58	12.4	25.56	23.72	14.5	63.78
Yellow×SFM0612	Yellow	15.69	5.76	11.55	26.87	23.46	16.67	67.00
Yellow×SFM0616	Yellow	57.89	15.31	13.45	13.35	0.00	0.00	13.35

Since 1990, it has taken 9 years to detect and analyze the cross progeny. Three male parents with the red gene, SFM0809, SFM1998, SFM0612, were identified. Based on the systematic evaluation of germplasm resources over 8-10 years, 79 female red breeding materials with large fruit, good fruit shape, high dry matter content, good storage performance, high content of VC, long shelf life, good taste, different ploidy and high grade of red performance were found. The red kiwifruit breeding technology system (such as collection, preservation and evaluation of red germplasm resources, paternal plant

breeding with red gene, hybridization, identification of genetic stability, cultivar comparison, production and cultivation, ecological adaptability test) was established.

Collection and preservation of the world's largest red-fleshed kiwifruit germplasm resource nursery has laid a solid foundation for the continuous cultivation of new red-fleshed kiwifruit cultivars.

1. Established the germplasm repository with the largest red-fleshed kiwifruit germplasm resources.

During more than 20 years of sustained collection across the country, 1,449 kiwifruit germplasm accessions were collected, and 29,490 genotypes were preserved, and the area of germplasm repository was 210 mu, which is the largest in the world. 132 red-fleshed kiwifruit germplasm accessions were collected and 5,280 genotypes were preserved, accounting for more than 90% of the preserved red-fleshed kiwifruit germplasm resources in the world (Table 5).

Table 5. Collection and conservation of kiwifruit germplasm resources.

Year	*A. chinensis*		*A. deliciosa*		*Actinidia sp.*		*A. chinensis* **var.** *rufopulpa and A. deliciosa* **var.** *coloris*	
	Accessions	**Plants**	**Accessions**	**Plants**	**Accessions**	**Plants**	**Accessions**	**Plants**
1997-2000 (Yangtze river basin)	0	0	66	1320	15	150	8	320
2001-2002 (Qinba Mountain)	0	0	89	1780	23	230	23	920
2003 (E'Mei Mountain, Henan)	135	2700	113	2260	40	400	36	1440
2004-2014 (Jiangxi, Fujian, Zhejiang)	324	6170	0	0	50	500	30	1400
2015 (Jiangxi, Guangxi province)	149	2980	0	0	30	300	25	1000
2016 (Jiangxi, Qinba Mountain)	223	4460	36	720	24	240	10	400
Total	*831*	*16310*	*304*	*6080*	*182*	*1820*	*132*	*5280*

A. specice: *Acitinidia arguta*, *purpurea*, *henryi*, *eriantha*, *macrosperma*, *maloides*, *melanandra*, *polygama*, *rubricaulis*, *teramera*, *henanensis*, *kolomikta*, *latifolia*.

A. chinensis var. *rufopulpa* and *A. deliciosa coloris*.

2. Evaluation system of germplasm resources was developed and a kiwifruit germplasm database was established.

Based on 86 key indexes, such as yield, fruit size, pulp color, resistance, nutrition, quality, storage tolerance and ploidy, an evaluation system for kiwifruit germplasm resources was developed. 29,490 genotypes have been evaluated one by one, more than 2.62 million data were preserved, and a database of kiwifruit germplasm resources was established.

3. Five breeding parents with high resistance to Psa were identified by laboratory and field trial evaluation of germplasm resources: 12 05-12 B, SF-0808, sf0828, 14-15-05B, and 14-04-19A.

The early-fruit, high-yield and high-efficiency planting technology of red kiwifruit and the green cultivation technology system of kiwifruit which is dominated by

prevention and control of Psa were established, and the whole industry chain technology of red kiwifruit was realized.

1. An early-fruiting, high-yielding and high-efficiency planting technology system for red-fleshed kiwifruit has been developed, including high orchard-building standard, planting grafted seedling in nutrient bag, precision formula fertilization by soil testing, integration of water and fertilizer, cultivating single-stem double-vine structures, mechanized pollination, flower thinning, fruit thinning, fruit bagging , shelter from rain in winter and early spring (Table 6).

Table 6. Comparison of crop yields with modified and conventional management.

	Year 1 (fruit kg mu^{-1})	Year 2 (fruit kg mu^{-1})	Year 3 fruit kg mu^{-1})	Year 4 (fruit kg mu^{-1})	Year 5 (fruit kg mu^{-1})	Average yield (fruit kg mu^{-1})	Commercial fruit packout rate (%)
Modified management	0	284	558	1069	1564	1063	81.1
Conventional techniques	0	0	268	859	1347	824	68.1

Large demonstration areas achieved harvest 2 years earlier, the average yield per mu increased by 239 kg when vines are mature fruiting, the rate of commercial fruit packout increased by 13%, and the net income per mu increased by 3483 yuan.

2. Main pests and diseases of kiwifruit were studied, and the strain of Psa in Sichuan was determined as PSA-3. Innovative methods for the rapid detection of Psa were developed. The technology system of "green prevention and control of Psa" was established, it involves the technology of "green greenhouse + winter protection + pharmaceutical control + healthy cultivation" (Table 7).

Table 7. Comparison between stereoscopic control and routine treatment of Psa.

Content	Incidence of diseased branches (%)	Incidence of diseased leaves (%)	Plant survival rate (%)	Bud		Amount of fruit (per plant)
				Rate (%)	Length (mm)	
Stereoscopic control	23.1	0	92.3	71.28	34.6	47.3
Routine treatment	60.8	93.2	33.1	71.32	21.63	3.2

Compared with the plants treated with routine treatment, the average incidence of Psa-diseased branches of the stereoscopic group was reduced by 62%, the incidence of diseased leaves was reduced to zero, the loss of vines was reduced nine-fold rate, the length of bud increased by 12.97 mm, and the amount of fruit was increased by about 14-fold, which basically solves the problem of vine death and orchard destruction caused by Psa. By promoting the green control technology of main diseases and pests, the average times of pesticide use has been reduced by two thirds, and the expenditure of pesticide has reduced more than 120 yuan mu^{-1}. The green high-end fruit produced in the green cultivation demonstration area meets the pesticide limit standard for export to the EU.

3. Harvest indexes, grading and packing standards and the technical parameters of controlled-atmosphere refrigeration of red-fleshed kiwifruit were determined.

From 2013 to 2016, the project group selected 6 commercial orchards of 'Hongshi No. 2' in Mianzhu and Shifang, Sichuan Province, three at each place, to study the harvest and storage periods of the fruit. The results are as follows: the optimum harvest index of red-fleshed kiwifruit ('Hongshi No. 2') is 150-170 days after full bloom, the dry matter should be around 17.9-20.9%, the soluble solids should be around 7.4-9.0%, the firmness of the fruit should be 5.0-6.0 kgf, the red grade should be 3-5 level. These five comprehensive

indexes are determined as the standard for harvesting red-fleshed kiwifruit. According to the indexes, the fruit quality will be good, the commodity consistency will be good, and the storage performance will be good.

The grading and packing standard is that fresh fruit should be packed in a single-layer tray with a net weight of 2.3-2.5 kg box^{-1}. It can be divided into five size grades: 36-grade, 60-70 g fruit^{-1}; 33-grade, 71-80 g fruit^{-1}; 30-grade, 81-90 g fruit^{-1}; 25-grade, 91-100 g kg^{-1} fruit^{-1}; 22-grade, 101-110 g fruit^{-1}. The storage temperature is 1±0.5°C, and the atmosphere composition is 2% O_2 and 5% CO_2. The storage period of fruit was prolonged for more than 2 months. Sixteen highly processed products in four categories have been developed, large-scale production has been achieved, and the industrial chain has been extended.

Promotion

1. Promotion.

From 2009 to 2015, 'Hongyang' was listed as the major kiwifruit cultivar in Sichuan province. The red-fleshed kiwifruit cultivars, mainly 'Hongyang', have been widely promoted and cultivated in 15 provinces (cities) such as Sichuan, Guizhou, Chongqing, Yunnan, Hunan, Henan, Shaanxi and Anhui for 20 years. In 2017, 1.18 million mu of the red-fleshed kiwifruit cultivars have been planted all over the country, accounting for 37% of the total area of kiwifruit in China.

2. Promotion and application prospect of the project results.

The ripening period of red-fleshed kiwifruit is about 1-2 months earlier than that of the green- and yellow-fleshed cultivars in China, which is half a year earlier than that of other major kiwifruit producing countries, such as New Zealand and Chile. It has the distinct advantage of different maturing time. Owing to its excellent quality and unique commercial characteristics, red-fleshed kiwifruit has become the third generation of the kiwifruit cultivars after the green- and yellow-fleshed cultivars from New Zealand, and has a broad market at home and abroad.

Social benefits

1. Red-fleshed kiwifruit is an important industry for poverty alleviation in poorer areas, and it plays an important role in lifting poor farmers out of poverty.

In Sichuan, Guizhou, Chongqing, Yunnan, Hunan, Henan and other poverty-stricken areas suitable for the development of red-fleshed kiwifruit, the technical backbone among farmers have been trained 50,000 times, and the fruit farmers have been trained nearly 100,000 times. The production skills of farmers were improved. The new red-fleshed cultivars were established over 1.18 million mu, and 590,000 rural poor people were lifted out of poverty, the annual net income was 6.793 billion yuan, and the annual income per capita was 11,500 yuan. At the same time, 350,000 people were engaged in the commercialization, preservation, refrigeration, processing, and sales of kiwifruit. The annual net income of related industries was 6.4 billion yuan, and the annual increase per capita 18,200 yuan. According to Xinhua News Agency, General Secretary Xi Jinping visited Xiangxi Tujia and Miao Autonomous Prefecture in Wuling Mountain to taste the locally produced 'Hongyang' on November 3, 2013. He said: "it tastes good, the texture is pretty good". The farmer informed him that the price of this kiwifruit is 12-15 yuan jin^{-1}, and the income mu^{-1} is 20,000-40,000 yuan. "That's a high added value", he said. Red-fleshed kiwifruit have become one of the most important industries in the poorer areas of China.

2. Promote technological progress in the industry.

The red-fleshed cultivar 'Hongyang', developed by the project group, has been widely used in the breeding of red-fleshed kiwifruit throughout the world. 90% of the world's red-fleshed kiwifruit cultivars have been bred using 'Hongyang', and this has promoted the

scientific and technological progress of red-fleshed kiwifruit breeding around the world. ‘Hongyang’ was the cultivar used to construct the first kiwifruit genome sequence in the world, which lays the foundation for genomics research. The red-fleshed cultivar cultivated by the project group accounted for more than 95% of the total cultivation area of the global red-fleshed kiwifruit cultivars, which promoted the development of the world red-fleshed kiwifruit industry. The innovative research results of the project have attracted wide attention both at home and abroad, and the China-New Zealand Kiwifruit Joint Laboratory has been established and formally opened by General Secretary Xi Jinping on November 21, 2014, and has significantly improved the global kiwifruit joint research and development level.

A new yellow-fleshed kiwifruit cultivar 'Nongda Jinmi'

Chunchao Yao[1], Jianjun Li[2], Junyi Yu[1] and Zhande Liu[1,a]

[1]College of Horticulture, Northwest A & F University, Yangling, Shaanxi 712100, China; [2]College of Plant Protection, Northwest A & F University, Yangling, Shaanxi 712100, China.

Abstract

'Nongda Jinmi' is a new yellow-fleshed kiwifruit cultivar, selected from the crossing of 'Jinnong 2' × Male of 'Jingyang 1'. The fruit shape is nearly cylindrical, while the fruit skin is green and covered with short hairs. The average fruit weight is 82.1 g, with yellow flesh color. The soluble solids content is 20.2%, the total sugars about 14.2%, total acids 1.42% and vitamin C 2.04 mg 100 g^{-1}. The fruit taste is delicious with balanced sweet and sour, and rich in aroma. The ripening period is in early September in Guanzhong Plain of Shaanxi Province. The yield is up to 30 t ha^{-2} in vines are fully mature.

Keywords: kiwifruit, yellow flesh, cultivar

INTRODUCTION

Kiwifruit is one of the wild fruit plants domesticated successfully in the 20th century (Ferguson and Bollard 1990). Since 1978, China has bred and selected over 100 cultivars of kiwifruit (Chen et al., 2009). 'Nongda Jinmi' is a new yellow-fleshed kiwifruit cultivar selected from offspring from crossing of 'Jinnong 2' × Male of 'Jingyang 1'. Crosses were carried out in April 1998 at the University's kiwifruit germplasm repository and over 6,000 hybrid plants were obtained in March 1999. Hybrid plants began to bear fruit in 2003. One of the plants was observed since it grew vigorously and performed well in resistance to stress, with high yields of early-ripening fruit. It was selected in 2007 as an early-ripening kiwifruit cultivar. Cultivar comparative experiments and regional trials were carried out in 2008. Through years' observation, it was concluded that the cultivar performs well with consistent high yields, was well-adapted, disease tolerant, especially more tolerant to PSA than the comparative cultivars such as 'Cuixiang' and 'Golden Fruit'. The cultivar was examined and approved in December 2012 by the Provincial Fruit Tree Cultivar Examination and Approval Committee as a new cultivar named 'Nongda Jinmi' (Figure 1).

CHARACTERISTICS OF THE CULTIVAR

The vines of the cultivar grow vigorously, one-year-old canes are greenish brown with grey-white hairs; fusiform lenticels off-white; mean internode distances 4.32 cm, laminate pith; oblate leaf with acute leaf apex and heart-shaped leaf base, upper surface glossy dark green, lower surface light green covered with white hairs, leaf 12.35 cm in length and 13.98 cm in width, crenate margin and light green stalk 9.67 cm on average; white flowers in cymes of three, oblong petals, usually six, rarely seven, per flower, corolla diameter 4.13 cm on average; six sepals; 36.5 pistils on average fused together; degenerated stamens with no viable pollen; cylindrical fruits of green skin with short hairs, on average 5.23 cm in vertical diameter and 4.76 cm in transverse diameter; fruit weight 82.1 g on average. The flesh of unripe fruit is greenish yellow in color, juicy and fine in texture, fragrant and sweet flavor. Soluble solids in fruit is 20.2%, total sugar 14.2%, total acid 1.42% and vitamin C 2.04 mg 100 g^{-1}.

[a]E-mail:dezhanliu@vip.sina.com

Figure 1. The new yellow-fleshed kiwifruit cultivar 'Nongda Jinmi'.

In the Guanzhong Plain of Shaanxi, sap bleeding occurs in February, budbreak begins the middle of March and flowering begins the middle of April. Flowers normally last 5~7 days and fruits ripen in early September after 120~130 days growth. Fruits can be stored for 15~20 days at room temperature and 90~120 days at 1±0.5°C. The vine goes into dormancy in November.

KEY CULTIVATION TECHNIQUES

In Shaanxi, the cultivar is suitable for growing on the northern and southern slopes of the Qinling Mountains and similar ecological regions. Planting density is 2 × 3~3.5 m matched with the designated male plant coded as number 10-6. The ratio of females to male is 8:1. T-bar trellis or pergola is adopted for orchards, with one stem and two main branches which support the canes. Thinning of the shoots and tipping are usually carried out in summer and thinning of crowded or weak or aged or disease-infected branches. Fruiting canes reserved should be robust and well-grown and cut back leaving 15~20 buds and 12 or 14 fruiting canes kept. After thinning of flowers and fruits, 3~5 fruits should be retained on long fruiting shoots, 2~3 fruits reserved on middle-length shoots and 1 fruit reserved on short fruiting shoots. In a well-established orchard, 35~40 fruits per m^2 of canopy should be retained. Organic fertilizer is preferred and is applied five times per year at the stages of budbreak, pre-blossoming, fruit swelling, fruit optimizing and post-fruiting.

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Kiwifruit breeding, new cultivars, and branding

A. Atak[a]

Atatürk Horticulture Central Research Institute, 77102 Yalova, Turkey.

Abstract

Kiwifruit are grown in more than 30 different countries. In addition, production is increasing every year. With increased production, consumers are demanding new cultivars. There is also a demand for cultivars resistant to diseases such as Psa (*Pseudomonas syringae* pv. *actinidiae*). Kiwifruit breeding has a history of about 100 years in the world but commercial cultivars have been obtained over the last 50 years. *Actinidia* (kiwifruit) germplasm collection provides a vital resource for breeding programmes. The genetic diversity provides an extensive library for genomics researchers to identify genes controlling key traits. At first new cultivars resulted from selection from the wild. Breeding studies are now carried out in the form of controlled hybrids and using marker-assisted selection.

An increasing number of the cultivars are being produced as a result of systematic breeding programs involving controlled/planned crosses, both intraspecific and interspecific. The most extensive breeding studies are carried out in New Zealand, Italy and China. Also breeding studies are continuing in Turkey, Chile, Japan, South Korea, Iran, USA and Greece. New cultivars always attract the consumers. These cultivars are sold at high prices with different flavours and attractive appearance. In breeding programmes, the initial aim was to obtain new cultivars with different fruit flesh colours (yellow and red). Other aims were self-fertility, chlorosis-resistance, and a lack of fan-shaped fruit. In recent years, resistance to Psa has become one of the most important aims of breeding programmes. Psa-susceptible cultivars should be grown in regions with low disease risk and in suitable soil/weather conditions. New cultivars are also beginning to be grown under plastic cover to protect plants from diseases in recent years.

Kiwifruit were introduced to Turkey in the late 1980s. The production of kiwifruit is increasing every year, is now close to 60,000 t and is increasing every year. Almost all the kiwifruit grown in Turkey are of the cultivar 'Hayward'. Half of the production in Turkey is from Yalova Province and the other half from the northern part of the country especially the Middle and Eastern Black Sea regions. In Turkey, kiwifruit breeding programmes began at the Atatürk Horticultural Central Research Institute in 2008. This breeding program is currently the only kiwifruit breeding programme in the country. Some genotypes have been selected as potential candidate cultivars. Observations and analyses with other genotypes and controlled hybrids are continuing. The first registration of a new cultivar was completed at the beginning of 2018.

Growers need to produce new cultivars in order to achieve high profits but that is not sufficient: it is also very important to make a high value brand. It is not possible to make high quality production and branding by itself. In these systems, growers are following the same production model and finally they produce high quality fruits with a certain brand value. Nowadays, production is being done with high quality under a single brand that will be the brand value. Today, consumers want to buy products that have brand name and know all production conditions from field/orchard to table. They can buy such products at a higher price. The competitiveness of other rivals is lower than those of the brand-name ones. A brand

[a]E-mail: arif.atak@tarim.gov.tr

should be created that will meet all the expectations and needs of consumers. It is expected that the brands that are producing in more than one country, not just in one country, will increase and they will dominate market in the near future.

Keywords: quality, selection, brand, Turkey, *Actinidia*

Reports on three new cultivars of kiwifruit 'Miliang 2', 'Xiangbiyu' and 'Beimu' selected from western Hunan Province, Central South China

Shibiao Liu[a], Xiaoqi Xiang, Qijian Tian, Jifu Cheng and Zeliang Shi

Kiwifruit Research Center of Wuling Mountains, Jishou University, Jishou 416000, Hunan, China.

Abstract

Three new cultivars of kiwifruit selected from Western Hunan province, central south of China were reported. 'Miliang 2' kiwifruit (*Actinidia deliciosa*) is a green-fleshed cultivar with long cylindrical fruit, a protruding beak at the stylar end, and dense grey-brown trichomes on the surface. This cultivar is suitable for growing in areas of altitude of 400-700 m, wherein the fruit matures in the last ten days of September. The average single fruit weight is 95 g and the best fruit yield is 22.5 t ha^{-1}. The fruit shelf life is about 10-15 days at room temperature after soft ripening. 'Xiangbiyu' (*A. deliciosa*) is a darkgreen-fleshed cultivar with short cylindrical fruit, an obvious sunken beak, and long dense, hard hairs. Areas at an altitude of 500-800 m are suitable for this cultivar, and the second ten days of October are the fruit maturation period. This cultivar has an average single fruit weight of 148 g and best fruit yield of 30-37.5 t ha^{-1}. The shelf life is about 7-10 days. 'Beimu' (*A. chinensis*) is a yellow-fleshed cultivar, with cylindrical fruit, slightly protruding beak and sparse or no pubescence. This vine is suitable for growing at an altitude of 400-700 m, where it could be harvested from the first ten days of October. The average single fruit weight is 100 g and the best fruit yield is 30.0 t ha^{-1}. The fruit has 7-10 days shelf life. All the cultivars are vigorous, fruitful and strongly resistant to diseases and pests. Cultivation techniques are briefly described.

Keywords: kiwifruit, new cultivar, 'Miliang 2', 'Xiangbiyu', 'Beimu', trait, cultivation

INTRODUCTION

Kiwifruit belong to the genus *Actinidia*, Actinidiaceae, which comprises more than 60 species native to large parts of China and some neighboring countries (Cheng et al., 2011). The western region of Hunan province (N 27°44'~29°38', E 109°10'~110°23') in Central South China, which administratively consists of 8 counties, is one of the natural distribution areas of kiwifruit in China (Huang, 2014), and is rich in wild kiwifruit resources. Three cultivars of kiwifruit including 'Miliang 1' (*A. deliciosa*), 'Xiangji' (*A. deliciosa*) and 'Xiangjihong' (*A. chinensis*) were selected from this region, and the fruit of the last two can be seedless if not pollinated (Pei et al., 2010). The present paper reports the selection and cultivation of three new cultivars of kiwifruit from the above region, namely 'Miliang 2', 'Xiangbiyu' and 'Beimu' that had been granted cultivar right by the Office of the Protection of New Varieties of Plants, MOA, P.R. China.

SELECTION PROCESS

A special vine of the cultivar 'Milang 1' (*A. deliciosa*) was reported by a kiwifruit grower, in Songbai Town, Yongshun County, Western Hunan Province in 2004, as having fruit which matured 10-15 days earlier and tasted sweeter than fruit of other vines of 'Miliang 1', though the shape and size were the similar. This was the mother plant of the

[a]E-mail: liushibiao_1@163.com

subsequent green-fleshed cultivar 'Miliang 2'. In the autumn of 2003, a rare vine with large, cylindrical and some redged fruits that were covered with long, hard hairs was found in Lvdong Mountain, Baojing County: this became the mother vine of the green-fleshed cultivar 'Xiangbiyu' (*A. deliciosa*). Interestingly, a plant that grew at an altitude of 600 m and bore glabrous, cylindrical, yellow-fleshed fruits was noted by the authors in the same site in 2007, and this was the mother vine of the new cultivar 'Beimu' (*A. chinensis*). Budwood of these three vines was collected the following spring and grafted as scions onto 30 seedlings grown from the seeds of native wild kiwifruit (*A. deliciosa*). The grafts grew vigorously in the nursery. Some of the vines flowered and bore fruits the subsequent year. The buds on the canes that bore maximum and typical fruits were selected and grafted onto seedlings again. Two generations later, the best vine was chosen from 100 vines of the descendants of each genotype according to a botanical and commercial evaluation and criteria, and buds of each were grafted on the stocks in a commercial garden in early spring. The vines began to flower and fruit on a small-scale the following year, and reached full cropping from year 4.

Having been observed and propagated for a number of years, the vines were confirmed to be distinctive new strains which had characteristics different from other published cultivars. So, the three strains were named as 'Miliang 2', 'Xiangbiyu' and 'Beimu' respectively. Applications for the protection of new cultivars of plants of the three strains were submitted to MOA, China in 2014. After the preliminary examination and substantive DUS examination (distinctness, uniformity and stability) according to the national guidelines on breeders' trials, cultivar rights were granted, issued, registered and published in 2017 by MOA, China (CNA20140291.8, CNA20140292.7, CNA20160071.2).

BOTANICAL CHARACTERISTICS

'Miliang 2'

The one-year-old canes are brown with many long spindle-shaped lenticels, larger bud bases, coarse bark and lamellate pith. The leaf is heart-shaped with an average length of 15.9 cm and width of 15.4 cm, serrulate margin and tapering blade tip. The upper surface is hairless or has rare hair, while the lower surface is densely covered with small trichomes. The slightly purplish petiole has an average length of 8.5 cm. The flowers gather into dichasia from nodes 1-5. The flower has a pedicel 4.5 cm long, 5-6 sepals, 5-6 petals, and the white corolla is 6.1cm in diameter. The flower has 41-45 stigmas and 55-56 degenerated stamens. The fruit is long cylindrical in shape, with round fruit shoulders at the flattened stalk end, protruding fruit beak at the stylar end and a stalk 5.6 cm in length. The fruit surface is brown, covered with dense, grey-brown trichomes and reddish-brown spots. The mature seed are orbicular-ovate and puce after air drying.

'Xiangbiyu'

This is a cultivar with strong vine vigor and resistance. The one-year-old canes are red-brown with long spindle-shaped lenticels, larger bud bases, dense hair and lamellate pith. The leaf shape is broad ovate with an average length of 18.7 cm and width of 17.5 cm, wavy margin and acute tip. The upper surface is sparsely pubescencent and the lower surface has dense trichomes. The slightly purplish petiole has an average length of 6.9 cm. The flowers gather into dichasia from nodes 1-4. The flower has a pedicel of 4.6 cm long, more than 5 sepals, 5-6 petals that form a white corolla, 6.4 cm in diameter. The flower has 40-42 stigmas, 50-60 degenerated stamens. The fruit is cylindrical and some prismatic in shape, slightly narrower at the flattened stalk end, and slightly wider at the stylar end. It also has square shoulders, a deeply sunken beak, and a stalk of 4.1 cm long. The brown surface is densely covered with long, hard, bronze trichomes and reddish-brown spots. The mature seed is orbicular-ovate and puce.

'Beimu'

The one-year-old canes are drab with many elliptical lenticels, smaller bud base, smooth bark and hollow pith. The leaf is heart-shaped with wavy margin and acute tip, and an average size of 17.8 cm in length and 12.5 cm in width. The upper surface is hairless or has sparse hairs, while the lower surface is covered with sparse hair. The slightly purplish petiole has an average length of 18.7 cm. The flower is solitary and white, at the basal nodes 1-5, and has a pedicel 5.1 cm long, 5-6 sepals, 5-6 petals that constitute a corolla 4.7 cm in diameter. The flower has 32 stigmas and about 60 degenerated stamens. The fruit is short, cylindrical in shape, with square shoulders at the flattened stalk end, slightly protruding beak at the stylar end, and a stalk 5.2 cm in length. The surface of the fruit is light brown, covered with sparse, easily shed pubescence and small reddish-brown spots. The mature seeds are orbicular-ovate and puce when dry.

MAIN ECONOMIC TRAITS

'Miliang 2'

The fruit of 'Miliang 2' is similar to that of 'Miliang 1' in shape and size. However, the transverse section of 'Milang 1' is elliptical, while that of 'Miliang 2' is almost round or only slightly elliptical. The diameter of the transverse section is 5.7 cm and the vertical length is 9.0 cm. The average single fruit weight is 95 g with a maximum of 163 g. The fruit flesh is green, at the centre of which is a light-green, smaller elliptical axile placenta (2.1 × 0.55 cm) (Figure 1). The fruit is convenient to eat with easily peeled skin and sweeter taste (15.5% of soluble solid content compared with that of 13.7% for 'Miliang 1'). The general nutritional components in mature fruit were as follows: starch 32.25 mg g^{-1}, soluble sugar content 45.87 mg g^{-1}, sucrose 12.04 mg g^{-1}, fructose 12.34 mg g^{-1}, organic acids 5.85 g 100 g^{-1}, vitamin C 324.20 mg 100 g^{-1}. The fruit performed very well in storage, 55% of the fruits retained considerable quality after storage at room temperature for 3 months, and the optimum edible period is more than 90 days when stored at a temperature of 3°C. The shelf life at room temperature after soft ripening is about 10-15 days.

Figure 1. New cultivar 'Miliang 2' (left), compared with 'Miliang 1' (right).

'Xiangbiyu'

'Xiangbiyu' fruit are typically short but large and with long, hard hairs on the skin. The diameter of the transverse section is 6.4 cm and the vertical length is 6.8 cm. The average single fruit weight is 148 g with a maximum of 225 g. The fruit transverse section is almost round with dark green flesh. At the centre of the fruit is a middle sized, grey-yellowish, elliptic axile placenta (2.0 × 0.9 cm) (Figure 2). The general nutritional components in mature fruit were: starch 42.55 mg g, soluble sugar content 43.74 mg g^{-1}, sucrose 11.23 mg g^{-1}, fructose 11.48 mg g^{-1}, organic acids 6.78 g 100 g^{-1}, soluble solid 12.5%, vitamin C 388.36 mg 100 g^{-1}. In terms of the storage quality, 50% of the fruits survived in good condition when stored at room temperature for nearly 3 months, but the optimum edible period would last for 70-80 days when stored at a temperature of 3°C. At room temperature, the shelf life is about 7-10 days after soft ripening.

Figure 2. New cultivar 'Xiangbiyu' (left), compared with 'Miliang 1' (right).

'Beimu'

The main traits of 'Beimu' fruit are the lack of hairs and the yellow flesh. The diameter of the round transverse section is 5.4 cm and the vertical length is 5.9 cm. The average single fruit weight is 100 g with a maximum of 182 g. At the centre of the flesh is a yellow-white, middle sized and irregular (triangular to elliptical) axile placenta (1.5 × 0.87 cm) (Figure 3). The fruit tastes fine with good balance of sweet and acid. The general nutritional components in mature fruit were: starch 40.82 mg g^{-1}, soluble sugar content 40.40 mg g^{-1}, sucrose 10.04 mg g^{-1}, fructose 10.59 mg g^{-1}, organic acids 7.85 g 100 g^{-1}, soluble solids 15.2%, vitamin C 367.20 mg 100 g^{-1}. 'Beimu' was of high storage quality, 50% of the fruits were of adequate quality when stored at room temperature for 3 months, while the optimum edible period is about 80-90 days when stored at 3°C. The shelf life at room temperature after soft ripening is about 7-10 days.

Figure 3. New cultivar 'Beimu' (left), compared with 'Miliang 1' (right).

CULTIVATION TECHNIQUES

Phenology and development

1. 'Miliang 2'.

This cultivar is suitable for cultivation at middle altitudes 400-700 m in western Hunan province. Budbreak is in the first ten days of March. Flowering is during the last ten days of April to the first ten days of May. The fruit growth period is mainly in June and July. The fruit maturation period is in the last ten days of September, about 10-15 days earlier than that of 'Miliang 1'. The leaves begin to fall in mid-December before the dormant period. 'Miliang 2' has a medium budbreak rate of 52%, higher cane-forming rate of 88% and fruiting cane rate of 85%, and the fruits are borne mainly on short shoots which make up 80% of all the shoots, allowing convenient pruning and canopy development. On average, each fruiting cane bore 2-5 fruits of consistent shape and quality, which makes 'Miliang 2' bear 5 kg and 10 kg of fruits per vine at the third and fourth years respectively after grafting and transplanting. It comes into the best fruiting period from the fifth year with more than 20 kg of fruits per vine, a yield of about 22.5 t ha^{-1}.

2. 'Xiangbiyu'.

Middle to higher altitudes of 500-800 m are the best planting area for this cultivar. Within this range, budbreak is in the first ten days of March, and flowering is in the first ten days of May. June and July are the main growth period of the fruits, and the second ten days of October is the fruit maturation period. The leaves fall in December followed by dormancy. 'Xiangbiyu' has a medium budbreak rate of 50%, higher cane-forming rate of 90% and fruiting cane rate of 90%, with fruits borne mainly on short shoots that make up 85% of all the shoots. Two to four fruits are set on each fruiting cane with consistent shape and quality. Each vine bore on average 10 kg of fruit at the third year and 20 kg at the fourth year after grafting. At the best cropping period, each vine could bear more than 30 kg fruit, a high yield of 30-37.5 t ha^{-1}.

3. 'Beimu'.

This vine is suitable for growing at middle altitudes, 400-700 m. Budbreak is in the first ten days of March, flowering is in the last ten days of April. Fruit growth is mainly in June and July. The first ten days of October is the best period for fruit harvest. The leaves begin to fall in the middle of December before dormancy. The higher budbreak rate of 'Beimu' is 60%, higher cane-forming rate is 92% and fruiting cane rate is 90%, with fruits borne mainly on short shoots which made up 86% of all shoots. Two to five fruits are set on each fruiting cane with consistent shape and quality. 'Beimu' bore 8 kg and 15 kg of fruits per vine at the third and fourth years respectively after transplanting. Twenty five to thirty kg of fruits per vine was recorded from the fifth year: that corresponds to a high yield of about 30.0 t ha^{-1}. The flesh color slowly changes from "green-green yellow-light yellow-yellow-deep yellow", which was mainly determined by the ratio of contents of carotenoid to chlorophyll, and the color-changing period was about 120 days after pollination (Zhang, 2017).

Cultural management

In order to obtain high-yields of good quality of fruits, great attention should be paid to the integrated management and agricultural procedures. First, we chose an appropriate design and planting density of the female vines, e.g., 1100 vine ha^{-1} for 'Miliang 2', 975 vine ha^{-1} for 'Xiangbiyu' and 1100 vine ha^{-1} for 'Beimu', with the male vines planted in separate rows. Second, management of soil, manure and irrigation should be enhanced to promote flowering and fruiting after grafting and transplanting. The three cultivars studied have good resistance to diseases and pests, so no pesticides were applied in orchards up to now, which made it easy to meet the requirements of non-polluting agricultural production. When vines begin to fruit, enough fertilizer should be applied to ensure yield and quality. Third, artificial pollination is necessary and thinning of unwanted fruits should be carried out early enough to encourage the growth of remaining fruits. Fourth, reasonable pruning and canopy management should be applied in winter and summer to promote the balance of vegetative growth and reproductive growth, especially for the vigorous cultivars. Fifth, the harvested date should be determined at a suitable period when the soluble solid content reaches 6.0-6.5% to ensure fruit quality.

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Breeding tomorrow's healthy and tasty kiwifruit

R.G. Atkinson[1], X. Chen[1], A. Currie[2], K. Gunaseelan[1], N. Ileperuma[1], K.B. Marsh[1], A.J. Matich[3], N.J. Nieuwenhuizen[1], E.A. Popowski[4], A.G. Seal[4], E.J.F. Souleyre[1], M.Y. Wang[1], R.A. Winz[1] and Y.-K. Yauk[1]

[1]The New Zealand Institute for Plant & Food Research Limited (PFR), Private Bag 92169, Auckland 1142, New Zealand; [2]PFR, 55 Old Mill Rd RD3, Motueka 7198, New Zealand; [3]PFR, Private Bag 11600, Palmerston North 4442, New Zealand; [4]PFR, 412 No 1 Road RD 2, Te Puke 3182, New Zealand.

Abstract

Good flavour and high health benefits are essential characteristics of any kiwifruit cultivar, but are not always the primary focus of traditional breeding programmes. *Actinidia* germplasm contains a wide diversity of flavour and health compounds so presents a number of opportunities in the development of new cultivars. Recently, significant advances have been made in our understanding of the biosynthesis of key flavour volatile compounds such as esters, terpenes and aldehydes as well as health components such as ascorbate and actinidin. This information will be essential as kiwifruit breeding moves towards developing high-flavour and high-health cultivars using marker-assisted and whole genome selection strategies.

Keywords: actinidin, aldehyde, ascorbate, ester, flavour, health, terpene

INTRODUCTION

For a fruit cultivar to appeal to consumers it must be convenient, tasty and healthy. Some consumers believe that modern cultivars (e.g., of apple, grape and tomato) all taste the same and that fruit aren't as nutritious as they once were. One reason for this situation is that traditional breeding programmes in these crops have a focus on improving grower traits for industrial production of fruit such as pest and disease resistance, high yield and appearance, and there is not always a focus on breeding for what the consumer wants (Tieman et al., 2017). It is also difficult to breed for flavour and health traits because they are highly complex and variable traits that are difficult to measure rapidly. With recent advances in genome sequencing, transcriptomics and transgenesis the process of identifying genes involved in flavour and health in non-model crops has accelerated and a more gene-centric approach can now be considered for breeding novel, high-flavour and high-health fruit. To do this in kiwifruit requires an understanding of the diversity of compounds underpinning the flavour and health properties in *Actinidia* germplasm, as well as knowledge of the biosynthetic pathways, key rate-limiting genes, and desirable alleles that produce these compounds.

DIVERSITY IN FLAVOUR AND HEALTH COMPOUNDS

The ~50 species in the *Actinidia* genus are known to produce fruit with a diverse range of sizes, shapes and colours (Ferguson and Huang, 2007). This diversity is also reflected in the types and concentrations of flavour and health compounds found in both cultivars and wild germplasm. Non-volatile flavour compounds such as sugars (glucose, fructose and sucrose) and acids (citric, quinic and malic) contribute sweetness and tartness to the fruit. However, the balance between sweetness and acidity is typically more important than individual components (Marsh and Harker, 2016). Bitterness and astringency caused by polyphenolics, tannins, catechins and triterpenoids may be present in undeveloped germplasm.

The main volatile flavour compounds found in most kiwifruit cultivars and accessions are esters, aldehydes and alcohols (Figure 1). Esters are associated with ripe,

fruity notes, whilst aldehydes are associated with green, fresh notes. Other compounds such as terpenes, sulfur compounds and furans are typically found at lower concentrations and in a more restricted range of material. Nieuwenhuizen et al. (2015) showed that the concentration of terpenes varied by three orders of magnitude in a survey across fourteen *Actinidia* species. Despite their low concentrations in fruit, many of these terpene compounds are likely to be important to fruit flavour as they have low odour threshold values.

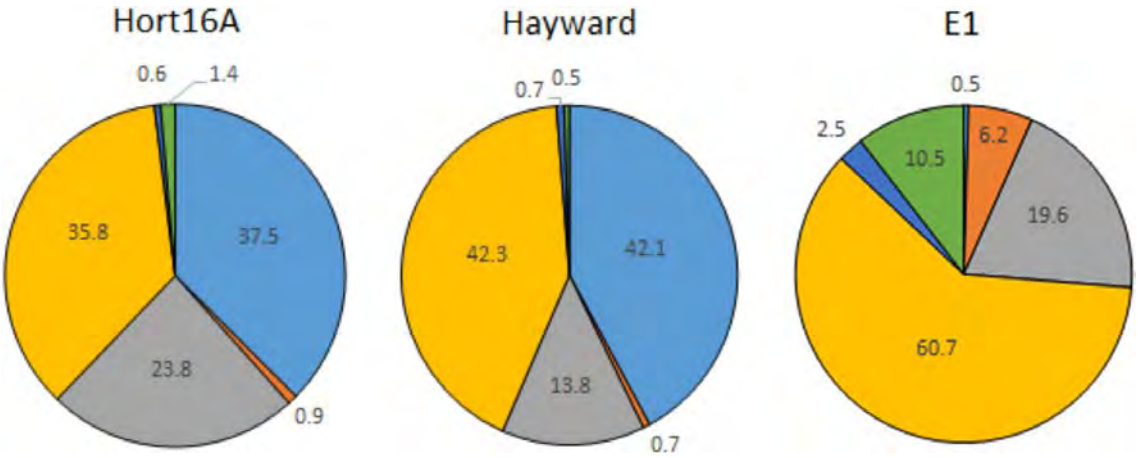

Figure 1. Diversity in kiwifruit volatile compounds. Headspace volatiles released from the ripe fruit of *A. chinensis* var. *chinensis* 'Hort16A', *A. chinensis* var. *deliciosa* 'Hayward' and the *A. eriantha* genotype E1 (Wang et al., 2006). Volatile data were obtained by direct thermal desorption and semi-quantitation by GC-MS. Esters (blue), aldehydes (yellow), alcohols (grey), acids (white), terpenes (orange) and other compounds (green) are represented as percentages of total volatiles detected.

Kiwifruit health components include folate, vitamin K, fibre, potassium, and polyphenolics (e.g. anthocyanins) (Boland and Moughan, 2013). However, kiwifruit are perhaps best known for their high concentrations of vitamin C (ascorbate) and actinidin. *A. chinensis* var. *deliciosa* 'Hayward' and *A. chinensis* var. *chinensis* 'Hort16A' fruit contain 85-110 mg ascorbate 100 g^{-1} FW while some *A. eriantha* fruit contains significantly higher concentrations (>800 mg 100 g^{-1} FW). Zespri Group Limited markets its fruit as containing "more vitamin C than an orange or two lemons" and states that "Zespri® SunGold kiwifruit ('Zesy002') contain more vitamin C than three lemons." Carr et al. (2012) demonstrated that the addition of kiwifruit to the daily diet significantly increased concentrations of vitamin C in plasma and suggested that kiwifruit was an excellent source of vitamin C in humans.

Actinidin is a soluble protein related to papain from papaya that is abundant in ripe kiwifruit (Nieuwenhuizen et al., 2007). It is a cysteine protease that aids digestion by hydrolysing proteins more completely than mammalian digestive enzymes alone (Rutherfurd et al., 2011) and is involved in increasing bowel movement frequency in both healthy and constipated individuals (Ansell et al., 2015). Conversely, studies have also shown that actinidin is the major kiwifruit protein responsible for allergic reactions in some, but not all, European populations (Lucas et al., 2007; Palacin et al., 2008).

KEY GENES IN THE BIOSYNTHESIS OF FLAVOUR AND HEALTH COMPOUNDS

Key genes involved in the biosynthesis of flavour volatiles such as terpenes, esters and aldehydes, as well as the health compounds vitamin C and actinidin, have been characterised in kiwifruit.

Terpene synthase (TPS) genes responsible for the production of terpene volatiles in flowers have been characterised from *A. chinensis* var. *deliciosa* (Nieuwenhuizen et al., 2009), *A. arguta* (Chen et al., 2010) and *A. chinensis* var. *chinensis* (Green et al., 2012). In fruit, Nieuwenhuizen et al. (2015) showed that terpinolene is the major terpene that accumulates in ripe *A. arguta* 'Hortgem Tahi' fruit and that accumulation correlates with ripening, softening and ethylene production. In contrast, ripe *A. chinensis* var. *chinensis* 'Hort16A' fruit produce 1,8-cineole, whilst immature 'Hort16A' fruit produce low concentrations of β-myrcene and geraniol. Biochemical analysis of recombinant enzymes in vitro showed that *A. arguta terpene synthase 1* (*AaTPS1*) makes mainly terpinolene (Figure 2), whilst *A. chinensis terpene synthase 1* (*AcTPS1*) makes primarily geraniol and β-myrcene. The *AaTPS1* gene is highly expressed in ripe 'Hortgem Tahi' fruit and *AcTPS1* is expressed at very low concentrations in immature 'Hort16A' fruit. The reason for the low expression in 'Hort16A' fruit was shown to be a mutation in the binding site of a ripening-related NAC transcription factor.

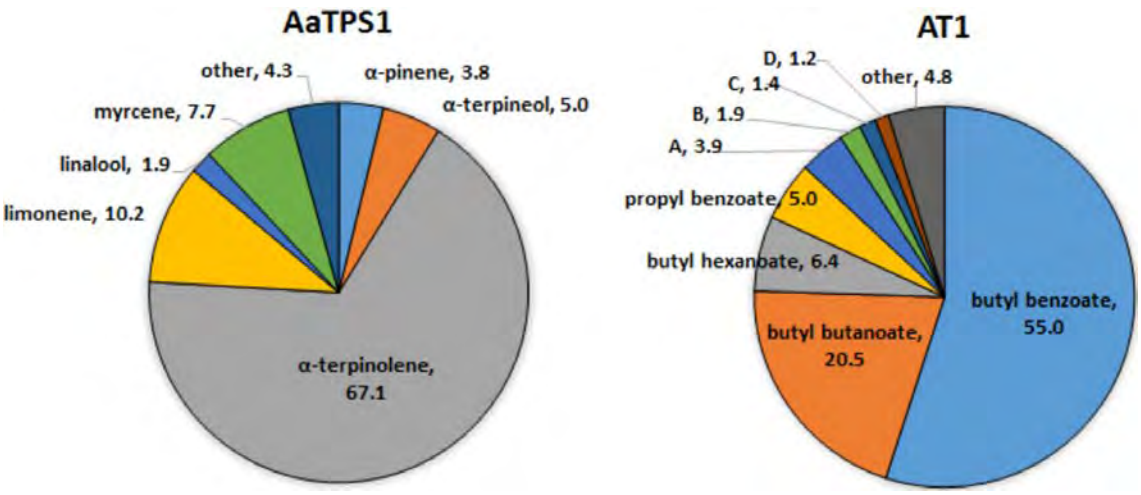

Figure 2. Biochemical characterisation of kiwifruit flavour enzymes in vitro. Left: Terpene volatiles (%) produced by recombinant *A. arguta terpene synthase 1* (*AaTPS1*) expressed in *Escherichia coli* (Nieuwenhuizen et al., 2015). Right: Volatile esters (%) produced by recombinant *alcohol acyltransferase 1* (*AT1*) expressed in *Saccharomyces cerevisiae* (Günther et al., 2011). A = butyl 2-(methylsulfanyl)acetate; B = propyl butanoate; C = butyl propanoate; D = butyl 3-(methylsulfanyl) propanonate.

Ester production in kiwifruit occurs late in fruit ripening and is strongly correlated with ethylene production (Atkinson et al., 2011). It is inhibited by cold treatment, which influences the flavour of fruit after cold storage; however, production of some esters can be restored by ethylene treatment before ripening (Günther et al., 2015). Alcohol acyl transferases (AATs) that catalyse the final step in ester biosynthesis have been identified in a range of *Actinidia* species (Günther et al., 2011). The ester profile in different cultivars is likely to result from the specificity of the AAT enzymes found in fruit (e.g., *AT1*; Figure 2), as well as substrate availability.

Aldehydes such as (*E*)-2-hexenal and hexanal appear to be ubiquitous in the fruit of kiwifruit species and are rapidly released upon wounding or chewing. Lipoxygenases (LOX) are key enzymes in the production of C6-aldehydes in kiwifruit and the expression of a number of genes has been studied in *A. chinensis* var. *deliciosa* 'Hayward' and 'Bruno' fruit (Zhang et al., 2006; Zhang et al., 2009). Two key genes appear to be *AdLox1* and *AdLox5*. Expression of these two genes increases as fruit develop to the climacteric stage, and both are up-regulated by ethylene treatment.

The biosynthetic pathway to vitamin C (ascorbate) production has been extensively characterised in kiwifruit and all genes in the L-galactose pathway have been cloned (Bulley and Laing, 2016). Regulation of ascorbate concentration is primarily through GDP-galactose phosphorylase (*GGP*), which converts GDP-galactose to galactose-1-phosphate. *GGP* gene transcription correlates well with ascorbate production along with GDP-mannose epimerase (*GME*) transcription. Translational regulation also plays a significant part in controlling ascorbate concentrations. Laing et al. (2015) have shown that the 5' untranslated region of *GGP* from kiwifruit (and other species) contains a highly conserved upstream open reading frame (uORF). The authors proposed that under high ascorbate concentrations, the uORF was translated and acted as an inhibitor of *GGP* translation. Under low ascorbate, the uORF was skipped and *GGP* was translated.

Actinidin proteins have been shown to be produced by a kiwifruit-specific clade of cysteine protease genes (Nieuwenhuizen et al., 2007). Some commercial cultivars such as 'Hayward' contain high levels of cysteine protease activity and high concentrations of actinidin protein, whilst others including 'Hort16A' and 'Hongyang' contain very low levels of cysteine protease activity and protein (Maddumage et al., 2013). Nieuwenhuizen et al. (2012) showed that a major quantitative trait locus for cysteine protease activity mapped to linkage group 16 in a segregating population of *A. chinensis* var. *chinensis*. This quantitative trait locus co-located with the gene encoding the major acidic form of actinidin in ripe 'Hayward' fruit encoded by the *ACT1A-1* allele. Cultivars may differ in their effects on digestion due to the expression of this single gene.

CONCLUSION

Significant progress has been made in understanding both the compounds and genes contributing to the flavour and health properties of kiwifruit. Marker-assisted and whole genome selection strategies are already successfully being applied to flavour and health breeding in apple, strawberry and tomato. Similar strategies have recently been initiated in kiwifruit and should lead to the more rapid development of novel, high-flavour, high health cultivars.

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Evaluation of eleven kiwifruit genotypes for tolerance to bicarbonate stress

Nan-nan Wang[a], Mengyuan Li, Haohao He, Zhande Liu, Chunchao Yao and Fengwang Ma[b]

State Key Laboratory of Crop Stress Biology for Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China.

Abstract

Bicarbonate stress is one of the main abiotic constraints in the production of kiwifruit (*Actinidia* spp.). Thus, screening for tolerant rootstocks is important. Here we evaluated the degree of tolerance to high-bicarbonate conditions by seedlings of 11 kiwifruit genotypes after culturing them in soil for 60 days with a nutrient solution supplemented with 0.5 g L^{-1} $CaCO_3$ + 0.84 g L^{-1} $NaHCO_3$. We then investigated the level of leaf chlorophyll (SPAD-meter value), plant height, root length, fresh weights (FWs), and dry weights of various seedling tissues. Principal component analysis (PCA) showed that the shoot FW, the ratio of root FW to shoot FW, SPAD value, plant height, and root length were the best indices to use for assessing bicarbonate tolerance by these seedlings. Based on the PCA as well as membership function and cluster analyses, we classified these genotypes into three groups: tolerant (seedlings of 'Qinmei' and 'Miliang No. 1'), moderately tolerant (seedlings of 'Yate', Huayinruanzao (*A. arguta* var. *giraldii*), Shanli (*A. rufa*), Zhouzhimeiwei (*A. chinensis* var. *deliciosa*), and 'Jinkui'), and sensitive (seedlings of Ruanzao (*A. arguta* var. *arguta*), 'Nongdajinmi', 'Jinxiang', and 'Xuxiang''). These results provide important information for further screening of these potential resources and improving our understanding about the physiological and molecular mechanisms for bicarbonate tolerance in kiwifruit.

Keywords: *Actinidia*, kiwifruit, bicarbonate stress, selection of tolerant genotype

INTRODUCTION

China is the largest producer of kiwifruit (*Actinidia* spp.) in the world, with yields in 2013 estimated to be 1.76 million t (FAO, 2016). The centers of production in China are in Shaanxi and Sichuan Provinces (Zhai, 2015), two regions where the calcareous soils are characterized by high bicarbonate concentrations and high pH values (Xiong and Li, 1987; Wu et al., 2010). Bicarbonate in calcareous soils is considered a main limiting factor that diminishes the bioavailability of nutrients such as iron, zinc, and potassium (Zhang et al., 2001; Liu et al., 2002; Song et al., 2003). This is due to the HCO_3^- buffer effect (Lucena, 2000), which dramatically reduces fruit quality and quantity, as well as vineyard longevity (Tagliavini and Rombolà, 2001; Abadía et al., 2011).

One strategy for alleviating this problem in kiwifruit is to apply chelates and physiologically acidic fertilizers, but this is expensive and carries potential environmental risks (Abadía et al., 2011; Covarrubias et al., 2014). An alternative means is to screen for bicarbonate-tolerant genotypes. In China, kiwifruit genetic resources are rich because most species naturally occur there (Huang et al., 2004). Except for 'Hayward' and 'Hort16A', most commercial cultivars, including 'Qinmei', 'Xuxiang', and 'Jinkui', were selected from China (Huang, 2014). These cultivars constitute a broad genetic range of native flora from several regions with different climatic and soil conditions. Each has been extensively investigated for variation in fruit traits (Huang, 2014), shoot growth (Clearwater et al., 2006, 2007), graft compatibility (Pang et al., 1989; Chartier and Blanchet, 1997; Ye et al., 2014), resistance to root-knot nematodes (Wang et al., 2001), and

[a]E-mail: wangnannan2004@163.com
[b]E-mail: fwm64@sina.com

adaptability to flooded environments (Mi, 2009). However, little is known about their tolerance to high-bicarbonate soils.

Therefore, the aims of this work were to assess the bicarbonate tolerance of various kiwifruit genotypes by measuring some plant growth parameters in soil culture, and to classify them into three categories using the principal component analysis and the membership function method, as well as cluster analysis.

MATERIALS AND METHODS

Plant material, growth conditions and treatments

The experiment was conducted in a greenhouse at Northwest Agriculture & Forestry University, Yangling, China. Seeds of 11 genotypes of kiwifruit (*Actinidia* spp.; Table 1) were stratified in moist sand for 60 d (60-70% relative humidity, 4°C) to break dormancy. After their radicles emerged, the seeds were broadcasted in sand-filled plastic trays (6×12 holes with 4×4×5 cm for each hole). They were initially irrigated with tap water for two weeks and then with a diluted nutrient solution for approximately three weeks. The solution contained 7.7 mM KNO_3, 5 mM $Ca(NO_3)_2$, 1.1 mM $NH_4H_2PO_4$, 1.6 mM $MgSO_4$, 23 µM H_3BO_3, 9 µM $MnCl_2$, 0.8 µM $ZnSO_4$, 0.3 µM $CuSO_4$, 0.01 µM H_2MoO_4, and 50 µM Fe-EDTA (Smith et al., 1989; Wang et al., 2016). When the seedlings had developed two to three fully expanded leaves, they were transferred to black plastic pots (9×9×9 cm; one plant per pot) containing a mixture of soil and substrate (1:1, v:v). The plants were watered with the nutrient solution until five to seven leaves had expanded from the shoots.

Table 1. Codes for tested kiwifruit genotypes.

Genotype code	Common name	Scientific name	Source[1]
1	Ruanzao	*Actinidia arguta* (Siebold and Zuccarini) Planchon ex Miquel	HEF
2	Huayinruanzao	*A. arguta* var. *giraldii* (Diels) Voroshilov	WCHC
3	'Nongdajinmi'	*A. chinensis* 'Jinnong' × 'Jinyang'	KES
4	'Jinkui'	*A. chinensis* var. *deliciosa* 'Emihoutao No. 1'	KES
5	'Jinxiang'	*A. chinensis* var. *deliciosa* 'Jinxiang'	KES
6	Zhouzhimeiwei	*A. chinensis* var. *deliciosa*	WCZC
7	'Miliang No. 1'	*A. chinensis* var. *deliciosa* 'Miliang No. 1'	KES
8	'Qinmei'	*A. chinensis* var. *deliciosa* 'Qinmei'	KES
9	'Xuxiang'	*A. chinensis* var. *deliciosa* 'Xuxiang'	KES
10	'Yate'	*A. chinensis* var. *deliciosa* 'Yate'	KES
11	Shanli	*A. rufa* (Siebold and Zuccarini) Planchon ex Miquel	HEF

[1] HEF, Horticultural Experiment Field of Northwest Agriculture & Forestry University; WCHC, Wild Community of Huayin City, Shaanxi Province; KES, Mei County Kiwifruit Experiment Station of Northwest Agriculture & Forestry University; WCZC, Wild Community of Zhouzhi County, Shaanxi Province.

Two bicarbonate treatments were initiated on 8 July 2016: control (normal nutrient solution) and high-bicarbonate (normal nutrient solution + 0.5 g L^{-1} $CaCO_3$ + 0.84 g L^{-1} $NaHCO_3$; Jelali et al., 2010). Each treatment was replicated five times, with seven plants per replication. Irrigation was applied at 5-day intervals. The experiment was terminated after 60 days, when the apical leaves of bicarbonate-treated plants displayed obvious yellowing.

Measurement of growth parameters

Chlorophyll concentrations were determined from the first fully expanded leaf on

each harvested plant, using a portable SPAD-502 meter (Konica Minolta Co. Ltd., Osaka, Japan). After their heights and taproot lengths were measured with a cloth tape, the plants were separated into leaf, shoot, and root portions. All samples were initially washed with tap water and then with distilled water for 30 s. They were quickly blotted before their fresh weights (FWs) were recorded. After the portions were oven-dried at 65°C for 72 h, their dry weights (DWs) were measured.

Statistical analysis

The relative value for each growth parameter was calculated as follows:

$$Xi = X_{\text{high bicarbonate}} / X_{\text{control}} \times 100$$

where X is the relative value of growth parameter i, $X_{\text{high bicarbonate}}$ is the value under high-bicarbonate stress, and X_{control} is the value determined under control treatment.

All statistical analyses were performed with the SPSS for Windows 16.0 software package (SPSS Inc., Chicago, IL, USA). Differences among genotypes were separated by Duncan's multiple range tests at $P<0.05$. To examine the relationships among parameters, we computed Pearson's correlations. Principal component analysis (PCA), cluster analysis by the Euclidean Distance-Ward method (Zhang et al., 2016) and a membership function method were also employed to evaluate the degree of bicarbonate tolerance for each genotype. Membership values were calculated as described by Zhang et al. (2007):

$$U_{ij} = (X_{ij} - X_{jmin}) / (X_{jmax} - X_{jmin}) \quad [1]$$

and

$$U_{ij} = 1 - (X_{ij} - X_{jmin}) / (X_{jmax} - X_{jmin}) \quad [2]$$

where i is the genotype, j is the growth parameter, U_{ij} is the relative membership value of growth parameter j for genotype i under bicarbonate stress, X_{ij} is the relative value of growth parameter j for genotype i, and X_{jmin} and X_{jmax} are the minimum and maximum relative values, respectively, for growth parameter j across all tested genotypes. The relative membership value was estimated with Equation [1] when an individual parameter and the stress effect were positively correlated, but Equation [2] was used when that correlation was negative.

RESULTS

Effect of high-bicarbonate treatment on plant growth

For purposes of comparison, we chose the relative value of each growth parameter to assess the differences in bicarbonate tolerance among seedlings of these kiwifruit genotypes. At the end of the experiment, plants that were seedlings of Genotype 6 (wild *A. chinensis* var. *deliciosa*) and 4 ('Jinkui') had the highest chlorophyll concentrations (SPAD-meter values) and were the tallest, respectively, while seedlings of Genotype 3 'Nongdajinmi') had the lowest values for those parameters (Table 2). Seedlings of Genotype 8 ('Qinmei') were associated with all of the highest values for leaf, shoot, and root FWs and DWs, while leaf FW and DW and shoot FW were lowest in seedlings of Genotype 1 (wild *A. arguta* var. *arguta*) (Table 2). Seedlings of Genotype 2 (wild *A. arguta* var. *giraldii*) had the highest ratios of root FW to shoot FW and root DW to shoot DW (Table 2). In contrast, the lowest ratio of root FW to shoot FW, and total root FW, were calculated for seedlings of Genotype 5 ('Jinxiang') while the root DW was lowest from seedlings of Genotype 9 ('Xuxiang') (Table 2).

Table 2. Relative values for growth parameters determined for seedlings of 11 kiwifruit genotypes under bicarbonate stress. Within a column, values not followed by the same letter are significantly different among genotypes at $P<0.05$, based on Duncan's test of five replicates.

Code[1]	X_1[2]	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
1	73.4c	79.7ab	88.3bc	50.5d	53.7d	41.1e	56.3c	58.7d	64.0de	39.7d	55.4bc
2	90.0ab	73.0ab	87.8bc	65.9c	60.2d	93.4ab	66.4bc	55.3d	87.2bc	75.0a	71.8a
3	49.6d	50.1c	114.6a	66.3c	58.7d	70.4cd	65.6bc	53.6d	68.6cde	56.2b	57.0bc
4	78.6bc	90.0a	78.1c	71.4c	79.4abc	65.6cd	77.1b	85.7b	86.0bc	43.9cd	53.2bc
5	89.7ab	76.8ab	93.6bc	64.2c	58.9d	39.9e	68.3bc	66.4cd	69.2cde	32.2d	51.4c
6	100.5a	75.7ab	83.8bc	71.1c	65.7cd	78.2bc	76.4b	68.2bcd	83.1bcd	57.5b	58.2bc
7	98.7a	89.5a	97.8b	86.6ab	79.1abc	91.4ab	96.0a	78.2bc	91.6b	55.2bc	52.7c
8	76.3bc	88.5a	98.2b	94.4a	87.8a	104.1a	100.0a	107.9a	113.8a	57.3b	55.1bc
9	82.9bc	75.6ab	84.8bc	67.9c	80.3ab	55.5de	72.8b	78.6bc	61.0e	37.5d	40.5d
10	74.0c	80.0ab	92.8bc	75.9bc	78.0abc	80.0bc	76.9b	80.2bc	92.9b	52.1bc	59.1bc
11	69.1c	68.0b	89.2bc	78.2bc	67.2bcd	90.8ab	78.4b	66.3cd	93.1b	62.7b	64.3ab

[1] Genotype codes are defined in Table 1.

[2] X_1, relative SPAD-meter value for chlorophyll concentration; X_2, relative plant height; X_3, relative root length; X_4, relative leaf fresh weight; X_5, relative shoot fresh weight; X_6, relative root fresh weight; X_7, relative leaf dry weight; X_8, relative shoot dry weight; X_9, relative root dry weight; X_{10}, relative ratio of root to shoot fresh weight; X_{11}, relative ratio of root to shoot dry weight.

Correlations among growth parameters

To examine the relationships among growth parameters, we used Pearson's correlations and found that 16 of the 55 data sets were significantly correlated, including the analyzed pairing of plant height with either shoot FW or shoot DW (Table 3). In addition, significant correlation was noted between root FW and the ratio of root FW to shoot FW (Table 3). Overall, most biomass-associated indicators were correlated with each other, especially with leaf FW (Table 3).

Comprehensive evaluation of tolerance to bicarbonate stress

For examining bicarbonate tolerance, we applied PCA. The first three components were retained by an Eigen value-one criterion and the screen test (Norm and Hatcher, 2013). Among them, Components I, II, and III accounted for 44.02, 26.73, and 18.88% of the total contribution, respectively, with a cumulative percentage of 89.63% (Table 4). This suggested that those three adequately explained most of the variance in our datasets.

Component I was mainly correlated with the weights of the above-ground plant portions, i.e., 0.934 for leaf FW, 0.925 for shoot FW, 0.954 for leaf DW, and 0.921 for shoot DW (Table 5). These indicators primarily reflected contrasts in shoot biomass production among genotypes. For Component II, the most closely correlated indicators were the ratios of root FW to shoot FW and root DW to shoot DW (Table 5), which demonstrated the balance in development between shoots and roots. For Component III, the strongest indicators were SPAD value, plant height, and root length (Table 5), which also reflected differences in plant phenotypes. All of these results showed that the best indices for screening bicarbonate-tolerant kiwifruit genotypes rely upon shoot FW, the ratio of root FW to shoot FW, chlorophyll concentration, plant height, and root length. When F-values were calculated by a system matrix, we found that the higher the value, the stronger the tolerance. Consequently, we were able to use those values to rank genotypes from high to low tolerance (Table 6).

Table 3. Correlation coefficients of growth parameters of seedlings of 11 kiwifruit genotypes under bicarbonate stress. * and **, within each pairing, values in bold font are significantly correlated at $P<0.05$ and $P<0.01$, respectively (n=11).

Growth parameter	X_1[1]	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
X_1	1										
X_2	0.307	1									
X_3	-0.508	-0.535	1								
X_4	-0.138	0.386	0.192	1							
X_5	0.363	**0.603***	-0.149	**0.795****	1						
X_6	-0.142	0.114	0.176	**0.798****	0.481	1					
X_7	-0.145	0.542	0.104	**0.971****	**0.825****	**0.703***	1				
X_8	0.290	**0.704***	-0.150	**0.752****	**0.913****	0.373	**0.805****	1			
X_9	-0.142	0.440	0.027	**0.851****	0.591	**0.862****	**0.795****	**0.644***	1		
X_{10}	-0.192	-0.227	0.170	0.374	-0.005	**0.850****	0.242	-0.134	0.579	1	
X_{11}	-0.301	-0.252	0.051	0.005	-0.386	0.520	-0.131	-0.383	0.419	**0.820****	1

[1] $X1$, relative SPAD-meter value for chlorophyll concentration; $X2$, relative plant height; $X3$, relative root length; $X4$, relative leaf fresh weight; $X5$, relative shoot fresh weight; $X6$, relative root fresh weight; $X7$ relative leaf dry weight; $X8$, relative shoot dry weight; $X9$, relative root dry weight; $X10$, relative ratio of root to shoot fresh weight; $X11$, relative ratio of root to shoot dry weight.

Table 4. Eigen values of three principal components and their contribution under bicarbonate stress.

Principal component	Eigen value	Contribution (%)	Cumulative contribution (%)
I	4.84	44.02	44.02
II	2.94	26.73	70.76
III	2.08	18.88	89.63

Table 5. Loading matrix of each component.

Principal component	X_1[1]	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
I	0.119	0.561	0.094	0.934	0.925	0.610	0.954	0.921	0.755	0.107	-0.254
II	0.066	-0.181	0.095	0.290	-0.140	0.770	0.161	-0.207	0.585	0.962	0.927
III	0.832	0.719	-0.881	-0.064	0.146	-0.076	0.072	0.183	0.096	-0.108	-0.006

[1] X_1, relative SPAD-meter value for chlorophyll concentration; X_2, relative plant height; X_3, relative root length; X_4, relative leaf fresh weight; X_5, relative shoot fresh weight; X_6, relative root fresh weight; X_7, relative leaf dry weight; X_8, relative shoot dry weight; X_9, relative root dry weight; X_{10}, relative ratio of root to shoot fresh weight; X_{11}, relative ratio of root to shoot dry weight.

Table 6. Ranking of bicarbonate tolerance by genotype (1=highest), based on F-values.

Code[1]	F-value	Order
1	-1.84	**10**
2	1.64	**3**
3	-3.32	**11**
4	0.68	**5**
5	-1.52	**9**
6	1.20	**4**
7	1.58	**2**
8	1.93	**1**
9	-1.37	**8**
10	0.42	**7**
11	0.61	**6**

[1]Genotype codes are defined in Table 1.

To confirm our PCA results, we assessed tolerance based on the membership function method and cluster analysis. These genotypes were ranked according to their average membership value (from high to low; Table 7) and then classified into three groups: 1) tolerant, seedlings of 'Qinmei' and 'Miliang No. 1'; 2) moderately tolerant, seedlings of 'Yate', Huayinruanzao (*A. arguta* var. *giraldii*), Shanli (*A. rufa*), Zhouzhimeiwei (*A. chinensis* var. *deliciosa*), and 'Jinkui'; and 3) sensitive, seedlings of Ruanzao (*A. arguta* var. *arguta*) , 'Nongdajinmi', 'Jinxiang', and 'Xuxiang' (Figure 1).

Table 7. Membership values of growth parameters for in kiwifruit genotypes. Ranking order is based on average of relative values of various growth parameters.

Code[1]	X_1[2]	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	$\overline{X}$[3]	Order
1	0.47	0.50	0.38	0.24	0.18	0.12	0.24	0.21	0.21	0.23	0.48	0.297	**11**
2	0.72	0.41	0.38	0.45	0.28	0.71	0.38	0.18	0.45	0.77	0.82	0.503	**4**
3	0.11	0.08	0.73	0.46	0.26	0.45	0.37	0.16	0.26	0.48	0.51	0.351	**10**
4	0.55	0.65	0.25	0.53	0.56	0.39	0.52	0.51	0.44	0.30	0.43	0.466	**7**
5	0.71	0.46	0.45	0.43	0.26	0.10	0.40	0.30	0.26	0.12	0.39	0.354	**9**
6	0.87	0.45	0.33	0.52	0.36	0.54	0.51	0.32	0.41	0.50	0.53	0.486	**6**
7	0.85	0.65	0.51	0.73	0.56	0.69	0.78	0.43	0.50	0.47	0.42	0.598	**2**
8	0.51	0.63	0.52	0.84	0.69	0.83	0.84	0.75	0.73	0.50	0.47	0.664	**1**
9	0.61	0.45	0.34	0.48	0.58	0.28	0.46	0.43	0.18	0.20	0.16	0.379	**8**
10	0.48	0.51	0.44	0.59	0.54	0.56	0.52	0.45	0.51	0.42	0.55	0.507	**3**
11	0.41	0.34	0.40	0.62	0.38	0.68	0.54	0.30	0.51	0.58	0.66	0.492	**5**

[1] Genotype codes are defined in Table 1.

[2] X_1, relative SPAD-meter value for chlorophyll concentration; X_2, relative plant height; X_3, relative root length; X_4, relative leaf fresh weight; X_5, relative shoot fresh weight; X_6, relative root fresh weight; X_7, relative leaf dry weight; X_8, relative shoot dry weight; X_9, relative root dry weight; X_{10}, relative ratio of root to shoot fresh weight; X_{11}, relative ratio of root to shoot dry weight.

[3] Average of relative value for parameters for each genotype.

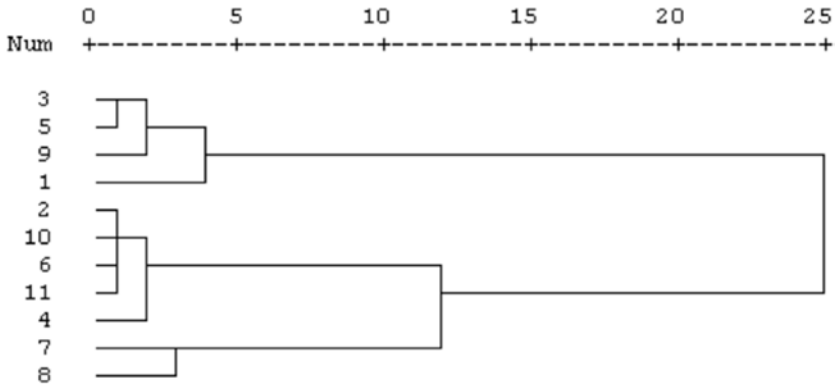

Figure 1. Dendrogram of bicarbonate-stress tolerance by tested genotypes of kiwifruit. Genotype codes are defined in Table 1. Cluster analysis was based on average membership values.

DISCUSSION

High-bicarbonate stress presents one of the most common challenges to fruit trees grown on calcareous soils (Tagliavini and Rombolà, 2001; Abadía et al., 2011). One strategy for coping with this problem is to select tolerant genotypes, as has already been done with apple (*Malus domestica*; Zhang et al., 2016), *Citrus* spp. (Byrne and Rouse, 1994), grape (*Vitis vinifera*; Ksouri et al., 2005; Assimakopoulou et al., 2016), *Prunus persica*, and *Cydonia oblonga* (Cinelli et al., 2003; Alcántara et al., 2012). However, variations in tolerance among kiwifruit genotypes within the same genus or species remain largely unknown. Our investigation of the seedlings of three *Actinidia* spp. and seedlings of eight kiwifruit cultivars indicated that seedlings of *A. chinensis* var. *deliciosa* 'Qinmei' and 'Miliang No. 1' have the greatest tolerance for high-bicarbonate stress and that levels differ significantly among groups of seedlings of the genotypes (Table 2, Table 7 and Figure 1).

Under high-bicarbonate conditions, seedlings of 'Nongdajinmi' exhibited yellowing in young leaves while seedlings of Ruanzao (*A. arguta* var. *arguta*) displayed a severe reduction in biomass, even though both are in the same "sensitive" group (Table 2 and Figure 1). These individual responses suggested that a multi-parameter evaluation is preferable to one based only on a single parameter. Our correlation analysis showed that 16 out of 55 data sets were significantly correlated (Table 3), thereby demonstrating that it is possible to reduce the observed variables into a smaller number of principal components. Subsequently, five growth parameters were recommended as most reliable by PCA (Table 5), providing a foundation for future screening of kiwifruit genotypes (including wild species) on a larger scale. Both PCA and the membership function methods are extensively used in assessing tolerance to abiotic stresses, such as to NaCl in apple (Fu et al., 2013); chilling in *Prunus armeniaca* (Zhang et al., 1999), grape (Zhang et al., 2007), and *Saccharum officinarum* (Zhang et al., 2015); and drought in *Triticum aestivum* (Bai et al., 2008) and *Setaria italica* (Meng et al., 2009). The results that we obtained from these two methods closely agreed with each other (Table 6, Table 7, Figure 1), again showing that our tolerance rankings are valid and reliable.

Overall, seedlings of the cultivars belonging to *A. chinensis* var. *deliciosa* appear to have the greatest capacity to endure bicarbonate stress, followed by *A. rufa* and *A. arguta*, while those of *A. chinensis* var. *chinensis* are the least tolerant. These findings also reveal the broad range of variability in *A. chinensis* var. *deliciosa*, probably because of its large number of cultivars and its wide geographical distribution (Huang, 2014). Furthermore, seedlings of Huayinruanzao (*A. arguta* var. *giraldii*) kiwifruit, which is native to calcareous soils, are better able to tolerate high-bicarbonate conditions when compared with seedlings of Ruanzao (*A. arguta* var. *arguta*), which occurs primarily on non-calcareous

soils (Huang, 2014). Donnini et al. (2012) have studied the adaptive strategies of a native plant species, *Parietaria diffusa* (M. & K.), to calcareous soils, and have demonstrated the necessity for screening numerous tolerant genotypes from communities of wild species growing on such soils. Therefore, researchers must also investigate a broader range of wild kiwifruit species for their bicarbonate-stress tolerance if we are to elucidate the mechanism for this tolerance and alleviate bicarbonate-induced problems in kiwifruit production.

CONCLUSION

Tolerance to high-bicarbonate conditions differed significantly among the seedlings of the kiwifruit genotypes tested here. We determined that the most useful indices for screening plants were shoot fresh weight; the ratio of root fresh weight to shoot fresh weight; values for chlorophyll concentration, as recorded with a SPAD meter; plant height; and root length. These parameters can be applied in future investigations of wild kiwifruit species that are also possibly tolerant to bicarbonate stress.

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The effects of different organic foliar fertilizers on the fruit and leaves of 'Hongyang' kiwifruit (*Actinidia chinensis*)

H.J. Gong[1,2,a], K.Y. Ye[1], F.M. Wang[1], Q.S. Jiang[1], J.W. Li[1,b], Q.H. Mo[1] and P.P. Liu[1]

[1]Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin 541006, Guangxi, China; [2]Guangxi University, Nanning 530004, Guangxi, China.

Abstract

To study the effects of different organic foliar fertilizers on the fruit and leaves of 'Hongyang' kiwifruit, we twice applied three different organic foliar fertilizers, Ji Sheng An, Bo Feng and Green Princess' and potassium dihydrogen phosphate (control) to six-year-old vines. Vitamin C, sucrose, total sugars, titratable acidity, soluble solids, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and anthocyanin contents of fruit, the thickness of ten leaves, the SPAD value and the nitrogen content of leaves were measured. The results showed that the total sugar content of the fruit of three organic foliar fertilizer treatments were all significantly higher than that of control fruit, titratable acidity of Ji Sheng An and Bo Feng treatments were significantly lower than control, vitamin C and soluble solids contents did not differ significantly between treatments. Leaf chlorophyll contents of three organic foliar fertilizer treatments were significantly lower than that of the control, while the carotenoids content was significantly higher than that of the control. The anthocyanin content in fruit of the Ji Sheng An and Green Princess treatments was significantly lower than that of the control and the Bo Feng treatment. The treatments had no significant effect on the SPAD value and the nitrogen content of the leaves. The leaves of the Ji Shang An treatment were significantly thicker.

Keywords: 'Hongyang' kiwifruit, organic foliar fertilizer, nutrition of fruit, pigment content

INTRODUCTION

The kiwifruit 'Hongyang' has become more and more popular with consumers for its radial red-fleshed core and high sugar content. In recent years 'Hongyang' has been developed in Guangxi as the main kiwifruit cultivar. But compared with other cultivars, the resistance of 'Hongyang' is weaker. Normally, potassium dihydrogen phosphate is sprayed on leaves as a foliar supplement to rhizosphere fertilization to improve the nutrition and the stress resistance of kiwifruit vines. There has been limited research on foliar fertilizer application on kiwifruit: Tu et al. (2013) and Zou et al. (2017) studied the impact of different spray concentrations of Yongye BIOS on shoot growth, fruit quality, maturity, yield and economic benefits of kiwifruit; Jun et al. (2015) studied the effect of four kinds of foliar fertilizer on 'Hongyang' kiwifruit; Li et al. (2009) studied the influence of a water-soluble, amino acid foliar fertilizer on yield, fruit weight and other economic traits of 'Hongyang' kiwifruit; Yao et al. (2002) studied the effect of five foliar fertilizers on fruit traits and storage tolerance of 'Qinmei' kiwifruit; Huang et al. (2015) studied the effect of spraying potassium fertilizer on the yield and quality of 'Guichang' kiwifruit.

Organic foliar fertilizers, especially amino acid foliar fertilizers, are being promoted for their remarkable improvement of tree nutrition and fruit quality. This study considers the effects of one amino acid foliar fertilizer and two new organic foliar fertilizers when applied to 'Hongyang' kiwifruit. The aim is to provide a scientific basis for the application

[a]E-mail: gongjian_3000@126.com

[b]E-mail: lijw@gxib@cn

of new organic foliar fertilizers on 'Hongyang' kiwifruit.

MATERIALS AND METHODS

Materials

Six-year-old 'Hongyang' kiwifruit vines planted at Ziyuan County, Guangxi of China were studied. They were at an orchard spacing of 3 × 3 m on a pergola support structure.

The foliar fertilizer tested were 'Ji Sheng An' More fruit purine (LVT WATER-SOLUBLE FERTILIZER), 'Bo Feng' Multiple high concentration liquid fertilizer (Beijing Kuang Zheng Bo Zhong Biological Technology Co., Ltd.), and 'Green Princess' organic foliar fertilizer (Guangxi Green Princess Biological Technology Co., Ltd.).

Experimental methods

1. Experiment design.

Treatments: 'Ji Sheng An' diluted 1:1500 times with water; 'Bo Feng' diluted 1:1000 with water; 'Green Princess' diluted 1:1500 with water and 0.2% potassium dihydrogen phosphate (control). Each treatment was twice applied to three vines, 12 May 2014 and 27 May 2014. After the second physiological fallen fruit determination of fruit set rate, leaf thickness and leaf nutrition, after fruit ripening determination of nutrients and fruit pigment content.

2. Determination of the thickness of leaves.

Up to ten leaves were picked from different positions on the vine, and the thickness of the leaves measured using a vernier caliper.

3. Determination of leaf nutrients.

Leafs nutrient status (SPAD and nitrogen) were measured by using plant nutrient speed measuring instrument (TYS-3N ZHEJANG TOP INSTRUMENT Co., LTD). Nine leaves from each sampling location were measured, with five values per leaf.

4. Determination of fruit nutrients.

After the fruit were ripe, vitamin C (Vc) was determined by iodine titration; titratable acid was determined by NaOH titration; total sugar was determined by anthrone colorimetry (Bai, 1993); sucrose was determined by the resorcinol method (Shanghai Plant Physiology Research Institute of the Chinese Academy of Sciences & Shanghai Institute of Plant Physiology, 1999).

5. Determination of fruit pigments.

The pigment contents in fruit flesh after ripening were determined. Chlorophyll and carotenoids were extracted and determined according to the method of Bai (1993) and Zhu (1990), after acetone extraction, the absorbance of the extract at 450, 645, 652, and 664 nm was determined by an ultraviolet spectrophotometer (UV757CRT, Shanghai Precision Instrument Co., Ltd.); anthocyanins were extracted and determined according to the method of Xiong (2003), anthocyanins by hydrochloric acid/methanol extraction, the absorbance of the extract at 530, 620, and 650 nm was determined by an UV757CRT spectrophotometer.

6. Data analyses.

Data analyses and graph were conducted by Statistica 6.0 package and Excel 2007 software.

RESULTS

Effects of different foliar fertilizer on fruit set rate

The results showed that application of 'Bo Feng' on 'Hongyang' kiwifruit vines increased fruit set over that of the control whereas the other two treatments lowered fruit set (Figure 1). However, analysis of variance showed that the differences were not significant ($p=0.234>0.05$).

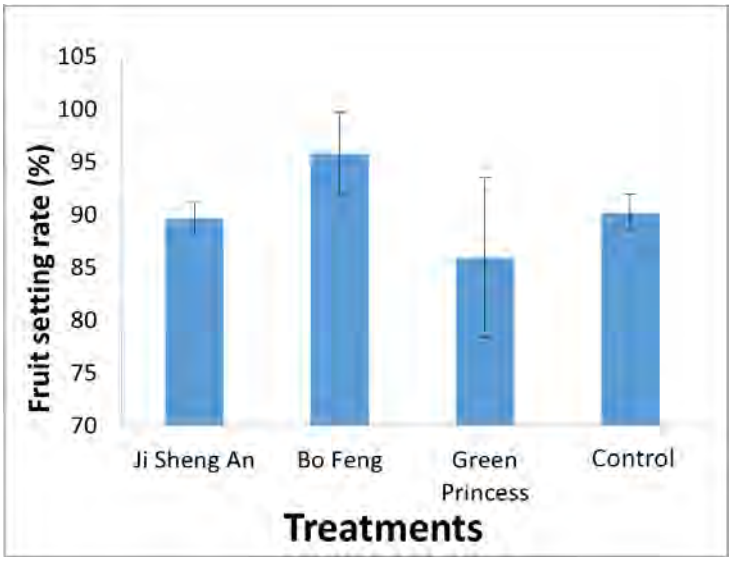

Figure 1. The fruit set rate of different treatments.

Effects of different foliar fertilizer on fruit nutrients content

According the Duncan multivariate variance analysis, total sugar content of organic foliar fertilizer treatments was significantly higher than in the control; sucrose content of 'Ji Sheng An' treatment was slightly higher than that of the control but both were much higher than the other two treatments; titratable acidity of 'Ji Sheng An' and 'Bo Feng' treatment were significantly lower than control; Vitamin C and soluble solids contents were not significantly different between treatments (Table 1).

Table 1. The effects of different foliar fertilizer on fruit nutrient content of 'Hongyang' kiwifruit.

Treatments	Total sugar (mg g^{-1})	Sucrose (mg g^{-1})	Titratable acidity (%)	Vitamin C (mg 100 g^{-1})	Soluble solids (%)
Ji Sheng An	117.85±0.83 aA	28.55±0.21 aA	0.78±0.00 bB	76.56±3.01 aA	15.92±0.86 aA
Bo Feng	90.96±0.98 cC	16.66±0.63 dC	0.78±0.00 bB	73.78±1.21 aA	14.73±0.73 bA
Green Princess	106.18±0.68 bB	17.96±0.24 cC	0.89±0.02 aA	77.60±4.22 aA	16.00±0.89 aA
Control	85.02±0.56 dD	26.92±0.86 bB	0.91±0.02 aA	79.00±1.21 aA	16.08±1.11 aA

Effects of different foliar fertilizer on pigments content

The results showed that pigment contents of flesh of 'Hongyang' kiwifruit of the three treatments were significantly different from control (Table 2). Chlorophyll contents of organic foliar fertilizer treatment were significantly lower than control, but chlorophyll content of 'Ji Sheng An' treatment was significantly higher than the other two treatments;

while the carotenoids content was significantly higher than control; the anthocyanin contents in fruit flesh of 'Ji Sheng An' and 'Green Princess' treatments were significantly lower than control and the 'Bo Feng' treatment.

Table 2. The effects of different foliar fertilizer on pigment content in flesh of 'Hongyang' kiwifruit.

Treatments	Chlorophyll a (mg 100 g^{-1})	Chlorophyll b (mg 100 g^{-1})	Total chlorophyll (mg 100 g^{-1})	Carotenoids (mg 100 g^{-1})	Anthocyanins (mg 100 g^{-1})
Ji Sheng An	0.828±0.155 bB	1.229±0.060 bB	2.056±0.204 bB	0.730±0.109 aA	2.076±0.074 cC
Bo Feng	0.546±0.070 cB	1.040±0.087 bcB	1.586±0.031 cC	0.857±0.063 aA	4.194±0.158 aA
Green Princess	0.555±0.091 cB	0.964±0.137 cB	1.518±0.221 cC	0.836±0.096 aA	2.369±0.119 bB
Control	1.386±0.075 aA	2.337±0.118 aA	3.722±0.111 aA	0.429±0.034 bB	4.065±0.021 aA

Effects of different foliar fertilizer on the leaves

The results (Table 3) indicated that there were no significant differences between treatments in relative chlorophyll content (SPAD) and N content of the leaves. Among the three treatments the SPAD and the N content of 'Bo Feng' treatment were higher than other two treatments and control (CK); while foliar fertilizer has an obvious effect on the thickness of leaves, spraying 'Ji Sheng An' the thickness of leaves was significantly higher than in 'Bo Feng'.

Table 3. Effects of different foliar fertilizer on the leaves of 'Hongyang' kiwifruit.

Treatments	SPAD value	Nitrogen content (mg g^{-1})	Thickness of ten leaves (cm)
Ji Sheng An	45.14±1.16 a	3.16±0.03 a	0.520±0.000 a
Bo Feng	46.49±1.52 a	3.42±0.13 a	0.460±0.020 b
Green Princess	46.30±2.75 a	3.21±0.20 a	0.497±0.035 ab
Control	44.60±0.42 a	3.21±0.17 a	0.477±0.040 ab

DISCUSSION

In this study, most consequences were consistent with those from previous research, for example, after spraying organic foliar fertilizer the fruit acid content declined and sugar content increased, the chlorophyll content, N content and thickness of the leaves increased, all consistent with the results of Xi et al. (2015). However, those authors found an opposite effect of Yongye BIOS on the acid content of 'Hongyang' kiwifruit. There appear to be no previous reports of the effects of organic foliar fertilizers on pigment content. Overall, application of organic foliar fertilizers significantly improved the nutrient level of the kiwifruit vine and fruit quality, although different foliar fertilizers could vary in their effect.

Treatment with 'Bo Feng' lowered the acid content and resulted in the highest SPAD value, N content, carotenoids and anthocyanin content; however, the fruit sugar content was lower than with the other two treatments. Treatment with 'Ji Sheng An' was good at increasing fruit sugar content and leaf thickness. So, using two types of foliar fertilizer together might be better.

CONCLUSION

Application of organic foliar fertilizer increased the fruit sugar content and decreased the organic acids; the chlorophyll content of the leaves was lower, the carotenoid content higher; the SPAD value and thickness of leaves increased. Compared to potassium dihydrogen phosphate the organic foliar fertilizers improved the nutrient level

of the kiwifruit vine and fruit quality.

'Bo Feng' treatments were the most effective but application of 'Ji Sheng An' at the same time might be even better.

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Evaluation of quality on six kiwifruit cultivars at developmental stage

Meiyan Tu[1,2,3,a], Shuxia Sun[1,2], Dong Chen[1,2], Jing Li[1,2], Guoliang Jiang[1,2,b], Ming'an Liao[3], Mingzhang Li[4], Chunyang Liu[1,2] and Haiyan Song[1,2]

[1]Horticulture Research Institute of Sichuan Academy of Agricultural Sciences, Chengdu 610066, Sichuan, China; [2]Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Southwest Region), Ministry of Agriculture, Chengdu 610066, Sichuan, China; [3]College of Horticulture, Sichuan Agricultural University, Chengdu 611130, Sichuan, China; [4]Sichuan Academy of Natural Resource Sciences, Chengdu 610015, Sichuan, China.

Abstract

Kiwifruit substance quickly accumulated and converted during fruit development resulting in different flesh color and fruit quality. The quality of six kiwifruit cultivars during fruit growth and maturity were evaluated in this study. Results showed that 'Hayward' had the highest fruit weight of 116.55 g. Soluble solids and soluble sugar contents of kiwifruits increased rapidly from S5 (95 days after anthesis) to S6 (at commercial maturity) stage. Vitamin C content exhibited a sharp increase and reached a peak at S3 (35 days after anthesis) stage, then declined continuously from S3 to S6 stage. The contents of chlorophyll, carotenoid and flavonoids in six cultivars rapidly decreased from S1 (5 days after anthesis) to S3 stage and maintained a steady level afterwards. ABTS, DPPH and •OH scavenging activity indicated that 'Jinshi 1' had higher antioxidant capacity. Nevertheless, 'Hongshi 2' was rich in TSS, vitamin C, starch, carotenoid and flavonoid. These quality parameters of kiwifruit were further evaluated by using grey correlation analysis and PCA analysis which showed 'Hongshi 2' had the best overall quality.

Keywords: kiwifruit, growth, physiological characteristics

INTRODUCTION

Kiwifruit (*Actinidia* spp.) originated in China and is widely cultivated across the world. In China, there are many new cultivars that have been bred and widely planted (Testolin and Ferguson, 2009). The fruit is tasty, nutritious and contains considerable amounts of acids, sugars, polysaccharides and minerals (Bulley et al., 2009; Krupa et al., 2011; Ma et al., 2017). Moreover, kiwifruit indicates a high antioxidant capacity. Not only phenolics, but also vitamin C significantly influence the antioxidant qualities of the fruits (Kalt et al., 1999). Consumption of kiwifruit offers health benefits including alleviating constipation and improving stool transit time and/or bulking (Chan et al., 2007).

Cultivar was the main factor influencing nutritional quality, though the product region could also affect the cultivar characteristics to some extent. However, kiwifruit comprises more than 70 species, of which only a few are of commercial importance, mainly *Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson and *A. chinensis* (Li and Zhu, 2017; Garcia et al., 2012). In recent years, consumers have plenty of choices and they have preferentially selected kiwifruit based on flesh color (Wen-Sheng et al., 2012). The flesh color can be green or golden, the taste and flavor of green and golden kiwifruit species differ from each other. In fact, approximately 90% of the kiwifruit on the global market belongs to the green-fleshed *A. deliciosa* 'Hayward' cultivar. The world demand for kiwifruit increased significantly the last 20 years and new markets, especially in Asia, have emerged (Bano and Scrimgeour, 2012).

[a]E-mail first author: hualelei@163.com

[b]E-mail corresponding author: jgl22@hotmail.com

Fruit size and quality are the most important characteristics affecting price and marketing of kiwifruit (Cruz-Castillo et al., 2014). Parameters that are important to achieve the best quality of kiwifruit at harvest are relatively large size and good shape, a high quantity of ascorbic acid and vitamins, and a good balance between soluble sugars and organic acids, which is responsible for its flavor (Sun-Waterhouse et al., 2013; Ainalidou et al., 2015). Additionally, kiwifruit flesh can have different colors derived from pigments such as chlorophyll, carotenoids and anthocyanins (Jaeger et al., 2003). Typically, 'Hayward' kiwifruit is larger, more cylindrical than a chicken's egg and usually weighs up to about 120 g. The flesh, which is light green with a white core, has a tart-sweet taste and the most common commercially available, green-fleshed kiwifruit is the 'Hayward' cultivar (Fiorentino et al., 2009; Ferguson, 1997). According to previous research (Gilbert et al., 1996), there is 150-200 mg vitamin C 100 g^{-1} FW in fruits of *A. arguta*. The significantly highest level of antioxidant activity was registered in 'Bidan' cultivar and the contents of polyphenols and ascorbic acids were also significantly higher in 'Bidan' cultivar, flavonoids in 'Hayward' and flavanols in 'Haenam' (Park et al., 2011). Ma et al. (2016) showed that the colored kiwifruit which had highest soluble solids (SS) content was 'Hongyang'. The highest content of organic acid was detected in 'Qinmei' (1.48 g 100 g^{-1}). 'Hayward' in China also had a high content but 'Hort16A' in New Zealand had lower contents. Therefore, regarding the region and cultivar, growth, quality and nutrient composition of kiwifruit showed significant differences.

Hence, in this context, the experiment was conducted to explore the nutritional quality of kiwifruit with three different flesh colors, and in total six widely planted cultivars in Sichuan province, China. Among the cultivars, we compared the differences of nutritional qualities among the different cultivars. The goal of this study was to explore the dynamic physiological characteristics of different kiwifruit, and to provide parameters regarding the nutritional quality of Chinese market kiwifruit to aid consumer selection of kiwifruit.

MATERIALS AND METHODS

Samples

The experiment was carried out on five kiwifruit cultivars, which were bred in China and 'Hayward' which came from New Zealand. These six cultivars have been widely cultivated in Sichuan province, China in recent years. They are 'Hongyang' and 'Hongshi 2', 'Hayward', 'Cuiyu', 'Jinyan' and 'Jinshi 1'. The plants grew in the Experimental Field of the kiwi fruit research base in Shifang City, the town of Sichuan Province Natural Resources Research Institute (mean temperature = 15.7°C; annual rain fall = 1053.2 mm). The cultivation conditions were the same for all the cultivars. The fruits were harvested at six different development stages (Stage 1: 5 days after anthesis; Stage 2: 20 days after anthesis; Stage 3: 35 days after anthesis; Stage 4: 65 days after anthesis; Stage 5: 95 days after anthesis; Stage 6: at commercial maturity). To prepare samples for analysis, the mean fruit weight, diameter and vertical diameter were determined. All fruits were initially washed in cold water of 5°C, then peeled and sliced. All chemical reagents used were of analytical grade. The experiment was replicated three times and physiological analyses were done on fresh fruit. For analyses of antioxidant qualities, fruit were frozen in liquid nitrogen (quick freezing) and stored at -80°C in preparation for the analyses.

Analytical methods

1. Total soluble solids (TSS), soluble sugars, titratable acids (TA) vitamin C (VC), soluble protein.

The TSS were determined using a digital display sugar meter. Soluble sugars were determined by anthrone colorimetry (Wang et al., 2017). The TA content was determined using an acid-base titration method based on CNS GB/T 12456-2008. The VC content was determined using a 2, 6-dichloroindophenol titration method based on CNS GB/T 6195-

1986. The soluble protein content was determined using a Coomassie Brilliant Blue method based on CNS GB 50095-2010 (Ma et al., 2017).

2. Chlorophyll, carotenoid, total phenol and total flavonoid.

Chlorophyll and carotenoid concentration by extraction from 1 g fresh flesh weight with 5 mL 80% acetone (v/v) and absorbance was measured by spectrophotometry (Tombesi et al., 1993) . The pigment concentrations were expressed in mg g^{-1} FW. One gram (g) of liquid nitrogen-ground kiwifruit pulp was ground and extracted in 100 mL of ethanol. The total phenolics content was determined according to the Folin-Ciocalteu colorimetric method (Du et al., 2009). One gram (g) of kiwifruit pulp was ground in 20 mL of 70% ethanol and subjected to ultrasonic-assisted extraction for 10 min. The total flavonoid content was determined according to a previously described protocol (Du et al., 2009). Results are expressed as mmol g^{-1} and mg g^{-1}, respectively.

3. Antioxidant activity in DPPH, ABTS and ·OH scavenging.

50 g kiwifruit pulp was extracted in 200 mL of ethanol and placed in a 50-mL centrifuge tube. The extraction was centrifuged for 10,000 g at 4°C for 5 min and stored at 4°C for analysis. DPPH scavenging activity and ABTS assay were based on a previous described method with slight modifications (Li et al., 2012, Engström and Nordberg, 2011). ·OH scavenging was determined according to Zhang et al. (2012).

Statistical analysis

The data were analyzed by the analysis of variance in randomized complete blocks using the DPS software. Each sample was replicated at least three times, with each replication measured twice. For those parameters for which significant differences were detected by the Duncan's mean comparison, were conducted using the LSD test at $P \leq 0.05$ level. Microsoft Excel was used to analyze the grey correlation analysis and PCA analysis method.

RESULTS AND DISCUSSION

Changes in appearance quality of kiwifruit during development stage

Flesh color is mainly determined by the genotype and copigmentation (Wang et al., 2015). In recent years, customers have been attracted by kiwifruit with different flesh colors, such as red and golden. In the present study, the dynamic changes of flesh color were observed clearly from Figure 1. We found that the color of red-fleshed cultivars ('Hongyang' and 'Hongshi 2') and green-fleshed cultivars ('Cuiyu') were pale red at S1 stage and then turned green. Moreover, at S4 stage, the color of 'Hongyang' and 'Hongshi 2' gradually deepened.

In respect to fruit development, the growth curve of kiwifruit was divided into three stages, namely I, II and III, based on fruit weight and growth rate (Salinero et al., 2009). The fruit shape index in five cultivars fluctuated during development stage but in 'Jinshi 1' there was no significant change. Fruit weight and dry matter content are the main parameters used for commercial grading of kiwifruit (Famiani et al., 2012). In all cultivars, fruit weight, diameter and vertical diameter of fruit gradually increased and the results also showed that the fruit weight of 'Hayward' and 'Cuiyu' (116.55 and 110.54 g, respectively) were higher than the other four cultivars (Figure 1 and Table 1). It has been observed that the growth curve of kiwifruit usually has two phases: the first one is of rapid growth and the second one of slower growth. Their boundaries were around 50 days after mid bloom (Bebbington et al., 2009). Hofman et al. (2000) showed that percentage of dry matter (DM) had the potential to be used as a maturity standard to determine the latest harvest time. It was found in this study that the amount of dry matter showed a rapid increase at S3 development stage and the highest dry matter for 'Hongyang' 'Cuiyu' and 'Hongshi' was 18.92, 18.90, and 18.57%, respectively.

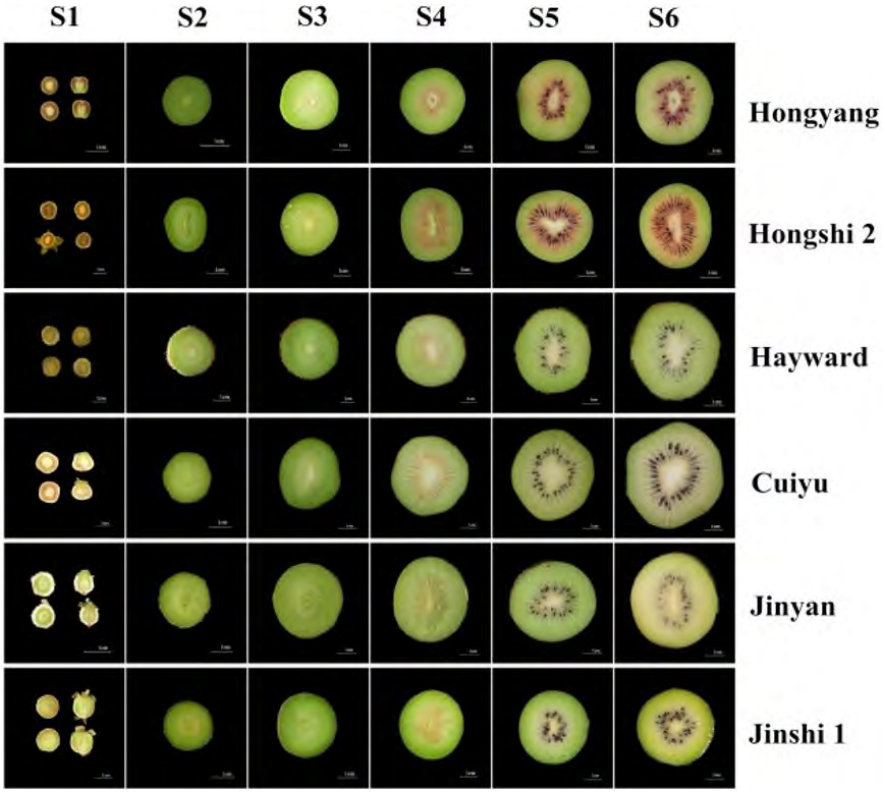

Figure 1. Profile of kiwifruits at developmental stages.

Table 1. The fruit weight, shape index and dry matter at developmental stages in different cultivars kiwifruits.

Cultivar	S1	S2	S3	S4	S5	S6
Fruit shape index						
Hongyang	1.18±0.01ab	1.26±0.02b	1.11±0.02d	1.08±0.01d	1.07±0.01b	1.08±0.01c
Hongshi 2	1.16±0.02b	1.31±0.02b	1.17±0.04cd	1.12±0.01cd	1.10±0.05b	1.08±0.01c
Hayward	1.23±0.0a	1.50±0.02a	1.43±0.02a	1.36±0.01a	1.32±0.01a	1.29±0.03a
Cuiyu	1.10±0.03c	1.17±0.01c	1.21±0.04c	1.16±0.03c	1.12±0.04b	1.23±0.02b
Jinyan	1.21±0.01a	1.29±0.03b	1.32±0.02b	1.23±0.01b	1.28±0.02a	1.24±0.01ab
Jinshi 1	1.06±0.02c	1.03±0.02d	0.98±0.01e	0.95±0.01e	0.95±0.02c	0.95±0.01d
Single fruit weight (g)						
Hongyang	0.81±0.04b	3.17±0.18d	28.65±0.35e	69.83±0.73c	75.29±0.96d	80.50±0.93d
Hongshi 2	0.84±0.04b	11.70±0.55a	34.33±0.50d	50.58±0.90d	50.70±1.48e	55.23±0.90e
Hayward	0.78±0.01b	12.34±0.43a	59.49±0.68a	82.46±1.00b	105.42±0.51a	116.55±0.82a
Cuiyu	0.76±0.04b	6.49±0.17c	48.09±0.67b	84.95±0.50a	101.01±0.55b	110.54±0.58b
Jinyan	0.83±0.02b	11.64±0.41a	44.56±0.38c	69.36±0.76c	84.99±0.49c	98.84±0.64c
Jinshi 1	1.08±0.07a	7.83±0.20b	18.55±0.50f	33.50±0.32e	37.22±0.43f	41.38±0.64f
Dry matter (%)						
Hongyang	10.87±0.15b	14.41±0.52a	7.25±0.17d	11.59±0.31c	18.63±0.51a	18.92±0.41a
Hongshi 2	9.07±0.28c	9.28±0.07b	10.21±0.08b	13.82±0.53a	18.03±0.40a	18.57±0.15a
Hayward	11.0±0.32b	7.63±0.33c	7.62±0.20cd	13.23±0.18ab	14.51±0.44b	15.20±0.91c
Cuiyu	10.70±0.25b	7.59±0.07c	8.13±0.21c	12.04±0.33bc	15.81±0.44b	18.90±0.28a
Jinyan	8.93±0.24c	7.61±0.11c	8.12±0.41c	9.71±0.39d	12.81±0.23c	14.24±0.20c
Jinshi 1	12.01±0.43a	9.61±0.01b	12.15±0.20a	13.54±0.58a	15.73±0.25b	16.62±0.25b

a Different letters indicate the statistically significant differences (LSD multiple range test, $p < 0.05$) among different kiwifruit cultivars.
b FI, fruit index; SFW, Single fruit weight; DM, Dry matter.

Contents of the main nutrients in the different kiwifruit cultivars

Total soluble solids (TSS) is an important value for evaluating the quality of fruits and vegetables, which comprises a number of components, such as sugars, organic acids, and vitamins (Ma et al., 2015). Among the different samples, the 'Jinshi 1' cultivar showed a very high content of TSS from S1 to S5 which was much higher than in any of the other five cultivars. In this study, there was a slowly increasing trend from S1 to S4 period but there were no significant differences among the other cultivars except 'Hayward', which had the lowest content (6.47%) (Figure 2A).

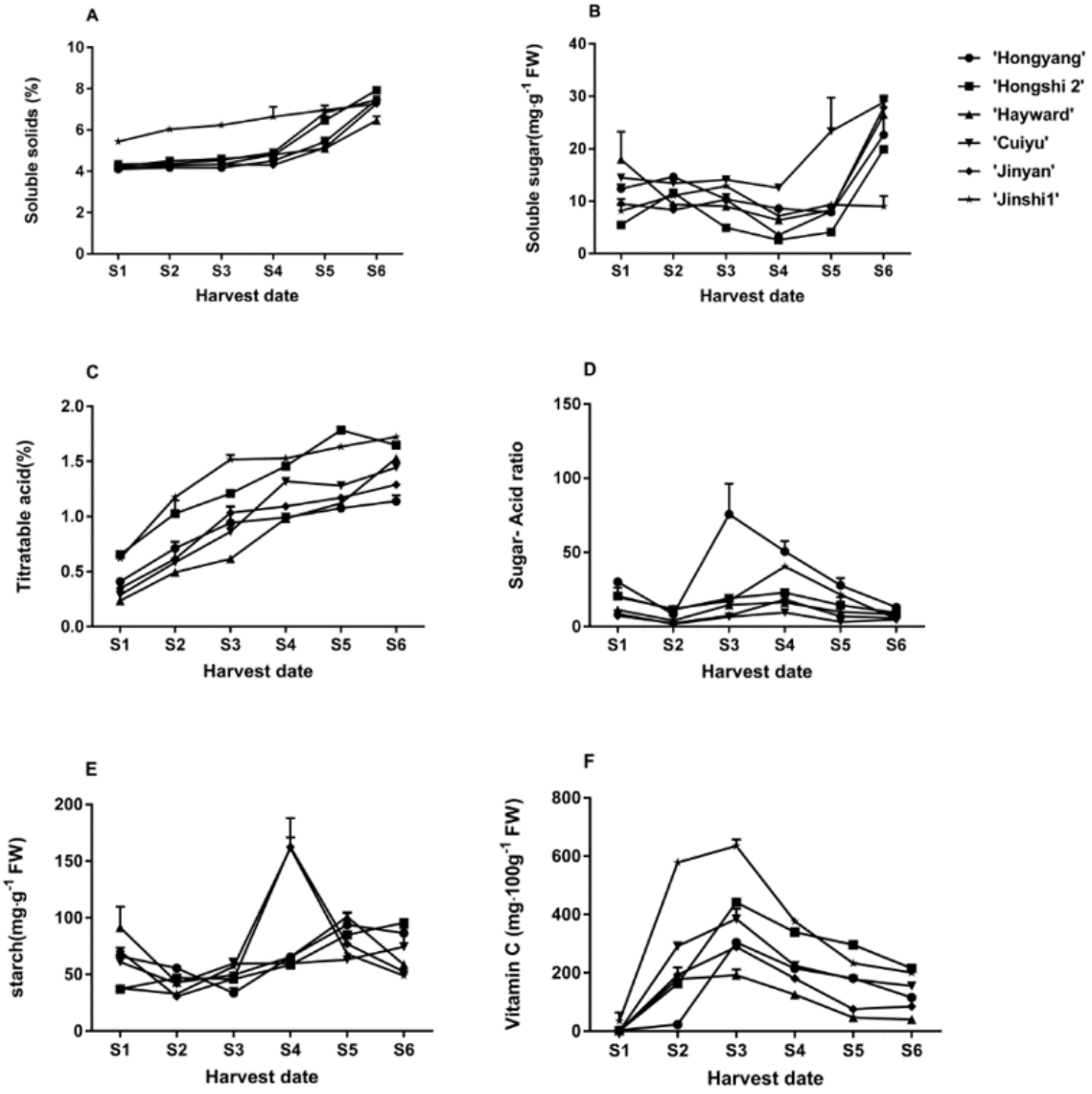

Figure 2. Contents of main nutrients during fruit growth and development of six kiwifruit cultivars. A) Soluble solids (%), B) soluble sugar (mg g^{-1} FW), C) titratable acid (%), D) sugar/acid ratio, E) starch (mg g^{-1} FW) and F) vitamin C (mg 100 g^{-1} FW).

The initial accumulation of soluble sugar and starch degradation is inconsistent; soluble sugar content was negatively correlated with the starch at later stages (Burdon et al., 2016). As shown in Figure 1B, the fruit had a similar level of soluble sugar of six cultivars, but there was a significant difference at S4 when 'Cuiyu' had a rapid increase and its content

ranged from 12.55 to 58.6 mg 100 g^{-1} fresh weight. 'Jinshi 1' had the lowest soluble sugar content (8.99 mg 100 g^{-1} fresh weight) and there were no significant differences among 'Jinyan', 'Hayward' and 'Hongyang', in accordance with previous report (Xie et al., 2014; Zhong et al., 2012). At the same time (Figure 2 E), the content of starch showed a slow increase after initially decreasing in 'Hongyang', 'Hongshi 2', 'Hayward' and 'Cuiyu', but the starch content of the 'Jinyan' and 'Jinshi 1' rapidly increased at S4 stage (to 162.41 and 161.34 mg g^{-1}, respectively) and then fell to the lowest (76.97 and 68.1 mg g^{-1}, respectively) in the next development stage.

Organic acids are the other important constituents in determining sensory qualitative characteristics (Marsh et al., 2004). In 'Hayward', the titratable acidity changes little or decreases during ripening, depending on growing location conditions (Crisosto and Crisosto, 2001). As shown in Figure 2C, the titratable acid of kiwifruits ranged from 0.236 to 1.722% and increased slowly during development stage in all cultivars. Significant differences among six cultivars were observed in titratable acid. The highest and lowest contents were in 'Jinshi 1' and 'Hongyang': 1.722% and 1.139%, respectively. Compared with other fruits, kiwifruit is rich in vitamin C, with higher contents than those determined in orange, strawberry, lemon and grapefruits (Landi et al., 2014). During development stages, the sugar/acid ratio in all cultivars fluctuated, declining from S1 to S4, and then increasing again at maturity (Figure 2D).

As shown in Figure 2F, 'Hongshi 2' and 'Jinshi 1' were characterized by a high content of vitamin C at S6 (215.91 and 200.96 mg 100 g^{-1}, respectively). In the fruit development period, vitamin C content decreased in all six kiwifruit cultivars after S3 stage when the vitamin C content reached a peak, the highest content was in 'Jinshi 1' 634.88 mg 100g^{-1}. Furthermore, Hunter et al. (2012) showed that vitamin C content of gold kiwifruit was around 105.3 mg 100 g^{-1}. Generally speaking, gold kiwifruit can be described as nutritious, providing a higher source of vitamin C than red- or green-fleshed cultivars (Ferguson and Ferguson, 2003).

Contents of the pigments in different kiwifruit cultivars

Chlorophylls and carotenoids are the main pigments that contribute to the characteristic bright green color of the flesh (Nishiyama et al., 2005). The potential beneficial health properties of carotenoids, in particular, such as anti-inflammatory and anti-oxidant effects have been widely recognized (Kaulmann and Bohn, 2014). The content of chlorophyll rapidly decreased from S1 to S3 stage and did not change significantly at the end of the growth period in red- and gold-fleshed cultivars. However, the chlorophyll content in 'Hayward' and 'Cuiyu' showed just a slight decrease. 'Hayward' and 'Hongyang' had the highest and lowest chlorophyll contents, respectively. The total chlorophyll content in the green-fleshed 'Hayward' was higher than that of other cultivars at S6 and there were no significant difference among 'Hongshi 2', 'Jinshi 1', 'Jinyan' and 'Cuiyu' (Figure 3A). At the beginning of the fruit developmental stage, Watanabe and Takahashi (1999) found that total chlorophyll contents decreased with fruit development to 28~31 μg g^{-1} in 'Hayward' at harvest.

The carotenoid content decreased significantly during S1 to S4 in all six kiwifruit cultivars. The results also showed that the carotenoid content maintained a stable level ranging from 0.668 to 2.197 mg 100 g^{-1}. The three highest contents were in 'Hongshi 2', 'Hongyang' and 'Jinshi 1', respectively at harvest. The results were consistent with the findings of previous workers (Benlloch-Tinoco et al., 2015). Carotenoid can accumulate to give attractive yellow, orange, and red pigmentation to some flowers and fruit, reducing the progression of diseases such as age-related macular degeneration, certain types of cancers, and cardiovascular diseases (Ampomahdwamena et al., 2009).

Plant phenolics have been shown to inhibit the formation of superoxide anion radicals generated by various enzymes (Davis et al., 2009). Phenolic compounds act as reducing agents and antioxidants. In this study, the phenolics initially increased then fell, the peak reached at S2 (Figure 3C). 'Jinshi 1' had the greatest abundance of phenolics (8.20 mmol

g^{-1}), which was a little higher than that of 'Hongshi 2' (7.91 mmol g^{-1}). The patterns of the changes in the content of the flavonoids were slightly different from those of total phenolics. As shown in Figure 3D, the contents of flavonoids decreased by more than 88.8% from S1 to S3. At maturity, 'Hongshi 2' had the highest flavonoid content, whereas 'Hayward' had the lowest. Flavonoids are the most common group of polyphenolic compounds in the human diet and are abundant in plants (Bursal and Gülçin, 2011).

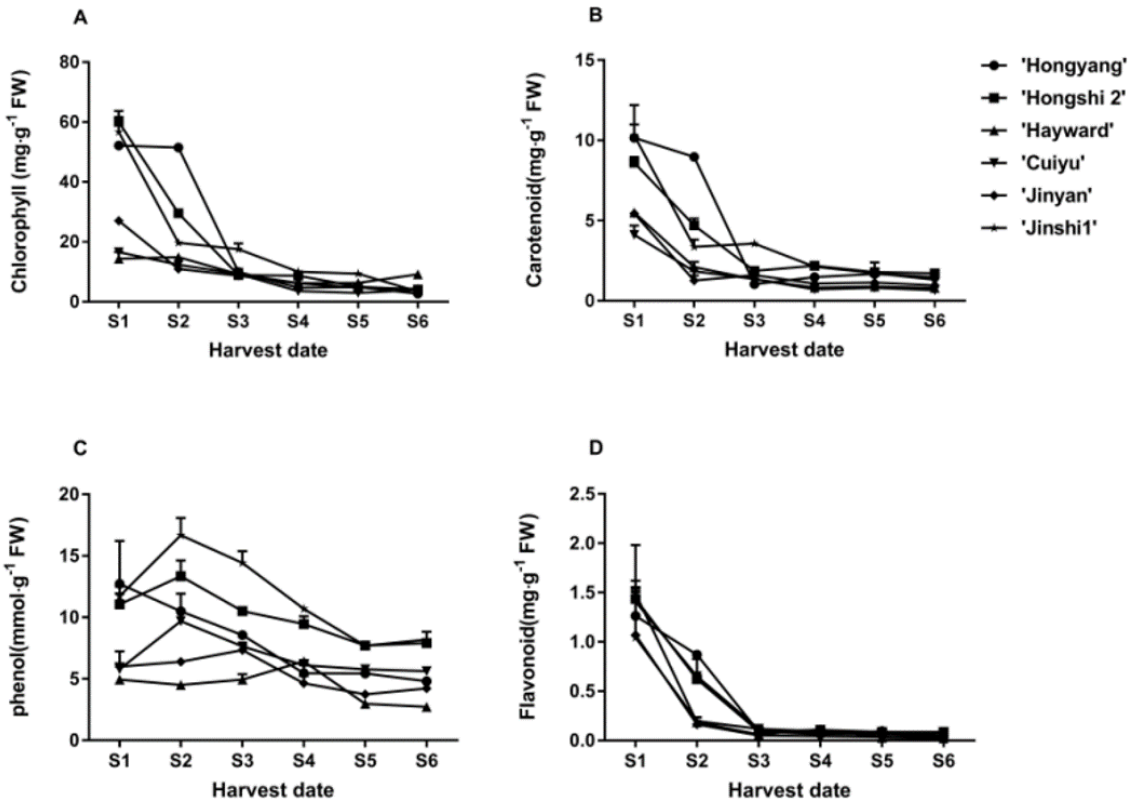

Figure 3. Contents of pigments, phenolics and flavonoids during fruit growth and development of six kiwifruit cultivars. A) Chlorophyll (mg g^{-1}), B) carotenoid (mg g^{-1} FW), C) phenol (mmol g^{-1} FW) and D) flavonoid (mg g^{-1} FW).

Antioxidant activities in different kiwifruit cultivars

Antioxidant activities varied among the kiwifruit samples as determined by the various assays used. In this study, the antioxidant activities in different kiwifruit cultivars were detected and three methods were used. As shown in Figure 4, the DPPH values and ABTS values showed the same descending trend generally but the hydroxyl radical scavenging rate increased. The highest content of DPPH values (76.23%) and ABTS values (85.71%) were observed in 'Jinshi' at maturity, whereas 'Hayward' showed the lowest content (16.41 and 13.72%, respectively). Additionally, the differences between the other cultivars were significant 'Hongshi' > 'Cuiyu' > 'Hongyang' > 'Jingyan'. This result was similar to the findings reported by Du et al. (2009b) who observed that the antioxidant capacity of 'Hongyang' and 'Hayward' was weaker than that of other genotypes of *Actinidia*, such as *A. eriantha* and *A. latifolia.* As shown in Figure 4C, the content of hydroxyl radical scavenging rate of 'Jingshi 1' was significantly higher than that of other five cultivars during developmental stage. In addition, 'Hongyang' had the lowest. The results have shown that comparison of all three methods gave different absolute values but the relationship for the same fruit was found in all methods, which was consistent with other reports (Park et al.,

2008). The results showed the antioxidant activities had the same order: 'Jingshi 1' > 'Hongshi 2' > 'Cuiyu'.

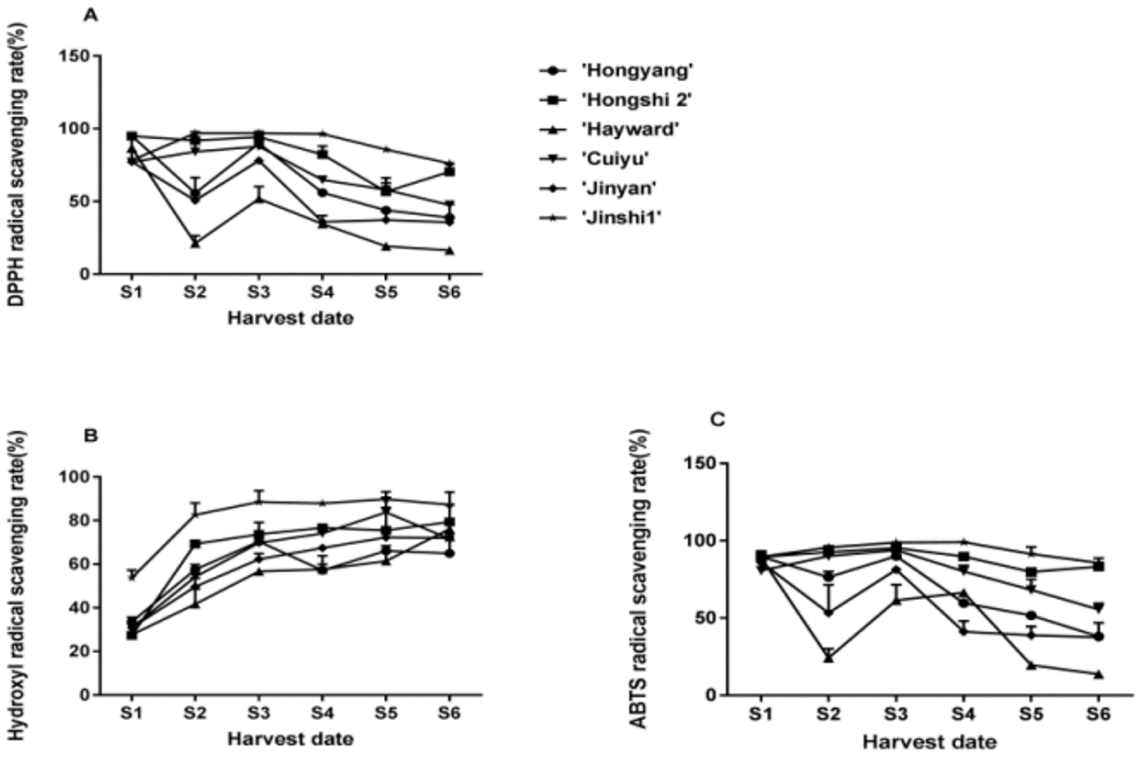

Figure 4. Antioxidant activities during fruit growth and development of different kiwifruit cultivars determined by A) DPPH radical scavenging rate (%), B) hydroxyl radical scavenging rate (%) and C) ABTS radical scavenging rate (%).

Grey correlation analysis and PCA analysis in different kiwifruit cultivars

To further understand the relationship of physiological characteristics to fruit quality, a grey correlation analysis was evaluated and is shown in Table 2. The highest correlation index was 'Hongshi 2' (0.799), because the content of TSS, VC, starch, carotenoid and flavonoid were ideal values. Furthermore, DPPH, ABTS, ·OH scavenging rates and TP content of 'Jinshi 1' were higher than those of other cultivars, although both 'Hongshi 2' and 'Jinshi 1' had the two lowest single fruit weight. On the contrary, 'Hayward' had the poorest fruit quality despite highest fruit weight, as nutrient compositions and antioxidant capacities were lower than those of other cultivars.

The first two factors (Fs) explained 71.29% of the total variability among the analyzed samples for all the investigated features. The contribution of each parameter to the two factors and the distribution of cultivars are shown in Figure 5. The first axis represents 50.73% of total variance and it was explained positively by carotenoid, Vitamin C, total phenol, TSS, while the second factor accounted for 20.56% of the total variation and it was positively correlated with starch, dry matter, soluble solids. Based on the PCA it can be stated that Vitamin C having the highest influence on fruit quality in kiwifruit.

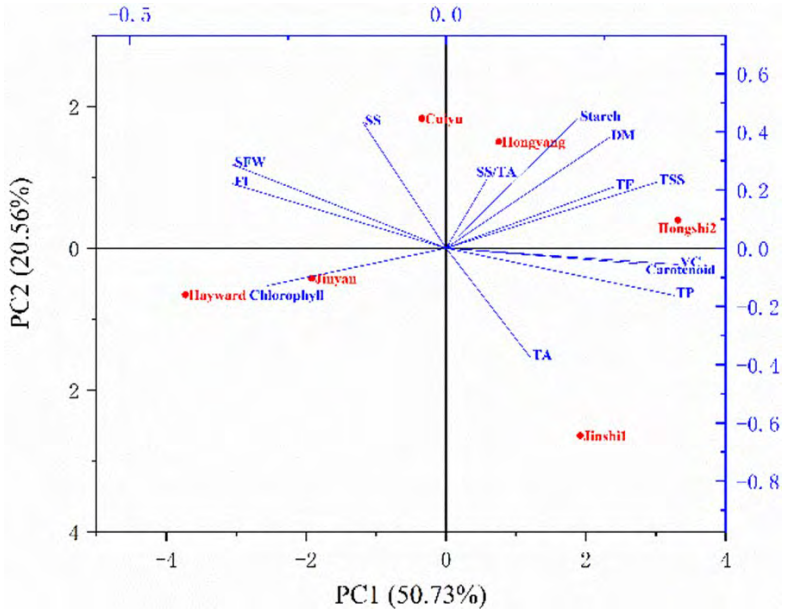

Figure 5. Biplot for results obtained from fruits of 6 cultivars of kiwifruit.

CONCLUSION

In conclusion, considering all of these data, to some extent the content of pigments and antioxidant activities in all cultivars have the same changing tendency during developmental stages, whereas there were significant difference in appearance quality and nutritional quality among these cultivars, cultivar was the main factor influencing the quality of kiwifruit. Additionally, some correlations between flesh color and nutritional quality were observed. The green-fleshed cultivars had better shape and fruit weight. The red fleshed cultivars had highest TSS, starch and carotenoid contents at maturity, especially 'Hongshi 2' had the greatest abundance of VC and flavonoid. As far as the quality in the two gold-fleshed cultivars there was a difference between soluble sugars and antioxidant activities which resulted in 'Jinyan' having a better flavor but 'Jinshi 1' had the highest radical scavenging ability. 'Hongshi 2' had the best overall quality particularly in terms of its nutritional composition.

Table 2. Grey correlation analysis in different kiwifruit cultivars at maturity.

Cultivar	SFW	FI	DM	TSS	TA	VC	TP	SS	Starch
Hongyang	0.568	0.712	1.000	0.874	1.000	0.467	0.495	0.653	0.810
Hongshi 2	0.436	0.712	0.956	1.000	0.476	1.000	0.920	0.567	1.000
Hayward	1.000	1.000	0.674	0.688	0.543	0.333	0.378	0.832	0.512
Cuiyu	0.887	0.888	0.997	0.874	0.601	0.591	0.564	1.000	0.648
Jinyan	0.728	0.913	0.622	0.829	0.755	0.402	0.456	0.920	0.474
Jinshi 1	0.387	0.607	0.770	0.829	0.443	0.855	1.000	0.371	0.454

	Carotenoid	SS/TA	Flavonoid	DPPH	ABTS	OH	GRG	Grade
Hongyang	0.750	0.834	0.398	0.454	0.422	0.614	0.670	4
Hongshi 2	1.000	0.480	1.000	0.844	0.936	0.818	0.810	1
Hayward	0.435	0.673	0.357	0.341	0.326	0.761	0.590	6
Cuiyu	0.400	0.842	0.778	0.520	0.537	0.694	0.721	2
Jinyan	0.484	1.000	0.398	0.433	0.420	0.700	0.636	5
Jinshi 1	0.635	0.349	0.467	1.000	1.000	1.000	0.678	3

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Kiwifruit plant growth and fruit yield and quality as influenced by fertilization with injection to the rhizosphere

Zhaobin Jing[1,2,3,a], Zhande Liu[3] and Chunchao Yao[3]

[1]Weinan Vocational and Technical College, Weinan 714026, Shaanxi, China; [2]Weinan Fruit Industry Institute of Shaanxi, Weinan 714026, Shaanxi, China; [3]College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China[a].

Abstract

The 6-year-old 'Hayward' kiwifruit was used as the experiment materials to explore the effect of fertilization either with traditional ring ditch or injection on growth, yield and quality of kiwifruit from 2015 to 2017. The results show that the advantages of kiwifruit orchard fertilization with injection to the rhizosphere are as follows: 1) the length and diameter of new shoots in the fertilization with injection treatments were significantly higher than traditional ring ditch fertilization, and the Chlorophyll A, Chlorophyll B and total Chlorophyll showed similar results except for treatment-2; 2) the fruit vertical and transect diameter, fruit volume and per fruit weight was maintained in higher level than the treatment of traditional ring ditch fertilization in the whole growing season (except for treatment-2); 3) the effect on fertilization with injection to the rhizosphere had different effects on the quality of kiwifruit. The liquid organic fertilizer, compound microorganism bacterium agent and water soluble fertilizer could improve the quality of kiwifruit. On the whole, combine fertilization with injection to rhizosphere and suitable water soluble fertilizer is an effective way to improve fertilizer and water use efficiency, yield and quality of kiwifruit, and it could be widely used as the modern high-efficient cultivation technology of kiwifruit.

Keywords: kiwifruit, vegetative characteristics, fruit weight, soluble solids, titratable acid, vitamin C, soluble sugar

INTRODUCTION

Kiwifruit is one of the most important fruits in the world, and it is widely used in food production due to its high nutritional value (Huang et al., 2007). Recently, the quality and production kiwifruit have been affected by some abiotic and biotic stresses. For example, low fertilizer utilization efficiency and drought has influenced kiwifruit orchards in almost all areas of its production in China. It has been identified that fertilization with injection to the rhizosphere (FIR), also be defined as alternate partial root-zone irrigation (APRI) is used broadly in many fruit plants of China. This technology was simple, low cost and overcome the problem of low fertilizer utilization efficiency. Some researches showed that APRI is efficient in saving water and improving water-use efficiency in some horticultural crops (Romero and Martinez-Cutillas, 2012). Water soluble fertilizer of different ingredients and conventional fertilizer were used in this experiment to study the effects of fertilization with injection to the rhizosphere on kiwifruit plant growth, fruit yield and quality in Shaanxi province, China.

[a]E-mail: jingzhaobin@nwsuaf.edu.cn; 353813638@qq.com

MATERIALS AND METHODS

Plant material and fertilization treatments

The 6-year-old 'Hayward' kiwifruit was used as the experiment materials, and the experiment was conducted at Zhouzhi county of Shaanxi province, China in 2015 to 2017.

The experiment includes five different treatments according to the fertilization type and ingredients:

- Treatment 1: leaf fertilizer (Fe+Mn+Zn+B≥20 g L^{-1}, amino acids≥100 g L^{-1}), punching fertilizer (organic matter≥30%, Fe+Mn+Zn+B≥20 g L^{-1}, amino acids≥100 g L^{-1}), major element water soluble fertilizer (30-I0-10≥50%, 18-8-34≥50%, 12-5-40≥50%, 20-20-20≥50%).
- Treatment 2: leaf fertilizer ($N+P_2O_5+K_2O$≥100 g L^{-1}), punching fertilizer ($N+P_2O_5+K_2O$≥100 g L^{-1}).
- Treatment 3: compound microbial fertilizer ($N+P_2O_5+K_2O$=6.0%), major element water soluble fertilizer (30-5-15≥50%, 12-30-8≥50%, 10-10-30≥50%).
- Treatment 4: major element water soluble fertilizer (20-20-10+1Mg+TE≥50%, 10-5-40+1Mg+TE≥50%), organic potash fertilizer (K_2O≥250 g L^{-1}), organic calcium fertilizer (Ca≥170 g L^{-1}, N≥50 g L^{-1}).
- Treatment 5(CK): organic fertilizer (organic matter≥45%), urea (N≥46%), calcium superphosphate (P≥16%), potassium sulfate (K≥52%).

Experiment design

The experiment was designed according to the agricultural industry standards of the People's Republic of China (NY/T1536-2007). This experiment was conducted in a randomized blocks design; two fertilization patterns were designed with three repeats. Treatments 1 to 4 were used by fertilization with injection to the rhizosphere (Figure 1), and treatment 5 was used by traditional ring ditch. The fertilizer was applied in five stages: germination stage, post-flowering, fruit puffing stage, barriers stage, and after harvest. Fertilizer rate was 5 kg at each stage by fertilization with injection to the rhizosphere. Fertilizer rate were urea (584 kg hm^{-2}), calcium superphosphate (250 kg hm^{-2}), and potassium sulfate (361 kg hm^{-2}).

Figure 1. Fertilization with injection to the rhizosphere technology.

Measurement of plant growth and fruit yield and quality index

New shoots and chlorophyll of kiwifruit were determined on June 30 using the ruler and the handled chlorophyll meter in field. Fruit vertical and transect diameter, fruit volume and per fruit weight were determined at each stages. Soluble solids, titratable acid, Vc and Soluble sugar were determined after harvest.

Statistical analysis

Each treatment included three replications. Analysis of variance was calculated using the SPSS 17 statistical software.

RESULTS AND DISCUSSION

Effects of FIR on vegetative characteristics of 'Hayward' kiwifruit

The new shoot length, new shoot diameter, chlorophyll a, chlorophyll b and total chlorophyll from water soluble fertilizer were significantly higher than conventional fertilizer. Compared to other treatments, treatment-1 showed the best results in four vegetative characteristics (Table 1).

Table 1. Effect of FIR on vegetative characteristics of 'Hayward' kiwifruit.

Treatments	New shoot length (cm)	New shoot diameter (mm)	Chlorophyll a ($mg \cdot g^{-1}$ FW)	Chlorophyll b ($mg \cdot g^{-1}$ FW)	Chlorophyll ($mg \cdot g^{-1}$ FW)
CK	35.72±0.04	6.75±0.06	1.45±0.16	0.62±0.08	2.07±0.25
T-1	40.99±0.04	7.15±0.19	1.76±0.15	0.79±0.02	2.54±0.17
T-2	45.36±0.02	6.92±0.11	1.45±0.13	0.58±0.03	2.03±0.17
T-3	46.75±0.05	6.95±0.07	1.72±0.21	0.68±0.09	2.40±0.31
T-4	42.50±0.03	6.91±0.09	1.63±0.16	0.64±0.05	2.27±0.22

Effects of FIR on fruit yields of 'Hayward' kiwifruit

The fruit vertical diameter, transect diameter, fruit volume, and fruit weight from water soluble fertilizer were significantly higher than conventional fertilizer except for treatment-2. Similarly, treatment-1 showed the best results in fruit yields of 'Hayward' kiwifruit (Figures 2 and 3).

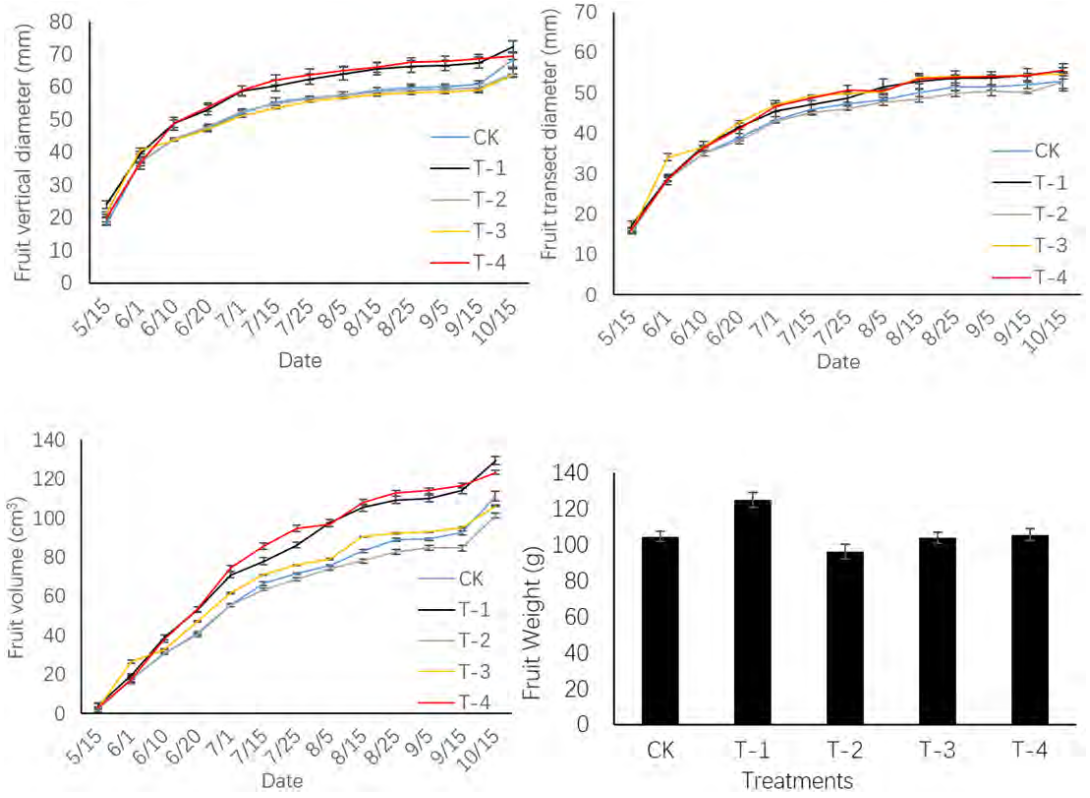

Figure 2. Effect of FIR on fruit vertical diameter, transect diameter, fruit volume, and fruit weight of 'Hayward' kiwifruit.

Figure 3. 'Hayward' fruits in this experiment on 30 June 2016.

Effects of FIR on fruit quality of 'Hayward' kiwifruit

The SSC, TA, Vc and SS of 'Hayward' kiwifruit from water soluble fertilizer were significantly different than conventional fertilizer. Similarly, treatment-1 showed the best results in fruit quality characteristics of 'Hayward' kiwifruit (Figure 4).

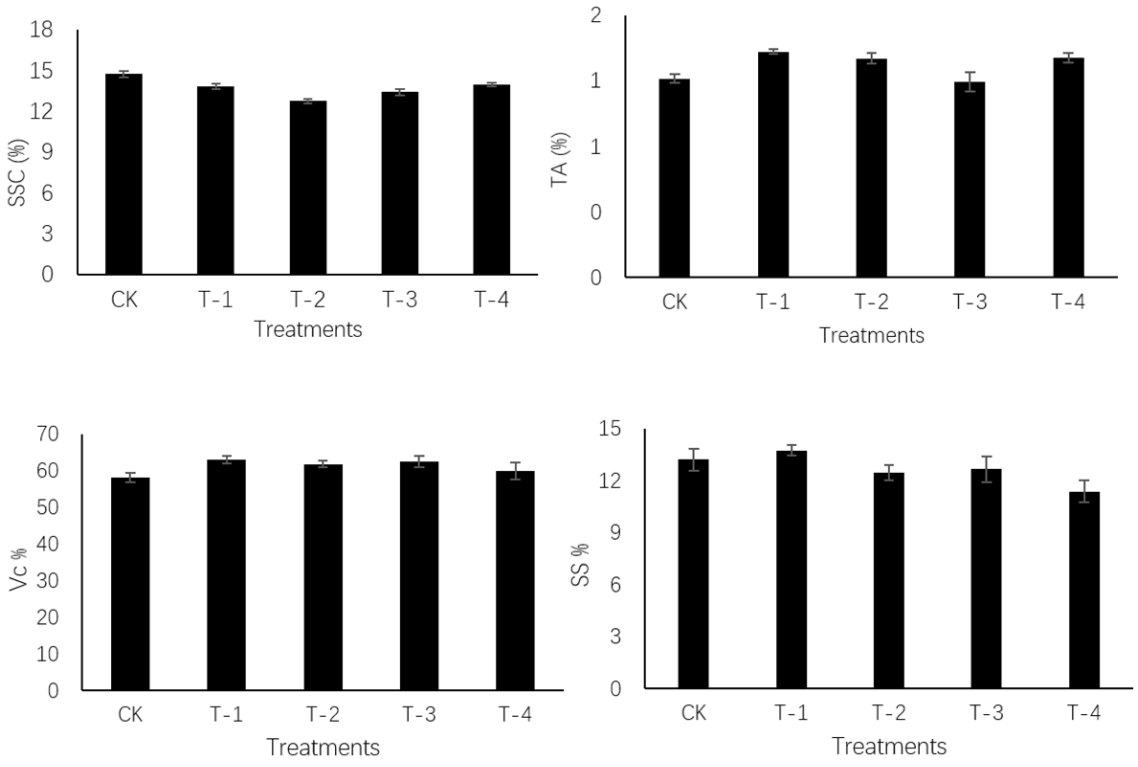

Figure 4. Effect of FIR on SSC (Soluble solids), TA (Titratable acid), Vc and SS (Soluble sugar) of 'Hayward' kiwifruit. Firmness: (CK:0.98, T-1:1.06, T-2:1.05, T-3:0.59; T-4:0.99).

It still remains debatable if the water soluble fertilizer by fertilization with injection to the rhizosphere could achieve the two goals of increasing fruit yield and quantity. Some researchers showed that both yield and quantity of crop are co-limited by water and nutrient availabilities (Wang and Xing, 2017). In this study, our results indicated that combining fertilization with injection to rhizosphere and suitable water soluble fertilizer is an effective way to improve fertilizer and water use efficiency, yield and quality of kiwifruit, and it could be widely used as the modern high-efficient cultivation technology of kiwifruit.

CONCLUSIONS

The following conclusions can be drawn from the study:

- The technology of fertilization with injection to the rhizosphere in China can increase kiwifruit plant growth and fruit yield, and improve some fruit quality characteristics significantly.
- Fertilization with injection is an effective way to improve the fertilizer and water use efficiency in dryland kiwifruit production.
- It is suggested that a kiwifruit specially fertilization with injection to the rhizosphere water soluble fertilizer (Fe+Mn+Zn+B≥20 g L^{-1}, amino acids≥100 g L^{-1}) should be applied in order to improve the plant growth and yield of kiwifruit.

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Optimising kiwifruit productivity in New Zealand

K.J. Patterson[a] and M.B. Currie

The New Zealand Institute of Plant & Food Research Limited, Auckland, New Zealand.

Abstract

Developments in cultivars and vine management practices for kiwifruit in New Zealand have increased productivity in commercial orchards substantially over the last 20 years. Emphasis on the production of fruit combining attributes of taste potential (equivalent to the proportion of dry matter in fruit at harvest), fruit size, fruit number and reduced harvest defects and postharvest losses has necessitated a more strategic approach to vine management to ensure dry matter allocation to fruit and standard yield attributes (fruit size and fruit number) are optimised, while ensuring the losses in the harvest and supply chain are minimised.

On-orchard productivity of kiwifruit grown in New Zealand can be considered as a combination of the three main components of taste potential (dry matter content of fruit at harvest; DMC or DM %), fruit size, and fruit number. Kiwifruit growing is characterised by an intensive approach, with considerable effort made by growers to maximise the productivity and financial return per unit area of land. A range of vine management approaches including girdling techniques, appropriate wood choice, strategic canopy management, control of competition from unnecessary vegetative shoot growth, and fruit growth stimulation are used in an integrated manner to optimise fruit yield and fruit quality.

INTRODUCTION

Kiwifruit production systems in New Zealand are currently based on the cultivars *Actinidia chinensis* var. *deliciosa* 'Hayward', marketed internationally as Zespri™ Green Kiwifruit and *A. chinensis* var. *chinensis* 'Zesy002', marketed as Zespri™ Sungold Kiwifruit. Production from another yellow fleshed cultivar, *A. chinensis* var. *chinensis* 'Hort16A' (marketed as Zespri™ Gold), reached a peak of 29 million trays in 2011. However, this cultivar is no longer in commercial production as a consequence of its high susceptibility to *Pseudomonas syringae* pv. *actinidiae* (Psa; Ferguson, 2017). Virtually all class 1 kiwifruit produced in New Zealand are exported, with 'Hayward' and 'Zesy002' comprising c. 71 and 29% of export volumes respectively in 2015-2016 (Anonym., 2016a).

Developments in vine management practices for both 'Hayward' and gold cultivars including 'Hort16A' and 'Zesy002' over the last 10-15 years have had a major influence on productivity expectations for kiwifruit grown under New Zealand conditions. Orchard systems and vine manipulation techniques are driven by the need for growers to achieve both high fruit productivity (yield of Class 1, export grade fruit per canopy ha) and superior fruit taste for consumers. Fruit dry matter content at harvest (DMC, commonly expressed as the percentage of dry matter in fruit) of 'Hayward' and 'Zesy002' kiwifruit is used as a proxy for soluble solids content in the ripe fruit (rSSC) and functions as a close indicator of consumer liking (Burdon et al., 2004).

Orchard production systems therefore need to focus on the attainment of high fruit numbers per unit area of canopy, adequate fruit size to achieve export grade requirements and high fruit DMC to meet consumer expectations. The understanding of how all three fruit components can be achieved in concert has required a systematic investigation into how vines and fruit of these cultivars respond to key vine manipulations such as girdling practices, canopy 'construction' and 'maintenance', vegetative growth control, crop

[a]E-mail: Kevin.Patterson@plantandfood.co.nz

loading principles, and fruit growth stimulation by growth regulators and biostimulants.

ORCHARD DESIGN AND VINE STRUCTURES

Kiwifruit vines are typically trained onto strong support structures capable of holding fruit and vine weights in excess of 60 t per canopy ha (4 t mu^{-1}) without significant damage of structures. The majority of the NZ industry now uses the pergola system to support vines, having moved away from the traditional T-bar system. The pergola system enables a more consistent canopy structure (even cane and leaf distribution) to be maintained across the orchard and is generally considered to be capable of higher yields per unit area than the T-bar system (Anonym., 2016b). An additional advantage of the pergola canopy is its self-sheltering nature, which improves the percentage of fruit that can be sold as class 1. Female vine planting distances are typically 5-6 m within row and 3.5-4.5 m between rows. In recent years there has also been a marked trend away from the matrix system of male and female vine layout (Sale and Lyford, 1990) to the strip or ribbon male system (male vines in alternate rows) which allows for focussed male vine management and improved bee access to assist pollination (Hunkin, 2013) as well as close proximity of males to more of each female vine. Vine structures typically comprise a single trunk with two opposing leaders oriented along the row axis. On female vines, fruiting canes are then borne on the permanent leader framework and are replaced annually. Female vines typically cover c. 20-30 m^2 of canopy. For strip male vines, shoot and cane growth is constrained to a narrow strip either side of the leaders, such that vine width (strip) may be no more than c. 1.0 m.

Kiwifruit orchards in New Zealand are intensively managed with average orchard areas of c. 2.3 ha for Gold and 3.5 ha for Green (Anonym., 2016b). Rapid conversion to new cultivars such as 'Zesy002' has been possible across the industry by the ease of reworking older 'Bruno' seedling rootstocks. Use of stringing techniques to encourage good leader growth during new vine establishment and during subsequent fruiting cane growth has been an important adjunct to quickly re-establish highly productive vines. Re-working of older stocks typically encourages vigorous initial scion growth and a rapid return to cropping, but such growth needs to be carefully structured to provide an appropriate vine framework for long-term production.

Implications for kiwifruit production in Shaanxi Province

Several green-, yellow- and red-fleshed kiwifruit cultivars are now being grown in Mei and Zhouzhi counties in Shaanxi province. At present the productivity focus is on fruit number and fruit size. In the future, it is likely that for some cultivars there will also be a focus on dry matter content and the percentage of class 1 fruit that can be produced, particularly as consumers in markets become more discerning about the taste and appearance of particular cultivars on offer. The general principle that dry matter at harvest is linked to SSC in ripe fruit is also expected to apply to fruit of such cultivars. However, taste metrics are likely to be cultivar-market specific and will need to be developed accordingly.

VINE MANAGEMENT AND THE CHOICE OF CANES FOR HIGH PRODUCTIVITY

A key vine management technique that is fundamental to high productivity of kiwifruit vines is the choice of cane types that impart both high flowering potential and moderate but manageable vigour. Attention to selective summer pruning to encourage the retention of less vigorous wood types is a critical aspect of kiwifruit productivity in New Zealand (Miller et al., 2001; Thorp et al., 2003) and recent studies confirm the importance of the principle for 'Zesy002' kiwifruit (Thorp et al., 2018).

For 'Hayward' production a high proportion of fruit stalk wood is retained during winter pruning and implementation of the system commonly called "the low vigour system" is fundamental to high productivity (Patterson and Currie, 2011).

For more vigorous cultivars such as 'Zesy002', retention of canes with smaller

diameters has been shown to contribute to high productivity with very high yields of 95 t per canopy ha now possible (Thorp et al., 2018). Targeting the retention of generally terminated canes requiring minimal pruning combined with the removal of excess large vigorous non-terminated cane is critical to high productivity. As pruning operations have the potential to damage fruit, particularly when skins are sensitive during development, reducing the requirement for summer pruning to manage growth can also improve fruit appearance.

Implications for kiwifruit in Shaanxi

The wide range of cultivars in production in Shaanxi demonstrates a range of vigour and propensity to produce different cane types. Appropriate shoot management and cane choice will be fundamental to help manage vine vigour and set the basis for high productivity and percentages of class 1 fruit. Management of cultivars should consider their individual growth characteristics and be cognisant of the need to utilise cane types that are highly fruitful, impart appropriate vigour potential and pruning techniques that avoid damaging fruit skins when they are particularly sensitive.

FRUIT THINNING AND CROP LOADING

Knowledge of fruit-size crop load relationships is critical to enable kiwifruit growers to set crop loads of fruit that will meet market expectations for fruit size. Commonly in fruit production systems, relationships between fruit number and fruit weight per unit area of canopy are derived to inform crop loading practice. For kiwifruit production in New Zealand, DMC of fruit must also be considered. Thinning practices are highly developed and ensure that lateral flowers of inflorescences, which tend to produce smaller fruit size and fruit that are misshapen, blemished and poorly pollinated, are removed as early as possible after fruit set. Studies on a range of high productivity 'Hayward' orchards suggest that for crop loads between 20 and 60 fruit m^{-2} of canopy, increases of c. 6 g FW occur for every 10 fruit m^{-2} reduction. By contrast the effect on fruit DMC is much more conservative, but with increases of c. 0.2 dry matter units still evident (Currie and Palmer, 2007). As a general principle it appears that compared with changes that occur in fruit weight, effects of crop load on fruit DMC in both 'Hayward' and 'Hort16A' are relatively conservative (Patterson and Currie, 2011).

CANOPY STRUCTURE

Canopy structure and its management during the growing season is the single most important component influencing kiwifruit productivity but surprisingly it is one of the least quantified attributes of vine management practice. Top-performing kiwifruit growers, however, have a very clear understanding of the principles required and are able to manage vines in ways that consistently achieve high productivity and high quality fruit. In particular, the management of "open" canopies throughout the growing season has reached a high level of skill, and combines techniques of appropriate cane choice and even cane spacing, removal of blind and unwanted shoots, and pruning techniques that minimise further unwanted extension growth. The combination of these approaches sets the basis of relatively open canopies that permit light penetration through the leaf layers of canopies that comprise large leaves and can be up to c. 1.0 m deep. A key indicator of the extent to which light is able to pass through the canopy is the degree of grass cover on the orchard floor. This simple measure is used by many growers as a key indicator of the required degree of "openness" in canopy structure (Patterson and Currie, 2011; Patterson, 2016).

Problematic canopies are characterised by excessively shaded lower leaves and leaf abscission in the latter stages of fruit development (Manning et al., 2010). Such canopies are now relatively uncommon and associated issues of fruit losses due to *Botrytis*, or poor quality fruit borne on leafless laterals under "dark" canopies are exceptional.

OPTIMISING THE POTENTIAL FOR FRUIT GROWTH

Relationships between seed number and fruit growth have been well documented for 'Hayward', 'Hort16A' and 'Zesy002' kiwifruit. With the advent of new cultivars of different ploidies being introduced into orchards it is essential to ensure compatibility between male and female cultivars. In this regard there is no substitute for evaluating all male × female combinations to ensure seed set and fruit development is optimal.

A second critical issue is the need to ensure that the male and female flowering coincides appropriately to ensure pollen can be transferred. Under New Zealand conditions pollination is achieved both with honey bees and through the use of several artificial pollen transfer systems.

Within Shaanxi province, a wide range of cultivars of different ploidies are in production. Because of the critical importance of seed number for fruit growth, attention to the requirement for suitable pollen sources to ensure fruit sizing potential is optimised is warranted. Issues of poor fruit sizing may simply be a consequence of insufficient compatible pollen being available.

MANAGEMENT OF VEGETATIVE GROWTH

Within kiwifruit vines competition for carbohydrate resources between shoot and fruit growth can significantly limit fruit growth and attainment of high fruit DMC. Research on 'Hayward' (Minchin et al., 2010; Snelgar et al., 2010) demonstrated the extent to which growth of vegetative shoots in spring and early summer can compete with fruit growth for carbohydrate resources. Minchin and co-workers used a model system comprising girdled shoots with four expanded leaves plus one fruit and compared fruit growth with or without vegetative growth from the terminal bud. Leaves were removed from the terminal shoot growth when they were no longer net importers of carbohydrate, so the direct effects of vegetative re-growth on the associated fruit development could be measured. When re-growth was stimulated c. 10 days before full bloom, fruit growth (FW) was reduced by as much as 51% and fruit DMC by as much as 20%. Stimulation of vegetative re-growth at c. 10 days after full bloom also reduced fruit growth but by a lesser amount (c. 20%) indicating that the effects on growth reduce once that fruit is actively growing. The study clearly demonstrated the large impairing effects that vegetative growth, particularly young expanding leaves, can have on fruit development.

At a practical vine management level, techniques such as removal of unwanted shoots, crush tipping and zero-leaf pruning at the right stage of development, as well as choice of canes that impart new shoot growth of a moderate vigour are essential. Attention to these will optimise canopy function and minimise the impacts that either vegetative growth or management at the wrong time can have on retarding fruit development and eliciting skin blemishes (Patterson and Currie, 2011).

GIRDLING PRACTICES

Girdling practices that temporarily disrupt phloem transport have historically been used from time to time in the culture of grapes, citrus, apple, peach and persimmon to improve fruit set, fruit size, and fruit quality attributes such as total soluble solids, colour, and fruit dry matter content (Goren et al., 2004). Girdling techniques for kiwifruit have been intensively developed in research and extension programmes and are almost universally used by kiwifruit growers in New Zealand. Girdling is primarily used to improve fruit size and to enhance carbohydrate allocation to fruit and thus increase the DMC of fruit at harvest (Currie et al., 2018).

Timing of girdling is important. Typically, at least two trunk girdles are applied to vines each growing season. A spring girdle is applied principally to increase fruit size at about 28 days after mid-bloom, just before the maximum rate of fruit expansion. The general view is that this girdle increases cell division in the fruit thereby leading to the potential for larger fruit (M.B. Currie, pers. Comm.) although changes to the availability of solutes for fruit expansion following girdling could conceivably have a role to play as well

(Hall et al., 2013).

A second trunk girdle to increase DMC is commonly applied around 80 days after mid-bloom during mid-summer. Phloem disruption at this time results in significantly higher carbohydrate accumulation above the girdle and the higher sugar concentration results in increased mass flow of carbohydrates to sinks such as fruit. Both girdling techniques are firmly embedded as kiwifruit vine management techniques for 'Hayward' and 'Zesy002'. Use of the summer girdling technique to improve DMC has had a major influence on average DMC of fruit at an industry level (Hall and Snelgar, 2016) and is now a fundamental technique to enhance the taste potential of kiwifruit in New Zealand.

FRUIT GROWTH STIMULATION

Attainment of an ideal size profile for kiwifruit cultivars is an important determinant of orchard profitability. In addition to the fundamental need to ensure adequate seed set, and adequate leaf area to drive fruit growth, a range of biostimulants is commonly used in New Zealand to enhance fruit growth and thereby optimise fruit size profiles at harvest. Minimum fruit sizes for export grade fruit in New Zealand are c. 75 g for 'Hayward' and 'Zesy002'.

The biostimulant Benefit-PZ was extensively used on 'Hort16A' to stimulate fruit growth and was generally able to alter the fruit size profile from a mean weight of c. 95 g to 125 g (Patterson et al., 2003). Although 'Hort16A' is no longer in production, the effects of Benefit-PZ use on the cultivar are discussed here as they provide an important insight into how excessive biostimulant use can stimulate fruit growth but also have negative effects on fruit DMC. Up to three applications of Benefit-PZ over the period of rapid fruit expansion were commonly applied to 'Hort16A' (Currie et al., 2005) and could result in large average fruit size (>140 g) but with diminished fruit DMC (DMC reduced by up to 1 unit). Clearly, while the fruit size benefits were highly desirable, the negative effect on DMC had implications for potential fruit taste in market (Currie et al., 2005).

The underlying mechanism of why fruit undergoing rapid expansion are unable to accumulate carbohydrate at rates sufficient to maintain high DMC has not been elucidated for Benefit-PZ although recent findings for fruit growth stimulation with forchlorfenuron (N_1-(2-chloro-4-pyridyl)-N_3-phenylurea, CPPU) provide some insight into a possible mechanism involving changes to osmotic potential of fruit during fruit expansion (Nardozza et al., 2017; Hall et al., 2013).

Benefit-PZ is not used on 'Zesy002' because of the naturally large fruit size characteristic of this cultivar. 'Hayward' is not as responsive to Benefit-PZ as 'Hort16A' and the product is not generally used commercially on this cultivar.

Implications

Fruit growth stimulants can be used to improve fruit size, and can be particularly useful for cultivars with smaller fruit size profiles (e.g., 'Hort16A'). Responses appear to be cultivar specific ('Hort16A' compared with 'Hayward'). Some caution in application is suggested to avoid situations where the fruit growth is stimulated to such an extent that dry matter accumulation and potential fruit taste is compromised.

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Autotrophic rooting, acclimatization and transplant of Kiwifruit tissue culture seedlings

Wei Li, Zhande Liu[a] and Zhiyao Ma

College of Horticulture, Northwest A&F University, 712100 Yangling, Shaanxi, China.

Abstract

Kiwifruit, which features high callus induction rate and differentiation frequency, is a suitable material for tissue culture. Due to less-refined technologies in tissue culture seedling rooting, acclimatization, and transplant processes, the cultivation and spread of tissue culture seedling of kiwifruit are limited. Speaking of the conventional method for tissue culture seedling rooting of kiwifruit which is performed on Ms medium, influenced by hormone combination and other uncertain factors, the callus becomes over-swelling and the survival rate of tissue culture seedlings plummets; the tissue culture period is relatively long because of long rooting period and acclimatization and transplant period after rooting, which generates huge production costs and is far from catering the market. To address the above issues, an autotrophic rooting method of tissue culture seedlings (with this method, the tissue culture seedlings of a certain height are cut and rooted in air-permeable polyethylene boxes with mediums in them. The selected seedlings root with the nutrition generated in the photosynthesis process of their leaves) is put forward. Combined with the acclimatization and transplant process, the culture process is simplified, the cultivation period is shortened, and production costs are reduced.

RESULTS

Selecting the rooting medium

The medium adopted in autotrophic rooting indeed plays a role. Mediums that can maintain moisture and heat and have the least influence on tissue culture seedlings are the best options. Therefore, in this experiment, the mediums were watered without any other treatment. Then, the most suitable medium for rooting was selected from four mediums-river sand, vermiculite, perlite, and complex mechanism based on the respective survival rate of seedlings (Figure 1).

Table 1 illustrates that among the four mediums, vermiculite has the least influence on tissue culture seedlings; in addition, vermiculite, followed by perlite and river sand, boosts the best heat conservation effect; and seedlings transplanted in compound medium rotted and withered after two days due to the fertilizer contained in the medium, based on which vermiculite was finally selected as the most suitable medium for autotrophic rooting of tissue culture seedlings.

Selecting the optimal concentration of root treatment hormone IBA

In order to accelerate autotrophic rooting process of tissue culture seedlings, the insertion end of the seedlings were subjected to IBA treatment (with the uniform duration of 2 h) before the insertion of vermiculite. The rooting initiation time, average rooting rate, average number of roots and average roots length (statistics after rooting for three days) after treatment with different concentrations were recorded so that the optimal IBA concentration can be determined (Figure 2).

[a]E-mail: dezhanliu@vip.sina.com

A. Inoculation box B. After inoculation

Figure 1. Seedlings were inoculated in polyethylene boxes with holes for heat and moisture conservation, ventilation, and light transmission.

Table 1. Relations between transplanting mediums and survival rates of tissue culture seedlings.

Transplanting mediums	Numbers of cuttings	Numbers of survival	Survival rate
River sand	20	7	35
Vermiculite	20	19	95
Perlite	20	14	70
Compound medium	20	0	0

A. Bud of seedlings B. IBA treatment

Figure 2. Bud of seedlings with appropriate lengths was selected for IBA treatment. Contact of leaves with IBA solution was avoided.

Table 2 illustrates that in the five IBA concentration gradients of 0.5~1.5 mg L^{-1}, when the concentration was 1.2 mg L^{-1}, the rooting time of the tissue culture seedlings was the earliest, the average rooting number was the highest, and the average length of the root was the longest. Although the initial rooting rate was not as high as that of the concentration of 1.2 mg L^{-1}, the comprehensive index was superior. Therefore, 1.2 mg L^{-1} was selected as the optimal root hormone concentration for autotrophic rooting of tissue culture seedlings.

Selecting the optimal root length for transplanting seedlings

While transplanting the seedlings, the root length is also an important factor affecting the success of transplant. When the roots are too short, their various functions are not yet perfect, so it is difficult to meet the needs of the seedlings for water and the seedlings are prone to wilting; when the roots are too long, they are easily damaged during the transplant process, which is also not conducive to the survival of the seedlings. Therefore, the following treatments were performed (20 seedlings for each treatment) to figure out the optimal root length range for transplanting seedlings (Figure 3).

Table 2. Influence of IBA treatments on autotrophic rooting of kiwifruit tissue culture seedlings.

IBA concentration (mg L^{-1})	Rooting initiation time (d)	Average rooting rate (%)	Average roots number	Average roots length (cm)
0.5	15	14.4	0.45	0.5
0.8	15	17.2	0.37	0.44
1.0	13	20.5	0.84	1.05
1.2	10	23.6	0.86	2.2
1.5	11	24.4	0.62	1.8

Figure 3. Survival rate of tissue culture seedlings with different root lengths through transplanting was counted.

Table 3 illustrates that, when the root length ranges from 1.5 to 2.5 cm, the survival rate of the seedlings is the highest after 10 days, which can reach over 90%; when the root length is about 0.5 cm, the survival rate is lower than the other two. Therefore, it is concluded that the optimal root length range for transplanting tissue culture seedlings is 1.5~2.5 cm.

Table 3. Statistics of transplant survival rate of rooting seedlings with different root lengths.

Root length (cm)	Survival rate (%)		
	3 d	7 d	10 d
0.5	85	75	75
1.5	100	95	95
2.5	100	90	90
3.5	95	95	85

Seedling transplanting and sound seedlings

Conventional tissue culture rooting seedlings requires seedling acclimatization before transplantation, that is, the inoculation boxes are gradually opened so that the seedlings can acclimate the external environment. In the autotrophic rooting process, the polyethylene boxes adopted have holes on them for ventilation. The rooting and acclimatization processes are combined, so the seedlings can be directly transplanted. Since the seedlings that are just removed from the boxes are too fragile to adapt to the new environment, it is suggested that the transplantation be performed on cloudy days so that the seedlings will not be exposed to hard lights.

Do not place the transplanted tissue culture seedlings in direct hard light. Take measures to maintain moisture. You can place arched sheds above the seedlings. The arched sheds should be put up with holes on them for ventilation. After 3 days, uncover the arched sheds and put the seedlings in scattered light to culture strong seedlings. When young leaves shoot out and old leaves become thick and glossy, move the seedlings out of the greenhouse and perform conventional management (Figure 4).

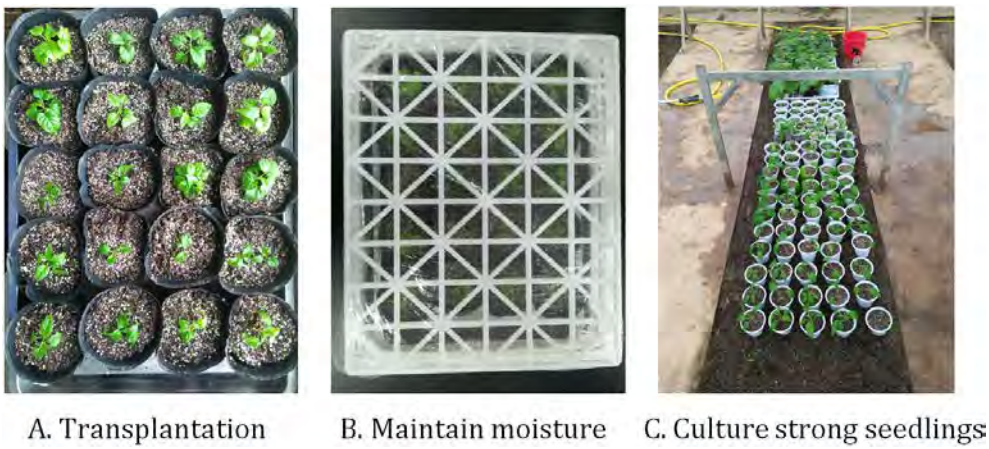

Figure 4. Take measures to maintain heat and moisture to improve the survival rate after the transplantation of tissue culture seedlings.

CONCLUSION

Compared with the conventional medium rooting method, autotrophic rooting of tissue culture seedlings is novel in the combination of rooting, acclimatization, and transplant processes. It boosts shorter tissue culture period, more simple production processes of tissue culture seedlings, and lower production costs, thus better meeting the requirements of production.

Antimicrobial activity analysis of extracts of *Scutellaria baicalensis* on kiwifruit fruit pathogens

H. Shi, R.C. Wang, Y. Wang, L. Pang, Q. Zhou, J.B. Qi and F.F. Wang

College of Horticulture and Landscape, Hunan Agricultural University, Changsha 410128, China.

Abstract

The aim was to study the antimicrobial activity of plant preservatives on kiwifruit soft rot pathogens (*Botryosphaeria dothidea*), in order to promote the healthy and sustainable development of its industry. The pathogen of soft rot disease in the red Yang kiwifruit of Hunan, Xiangxi and the Chinese medicine *Scutellaria baicalensis* Georgi were as experimental material, the inhibitory circle was used as a measure, the extraction conditions of the active substances and the determination of inhibitory properties of active substances were determined. Experimental results show that, when 80% ethanol is used as extraction solvent, the extract has strong inhibitory effect. At 1:20 (g:mL), the ratio of material to liquid, 2 h ultrasonic extraction time, 70°C extraction temperature, and 1000 W ultrasonic power, the extract has strong inhibitory effect, the diameter of the inhibitory circle was more than 26.50 mm. Compared with other conditions, the inhibitory activity of the extracts increased by more than 50%. UV irradiation over 10 h, more alkaline environment, Ag^{+}, $Fe3^{+}$, Cu^{2+} metal ions on the activity of extracts have some negative side effects. The diameter of inhibition zone decreased by 20-60%. The activity of extracts was less affected by storage at less than 25°C for about 40 days. The above extraction method is adaptable, cost saving, efficient and extraction rates are high, and the inhibitory effect is good. The optimum activity conditions of the extracts were determined. *Scutellaria baicalensis* extract is a new plant source preservative. It provides the direction and practical significance for the long-term with an efficient, green and environmentally friendly storage of kiwifruit.

Keywords: *Scutellaria baicalensis* extract, kiwifruit, pathogen, antibacterial activity analysis

INTRODUCTION

Actinidia is a vine with fruit rich in vitamin C (VC). It is a perennial dioecious deciduous climber. It has the reputation of being "the king of fruit". The fruit is delicate, juicy, delicious, sweet, and rich in nutrients (Li et al., 2017; Drummond, 2013). Many kinds of organic substances, such as sugars, proteins, amino acids, and a variety of essential minerals are in the fruit which are highly nutritious and have some health benefits. It is always the favorite of the vast majority of consumers (You et al., 2014; Salzano, 2018). China now has an area of about 250,000 ha of kiwifruit orchards, accounting for 71.4% of the total 350.000 ha of cultivated kiwifruit plantings in the world. In the world's annual output of 2,733,800 t of kiwifruit, China's annual output is about 1,216,700 t, accounting for 44.5% of the total (Wang et al., 2015). The development of kiwifruit industry plays an important role in the development of the agricultural economy in China, but problems in the storage and transport of its fruit have restricted the development of the industry. The harvest period of kiwifruit from September to October, for many places, is still in the season of higher temperatures. In addition, kiwifruit is a climacteric fruit. It is very easy to soften and rot and deteriorate at normal temperatures (Han et al., 2003; Wu, 2014), largely due to the invasion of the rot pathogenic fungus *Botryosphaeria dothidea* (Yang et al., 2018). Therefore, the fruit should be placed in suitable storage conditions as soon as possible and effective preservation measures should be taken. At present, many

preservatives on the market are mixed with some chemical reagents. Often using chemical reagents will bring great safety risks to people's health. A natural fruit preservative which is efficient, safe, non-toxic and stable in performance, has attracted more and more attention (Santagata et al., 2018). The use of natural extracts as preservatives has been reported, but such application to kiwifruit preservation is very rare. The extraction of plant extracts is easy, economical and effective, and does not harm the human body and the environment. Therefore, the study of the application of natural extracts to fresh fruit of kiwifruit has become of considerable interest (Manso et al., 2011). The purpose of this study is to use the natural inhibitory substances produced by the plant *Scutellaria baicalensis* to replace the chemical preservatives and to develop a plant derived preservative suitable for fruit of *Actinidia*.

MATERIALS AND METHODS

Test materials and reagents

Botryosphaeria was isolated and identified in my own laboratory and *Scutellaria* was bought from Hunan Jiu Chi Tang Pharmacy. Glucose, agar powder, Tween, ethanol, acetone, ethyl acetate, chloroform, petroleum ether, hydrochloric acid, sodium hydroxide, sodium chloride, magnesium chloride, ferric chloride, copper chloride and silver nitrate. Organic reagents are analytical grade, National Medicine Chemical Reagent Production Co., Ltd.

Test instruments

DHG-9246A electrothermal thermostatic drying box Shanghai Jinghong Test Equipment Co., Ltd.; THZ-92B adjustable temperature controlled shaking incubator Shanghai Pudong Physical Instrument Factory; ZW1105051705 ultraviolet visible spectrophotometer Shanghai Spectral Instrument Co., SB-3200D ultrasonic instrument Ningbo Shinozhi Biological Polytron Technologies Inc; DR-1001 rotary evaporater Zhengzhou The Great Wall Industry and Trade Co., Ltd.; G16 desktop high speed centrifuge Changsha Yingtai Instrument Co., Ltd.; DK-98-IIA electric thermostatic water bath pot tester Instrument Co., Ltd.; sterilizing pot HVA-85 automatic high pressure steam sterilizing pot Tianjin Yongda Chemical Reagent Co., Ltd.; PHS-25 pH meter Shanghai Instrument Electric Science Instrument Stock Co Ltd.

Preparation of plant extract

The plant sample powder, 10 g, was added to a 350 mL triangle bottle; to which 200 mL of extraction solvent was added, giving a ratio of solid to extraction solvent of 1:20. The bottle was placed in the ultrasonic apparatus; ultrasonic extraction of 2 h at 70°C; then the extract was filtered; the filtrate was concentrated to dryness and taken up in acetone, 1.0 g original sample mL^{-1}; put into a 10 mL capacity bottle. The sample was labelled and placed in the refrigerator at 4°C for storage.

Preparation of test culture

After activation, the strain was inoculated in PDA culture medium; cultured at 28°C for 60 h; rinsed with tween aseptic saline; broken with glass beads; shock, centrifuged and filtered. Determination of suspension concentration by turbidimetry. Concentration was adjusted to 106~107 cfu mL^{-1} extract.

Determination of inhibitory activity of extract

First the base medium of about 8 mL was poured into the Petri dish. After its solidification, the mixture of 9 mL medium that had been melted around 40°C and 1 mL microbial suspension, shaken well, controlling the concentration of microbe liquid between 105~106 cfu mL^{-1}, was added to the plate. When the upper medium was rapidly solidified, the sterilized Oxford cup was placed in the upper culture base, four Oxford cups were placed in each Petri dish, adding 100 uL extract of *Scutellaria baicalensis* to each cup

(removal of bacteria by 0.22 um organic filter membrane), the Petri dish was placed in a constant temperature incubator at 28°C to avoid light exposure. There were three replications to each treatment. The diameter of inhibition was measured with Vernier caliper after 72 h (Cross method).

Single factor extraction process of inhibition

Inhibition diameter as a reference index with individual factors changed one at a time, the others remaining constant.

1. Influence of different extraction solvents.

Water, ethanol, acetone, ethyl acetate, chloroform and petroleum ether were used as extraction solvents. Determination of inhibition by cup and dish method. The experiment was replicated three times per treatment. The inhibitory ability of the extracts against *Botryosphaeria* was evaluated.

2. Influence of different solvent concentration.

It is known from the experimental results that ethanol as an extraction solvent inhibits strongly. Therefore, ethanol with concentrations of 20, 40, 60, 80, 90 and 100% was selected as the extraction solvent. Determination of inhibition by cup and dish method. The experiment was replicated three times per treatment. The inhibitory ability of the extracts against *Botryosphaeria* was evaluated.

3. The influence of the ratio of plant material to liquid.

Varying rartios ofof plant material: extraction solution were evaluated: 1:5, 1:10, 1:20. Determination of inhibition by cup and dish method. The experiment was replicated three times per treatment. The inhibitory activity of the extracts against *Botryosphaeria* was evaluated.

4. Influence of extraction temperature.

After extraction at temperatures of 25, 40, 60, 70, 85 and 100°C, the inhibitory effect was determined by cup and dish method. The experiment was replicated three times per treatment. The inhibitory activity of the extracts against *Botryosphaeria* was evaluated.

5. The effect of ultrasonic extraction time.

The extraction time was 1, 1.5, 2, 2.5, 3, and 4 h. Determination of inhibition by cup and dish method. The experiment was replicated three times per treatment. The inhibitory ability of the extracts against *Botryosphaeria* was evaluated.

6. The influence of ultrasonic power.

Under the condition of ultrasonic power of 300, 500, 700, 1000, 1200 and 1500 W. Determination of inhibition by cup and dish method. The experiment was replicated three times per treatment. The inhibitory activity of the extracts against *Botryosphaeria* was evaluated.

Determination of inhibitory properties of extract

1. Effects of light and ultraviolet irradiation on inhibitory activity of extracts.

The extract was divided into six portions and placed in a transparent glass tube, they were irradiated at 15 W, 40 cm for 5, 10, 20, 35, 50, 70 h, respectively. Referring to the above methods, 100 µL extract solution in each Oxford cup, cultured for 72 h at 28°C. The experiment was replicated three times per treatment. The diameter of the inhibition was measured with a Vernier caliper, the inhibitory effect being the average value.

2. Effect of temperature on inhibitory properties of extract.

The extract was divided into six portions, placed in a transparent glass tube, they were placed at -20, 4 and 25°C for 1 day, respectively. Treatment of 2 h under the conditions of a water bath at 70, 80, and 100°C, the inhibitory effect was measured by the cup and disc method. The experiment was replicated three times per treatment.

3. Effect of pH on inhibitory properties of extract.

The extract was divided into six parts, and the pH adjusted using sodium hydroxide or hydrochloric acid 3, 4, 6, 7, 8 and 12, respectively. After 24 h of balance, the inhibitory effect was measured by the cup and disc method. The experiment was replicated three times per treatment.

4. Study on storage performance of extract.

In order to understand the storage properties of *Scutellaria baicalensis* extracts at different temperatures, extracts were stored at 4 and 25°C, respectively. Determination of inhibitory activity every 20 days, six times for measurement. Determination of inhibitory effect was with *Botryosphaeria* as the test organism. The inhibitory effect was measured by the cup and disc method. The experiment was replicated three times per treatment.

5. Effect of metal ions on inhibitory activity of extracts.

The aqueous extracts were divided into five parts. To the extracts, sodium chloride, magnesium chloride, ferric chloride, copper chloride and silver nitrate were added at a final concentration of 10, 30, 50, 70, 90 and 100 mmol L^{-1}, respectively. The inhibitory effect was measured by the cup and disc method. The experiment was replicated three times per treatment.

Data analysis and graphic production

Excel 2010 and Origin8 software are used for data processing and graph production. SPSS18.0 software was used for statistical analysis of test data. Different capital letters indicate the level of the extreme significance of alpha = 0.01. $P<0.01$ indicates that differences are statistically significant.

RESULTS AND ANALYSIS

Extraction of active principle from *Scutellaria baicalensis*

1. Effects of different extraction solvents on inhibition.

Six extraction solvents were selected according to the polarity of the reagents. Ethanol, acetone and ethyl acetate were used as extraction solvents for effective components of medicinal materials. The activity and content of the inhibitory components were relatively high with good inhibitory effects. The inhibition diameter reached 26.13 mm. Chloroform and petroleum ether as extraction solvents: the inhibitory effect of the extract was poor (about 15.00 mm) which may be because the polarity of the two solvents is too weak, the result of the low total content of the extract. Water was used as a solvent but the inhibitory was also poor, probably because the extract contained many impurities, such as sugars, proteins and inorganic salts. This extract was difficult to concentrate, underwent changes and mouldy during storage (Liao et al., 2013). Therefore, considering the cost, the inhibitory effect and the effects on the environment, etc., ethanol is a relatively good extraction solvent.

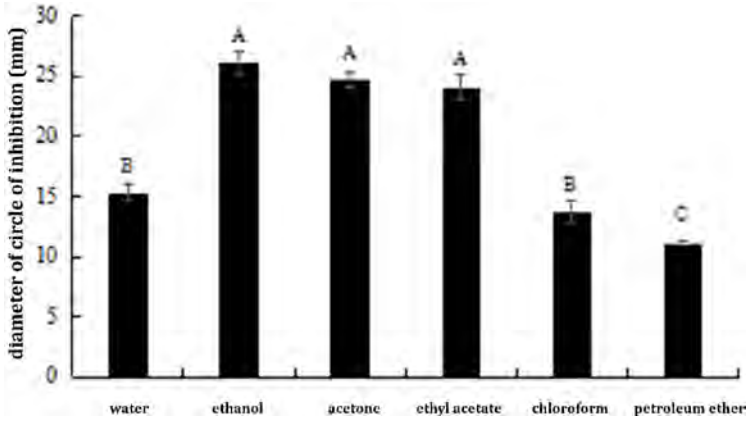

Figure 1. Effect of different extraction solvents on inhibitory activity.

2. Effect of ethanol concentration on inhibitory effect.

The results obtained from the above experimental results. The ethanol extract of *Scutellaria baicalensis* has a strong fungicidal. Different concentrations of ethanol as extraction solvent: the results of the experiment are shown in Figure 2. Six concentrations of ethanol were selected for the experiment. When the concentration was low (20-60%), the inhibitory effect on *Botryosphaeria* was poor, and the diameter of the inhibition was less than 20 mm. This may be because the polarity of the solvent is too large, there is a low leaching rate of finite element, there is a high molecular leach rate of sugar, protein and so on is high. When the concentration of ethanol was more than 80%, the inhibitory effect of the extract was greatly improved. It shows that the inhibitory activity of the effective components is stronger at this time. The extracts prepared with 80 and 100% ethanol had similar inhibitory effects. Therefore, it is better to choose 80% ethanol as the solvent for cost reasons.

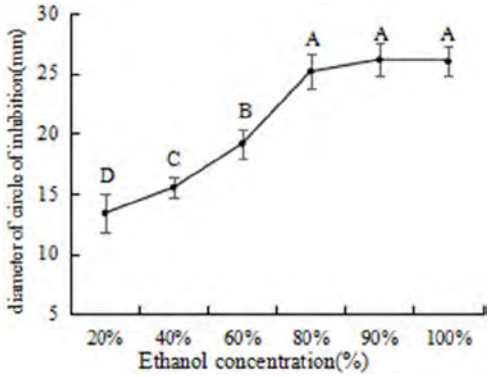

Figure 2. Effect of ethanol concentration on antimicrobial activity.

3. Effect of the ratio plant material: solvent on inhibition.

80% ethanol was selected as extraction solvent. The inhibitory effect under different ratios of plant material to solvent is shown in Figure 3. Within the range of material and liquid ratio up to 1:20 (g:mL), and using the diameter of inhibition as a measure, it can be determined that the extraction rate of effective components increases with the increase of ethanol consumption. When the amount of ethanol is greater than 20 mL, the extraction rate of the effective substance has reached a maximum. With the increase of alcohol volume, the extraction rate does not increase all the time. This may be because when the amount of ethanol per g is greater than 20 mL, the effective substance has been able to

dissolve completely (Gutiérrez et al., 2018). Therefore, the consideration of cost and experimental intensity, it is suitable to choose the 1:20 ratio.

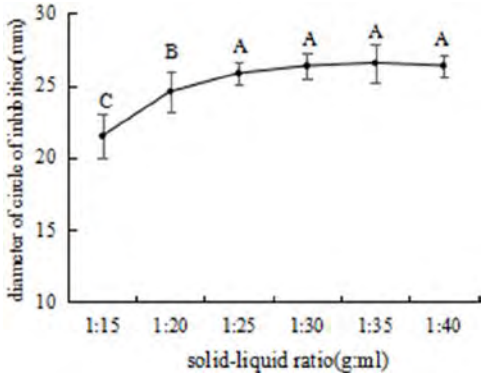

Figure 3. Effect of ratio of plant material: solvent on inhibitory activity.

4. Effect of extraction temperature on active principle

Temperature is one of the main factors affecting the extraction of effective components from plants. Inhibitory effect of different temperatures as shown in Figure 4. When the extraction temperature was up to 70°C, with the increase of temperature, the inhibitory activity of the extracts increased. This may be due to the gradual increase in the dissolution of active ingredients. When the extraction temperature was higher than 70°C, the inhibitory effect of the extract gradually decreased. When the extraction temperature was 100°C, the diameter of the inhibition circle is only 24.23 mm. This may be because excessive temperature will lead to lower activity of active ingredients (Shi et al., 2015). Therefore, considering the dissolution rate and activity of the effective substance, it is more appropriate to select the extraction temperature of 70°C.

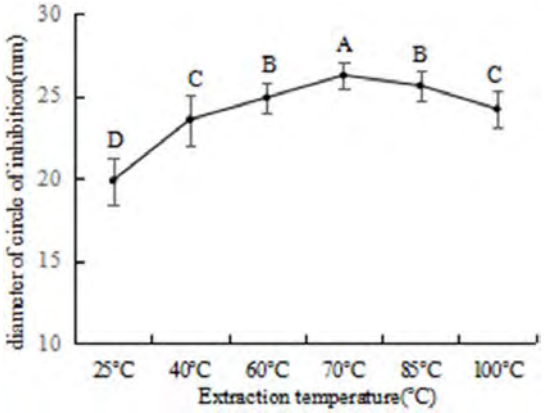

Figure 4. Effect of extraction temperature on antimicrobial activity.

5. Effect of extraction time on inhibitory activity.

Extraction time is also one of the main factors affecting the extraction efficiency of effective components in plants. Under the condition that the ratio of material to liquid is 1:20, the extraction temperature is 70°C. The inhibitory effect of extracts from the same extraction time on *Botryosphaeria* is shown in Figure 5. Taken the diameter of inhibition as an indicator, it can be seen that the *Scutellaria baicalensis* is in the extraction of 2 h. The dissolution of the effective component has almost reached the maximum. At this time, the diameter of the inhibition circle has reached about 25 mm. With the prolongation of the extraction time, the dissolution of the effective component was slightly improved, but it is

not obvious. The effective components of *Scutellaria baicalensis* were extracted by 2 h, almost completely dissolving out. Therefore, take into account the efficiency of the experiment, textraction time of 2 h is more suitable (Ferreira et al., 2018).

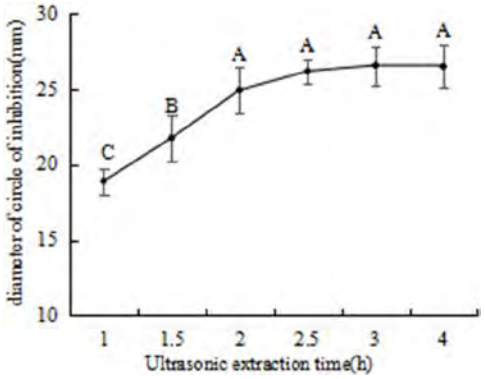

Figure 5. Effect of extraction time on antimicrobial activity.

6. Effect of ultrasonic power on bacteriostasis.

Ultrasonic extraction is one of the effective methods to speed up the rapid dissolution of plant efficacy components. The bacteriostasis effect of effective components under different ultrasonic power conditions on *Botryosphaeria* is shown in Figure 6. In the range of ultrasonic power of 1000 W, with the increase of power, the dissolution of plant active ingredients is gradually increased, and the corresponding bacteriostasis effect is gradually strengthened. When the ultrasonic power is 1000 W, the diameter of the bacteriostasis circle is 25.30 mm, which is increased by 73.76% compared to 300 W. When the ultrasonic power is greater than 600 W, the dissolution of the effective component increases slightly, but it is not obvious, but slightly decreased at 1500 W. It may be that the higher ultrasonic power breaks the activity of the active component (Prakash et al., 2018). Therefore, considering the influence of experimental efficiency and experimental intensity, the ultrasonic power of 1000 W is more suitable.

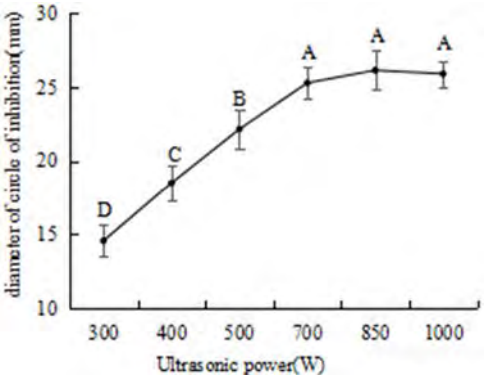

Figure 6. Effect of ultrasonic power on antimicrobial activity.

The stability of inhibitory active components

1. Effect of light on inhibitory active components of extracts.

The effective activity of the extract is determined by its own properties and is influenced by the external environment to a certain extent. The effect of the light irradiation time on the inhibitory activity of the effective substances is shown in Figure 7. On the whole, the effect of UV irradiation on the active component activity is greater than

that of sunlight, which may be because ultraviolet light can destroy the structure of effective substance and affect its active (Kumarasamy et al., 2018). With the prolongation of light duration, the activity of active substances decreased gradually under two conditions, but the decrease of UV irradiation was more obvious. After UV irradiation for 70 h, the diameter of the inhibitory ring is only 18.91 mm, and the inhibition zone diameter is reduced by 28.06% compared to 5 h. Sunlight irradiation also affected the activity of extracts, but the effect was not significant. Therefore, when storing the extract, try to keep it away from the light and keep away from ultraviolet light.

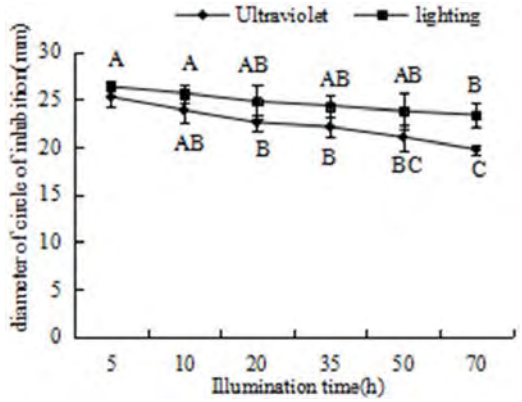

Figure 7. Effect of light time on the activity of extracts.

2. Effect of temperature on inhibitory components of extracts.

The storage temperature of the extract is also one of the main factors affecting its activity. The effect of different storage temperatures on the bacteriostatic effect of the effective ingredients is shown in Figure 8. The bacteriostasis effect of extract on *Staphylococcus* was determined at -20, 4 and 25°C for 1 day. It was found that the bacteriostatic effect was not significant under three conditions, and all of them had high bacteriostatic activity. After treating 2 h in a water bath at 50, 80 and 100°C, the activity of the extract decreased, and the activity of the extract decreased gradually with the increase of temperature. After 2 h at 100°C, the diameter of the bacteriostatic circle was 21.81 mm relative to 25°C for 1 day, and the diameter of the inhibition ring was reduced by 19.45%. Therefore, low temperature had little effect on the activity of extract, and the effect of high temperature was greater. It was more suitable to refrigerate at -20 and 4°C.

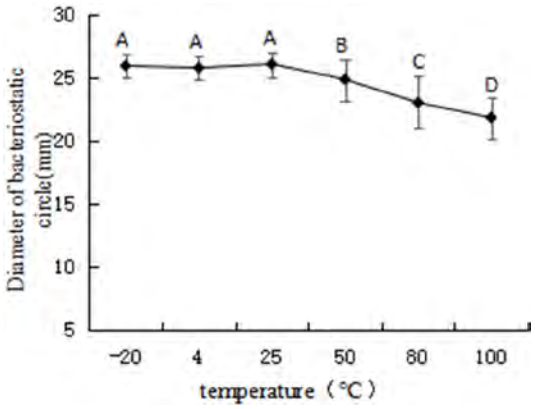

Figure 8. Effect of temperature on the activity of extracts.

3. Effect of pH on inhibitory properties of extract.

The pH of the extract has a certain effect on its activity. The effect of different pH the antibacterial activity of the extract is shown in Figure 9. When the extract is acidic, its bacteriostatic effect is good, and the pH value has a strong bacteriostatic effect between 3-6. When the extract was neutral and alkaline, its bacteriostatic effect gradually decreased, and with the enhancement of alkalinity, the bacteriostatic effect decreased obviously. At pH=12, the diameter of the bacteriostasis circle was only 14.51 mm and the bacteriostatic effect under other conditions had significant difference ($P<0.01$), indicating that under the condition of acid or weak acid, the extract had high activity and had strong bacteriostatic effect.

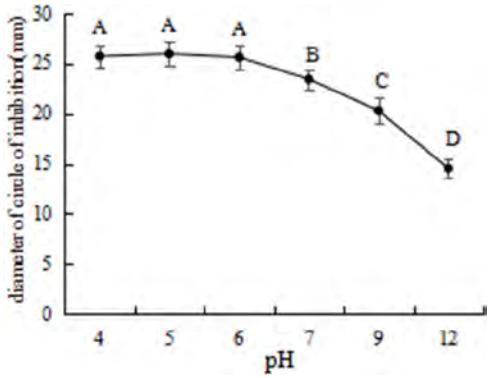

Figure 9. Effect of pH on the activity of extracts.

4. Effect of extraction time on its antibacterial activity.

The storage time had a certain effect on the activity of the extract, when stored at 4 and 25°C, respectively. The effect of storage for 120 d on its bacteriostasis is shown in Figure 10. At two temperatures, with the prolongation of storage period, the bacteriostasis effect of the extract decreased to a certain extent, but the activity of the extract had little influence on the storage conditions at 4°C. The diameter of the bacteriostasis circle could reach 22.94 mm when the extract was stored for 120 d, indicating that the low temperature storage at 4°C could effectively prolong the activity of the extract. Under 25°C storage conditions, the activity of the extract was greatly affected. After 60 d storage, the activity decreased obviously. At 120 d, the diameter of the bacteriostasis circle was only 20.25 mm, which was 22.47% lower than that of the storage 20 d. Therefore, the storage period at room temperature is preferably less than 40 d, and the storage period at 4°C is preferably less than 100 d.

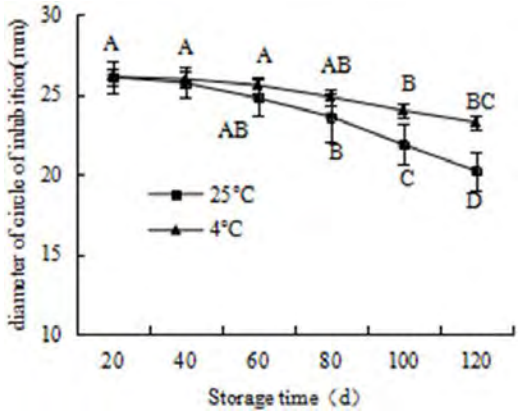

Figure 10. Effect of storage time on the activity of extracts.

5. Effect of different cations on antimicrobial activity of extracts.

Different metal ions have a certain effect on the activity of extracts. The inhibitory effect of different metal ions on grape cavity bacteria under different concentrations is shown in Figure 11. On the whole, the inhibitory effect on the inhibitory effect of the extract was $Ag^{+} > Cu^{2+} > Fe^{3+} > Mg^{2+} > Na^{+}$. When the concentration of metal ions was less than 10 mmol L^{-1}, the effect on the extract was small. When the ion concentration was greater than 30 mmol L^{-1}, Ag^{+}, Cu^{2+} and Fe^{3+} had a great influence on the extract, and the ability to inhibit bacteria began to decrease obviously when the concentration was Ag^{+}. When reaching 90 mmol L^{-1}, the inhibition zone diameter is only 19.67 mm. Mg^{2+} and Na^{+} had almost no effect on the activity of extracts at low concentrations, and had a slight effect at more than 70 mmol L^{-1}. Therefore, it is known that heavy metals with high oxygen ability have great influence on the activity of the extract, which may be because the strong oxidizing ability destroys the structure of the active substance, or combines the complex with the extract, thus reducing the caused by its activity (Chu et al., 2014).

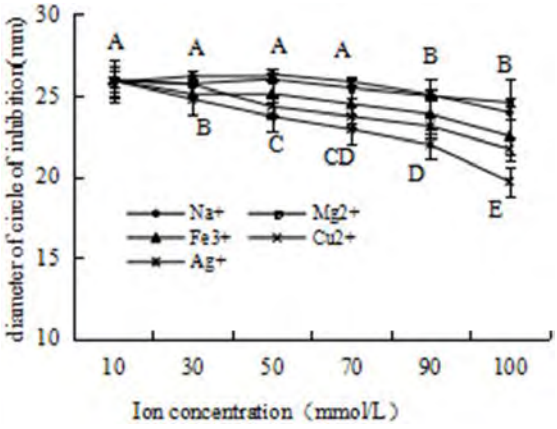

Figure 11. Effect of metal ion on the activity of extracts.

DISCUSSION

(1) The extract of *Scutellaria baicalensis* Georgi has strong inhibitory effects on *Botryosphaeria*. Mid-polarity solvents such as 80% ethanol, acetone, and ethyl acetate are used as extraction solvents for effective components of medicinal materials. The antimicrobial effect of the extract is better. The ratio of material to liquid at 1:20 (g:mL), 2 h ultrasonic extraction time, at a temperature of 70°C, under the condition of 1000 W ultrasonic power. The extract has strong inhibitory effects. It could be due to the relatively high activity and relatively high content of active ingredients (Bacon et al., 2017). Suitable polarity solvents will allow rapid and efficient dissolving of functional ingredients, assuming that appropriate extraction temperatures and ultrasonic power do not affect the solution of the effective components, and that the activity of the extract is not destroyed.

(2) Stability test shows that the external environment has certain effects on the activity of *Scutellaria baicalensis* extracts. Exposure to light and UV irradiation for more than 20 h will have a certain effect on the activity of the extract. The extract has a great influence on its activity in alkaline environment. It may be that the alkaline environment destroys the structure of the extract, thereby affecting its activity. Similarly, Ag^{+}, Fe^{3+} and Cu^{2+} metal ions have great influence on the activity of extracts, On the one hand, it may be due to strong oxidation that destroys the structure of the extract, on the one hand, the production of complexes with extracts decreases their activity. The extract was stored at below 25°C for 40 days, with little effect on inhibition (Wang al., 2018; Kitzler et al., 1986).

CONCLUSIONS

Scutellaria baicalensis extract is a new plant-derived preservative for kiwifruit fruit, highly efficient, green and not harmful to the environment. It has certain practical significance. But the extract of *Scutellaria* is a crude extract, it is not possible to determine the active principle. In future, it is necessary to further purify the extract, determine the structure, and study the mechanism of inhibition.

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Determination and analysis of aroma substances in four different kiwifruit cultivars by GC-MS

Jiannan Chen[1], Zhande Liu[1,a], Haohao He[2] and Zhiyao Ma[1]

[1]Horticultural College, Northwest A&F University, Yangling, Shaanxi Province, China; [2]Fruit Technology Promotion Service Center, Meixian, Shaanxi Province, China.

Abstract

The aroma substances of four different kiwifruit cultivars were studied, and the principal components and the similarities and differences of aroma substances among these four cultivars were explored. The aroma substances of the kiwifruit cultivars were determined by GS-MS and the quantitative analysis of aroma substances was carried out by the internal standard method. At the same time, principal component analysis was used to analyze the similarity between cultivars and the main aroma substances contributing to the differences between cultivars. The results showed that 83 aroma substances were detected, 33, 39, 31 and 34 aroma substances were detected respectively in 'Qihong', 'Xuxiang', 'Hayward' and Zespri® SunGold ('Zesy002'). The kiwifruit cultivars has similar main aroma substances, such as alcohols, aldehydes, ketones and phenols. Together, they contribute fragrance to kiwifruit. The acids and esters are the different aroma substances of the four cultivars, which endow odour and sweet aroma and also enrich the flavor of kiwifruit. 'Qihong' has an aroma of a mixture of delicate fragrance and sweetness; 'Xuxiang' and 'Hayward' are similar in aroma, a mixture of delicate fragrance and fruit odour, and SunGold has an aroma of delicate fragrance.

Keywords: GS-MS, principal component analysis, kiwifruit

CONCLUSION

Classification and comparison of the aroma substances in four different kiwifruit cultivars

33 aroma substances were detected in 'Qihong', including 5 alcohols, 4 aldehydes, 4 ketones, 2 phenols, 6 acids, 5 esters and 7 other aroma substances.

39 aroma substances were detected in 'Xuxiang', including 8 alcohols, 4 aldehydes, 2 ketones, 4 phenols, 4 acids, 8 esters and 9 other aroma substances.

31 aroma substances were detected in 'Hayward', including 4 alcohols, 6 aldehydes, 1 ketone, 2 phenols, 3 acids, 10 esters and 5 f other aroma substances.

34 aroma substances were detected in Zespri® Sungold ('Zesy002'), including 3 alcohols, 6 aldehydes, 3 ketones, 2 phenols, 3 acids, 10 esters and 7 other aroma substances. Under the same conditions, there were some differences in aroma substances among different kiwifruit cultivars.

The contents of alcohols, aldehydes and phenols in the four cultivars were higher than 1100 μg L^{-1}, and the contents of alcohols in 'Xuxiang' and 'Hayward' were similar and higher than those of other cultivars (Table 1). The contents of ketones and other aroma substances in the four cultivars were lower than 1100 μg L^{-1}, the content of acids in 'Qihong' was 7 times as much as that in 'Xuxiang', and almost 4 times as much as that in Sungold. The content of esters was the highest in 'Xuxiang' and 'Hayward'. Aldehydes, phenols and acids are the main aroma substances in 'Qihong', and aldehydes and phenols are the main aroma substances in SunGold and 'Hayward', while in 'Xuxiang', the contents of alcohols, aldehydes, phenols and

[a]E-mail: dezhanliu@vip.sina.com

esters are similar. The uniform distribution of aroma substances may contribute to the rich flavor of 'Xuxiang'.

Table 1. Contents of aroma substances in four kiwifruit cultivars.

Aroma substances	Contents (μg L^{-1})			
	'Qihong'	'Xuxiang'	'Hayward'	SunGold
Alcohols	1119.62	2661.14	2706.71	1829.51
Aldehydes	2709.47	2619.24	7738.52	5818.86
Ketones	764.03	533.69	602.51	1038.84
Phenols	3948.34	2509.82	4173.80	4727.15
Acids	4038.74	561.62	621.34	1096.11
Esters	384.02	1577.24	1994.36	1021.64
Others	516.57	235.08	173.90	884.32
Total	13480.80	10697.83	18011.13	16416.43

Common aroma substance in the four kiwifruit cultivars

There are 13 common aroma substances in the four kiwifruit cultivars, which includes 2 alcohols: 1-hexanol (227.03-568.51 μg L^{-1}) and 2-hexen-1-ol (548.13-1905.46 μg L^{-1}); 4 aldehydes: caproaldehyde (117.54-998.35 μg L^{-1}), 2,4-dimethyl-benzaldehyde (892.24-2502.52 μg L^{-1}), 4-methoxysalicylaldehyde (12.68-15.61 μg L^{-1}), and trans-2-hexenal (143.13-4722.97 μg L^{-1}); 1 ketone: 2-hydroxy-2-methylpropiophenone (521.767-689.98 μg L^{-1}); 2 phenols: phenol (12.67-18.39 μg L^{-1}), and 2,4-di-tert-butylphenol (2463.91-4708.76 μg L^{-1}); 1 acid: palmitic acid (456.30-2857.99 μg L^{-1}); 2 esters: methyl hexadecanoate (85.64-177.98 μg L^{-1}) and methyl stearate (49.79-107.49 μg L^{-1}); 1 other aroma substance: methyl sulfone (75.53-257.63 μg L^{-1}). In summary, there are common aroma substances in the four kiwifruit cultivars, such as alcohols, aldehydes, ketones, phenols, acids, esters and other aroma substances, but their contents are different.

Principal component analysis of aroma substances

For the first two principal components of the four kiwifruit cultivars, the first principal component value was taken as the horizontal coordinate and the second principal component value was taken as the vertical coordinate. (Figure 1). It can be seen from the graph that the four cultivars are concentrated on the positive axis of the first principal component. The distance between 'Xuxiang' and 'Hayward' is small, indicating that the aroma substances of the two cultivars are similar, but the difference between the other cultivars is greater.

The scatter plot (Figure 2) takes the first principal component value as the X-coordinate and the second principal component value as the vertical coordinate. Combined with Figures 3 and 1, it shows that the aroma substances affecting 'Qihong' mainly concentrate on the positive half axis of the second principal component and the negative half axis of the first principal component. According to the influence, the order is methyl3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (53), 1-hexanol(1), trans-2-hexenol (3), and butyl formate (47). The aroma substances affecting SunGold are mainly concentrated in the positive half axis of the first principal component, according to the influence, the order is 2-hydroxy-2-methylpropiophenone (21), methyl stearate (50), and methyl palmitate (49). The aroma substances affecting 'Xuxiang' and 'Hayward' are mainly concentrated in the negative semi axis of the main components. According to the influence, the order is 2-propyl-1-heptanol (6), isobutyric anhydride (40), 2-methyl-2-butanol (10), ethyl ethynyl carbinol (11), linoleic acid (41), palmitoleic acid (42) and palmitic acid (34).

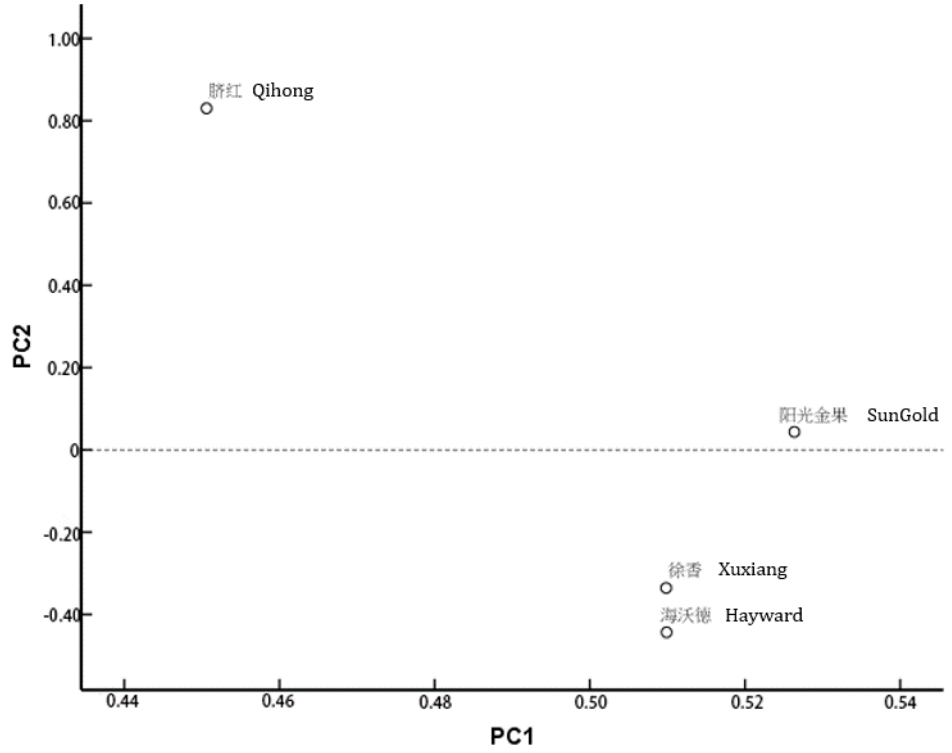

Figure 1. Scatter plot of the principal component in the 4 kiwifruit cultivars.

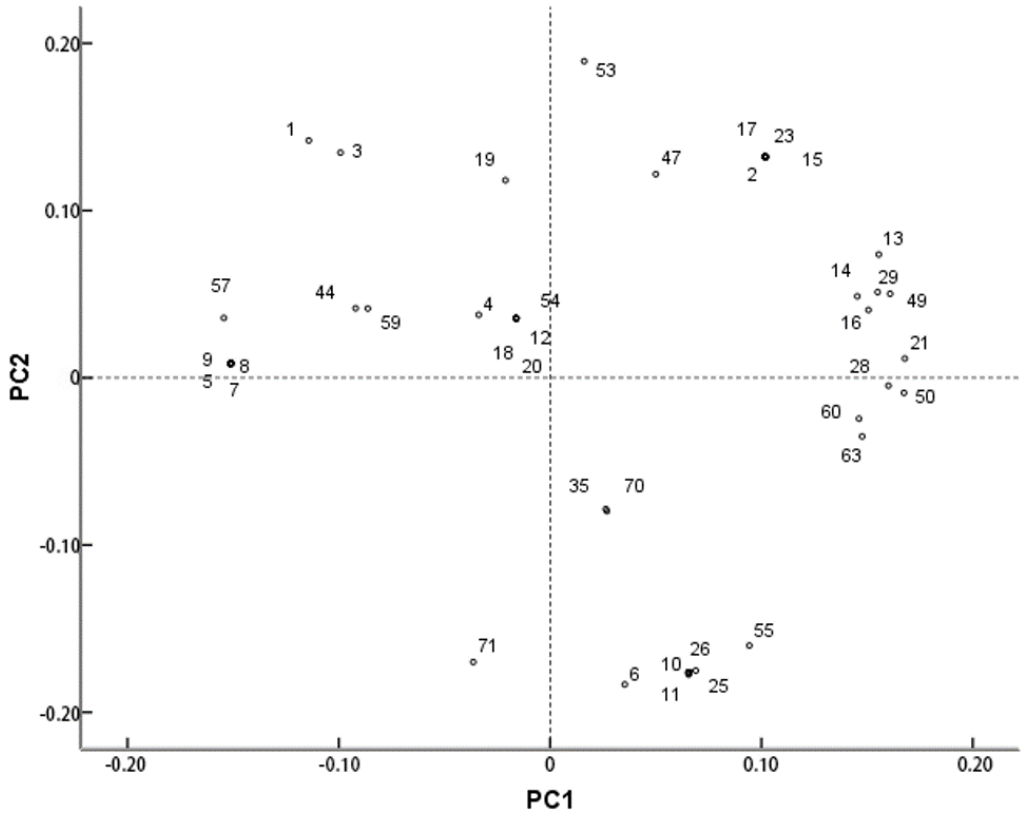

Figure 2. Scatter plot of the main aroma substances.

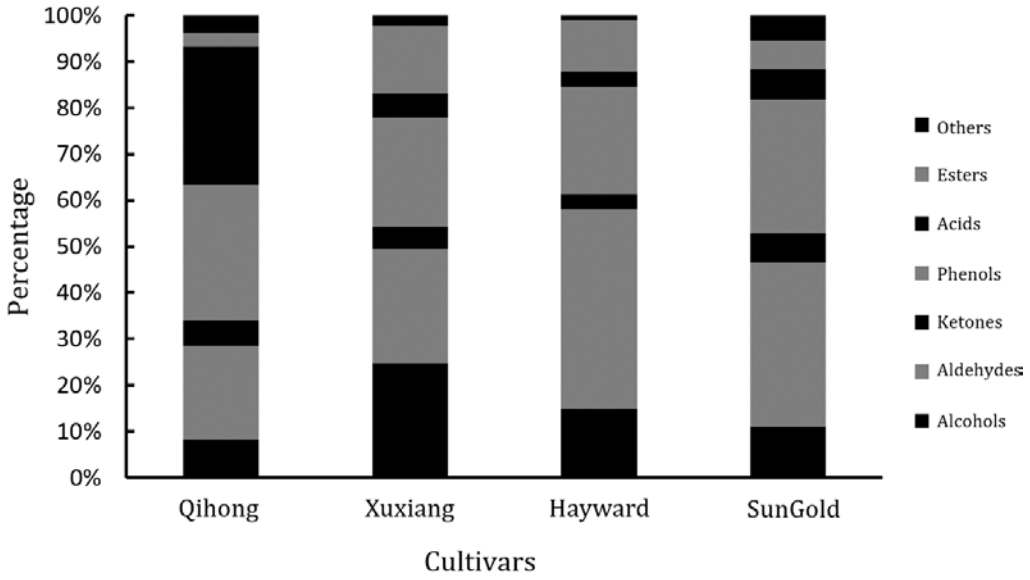

Figure 3. Percentage of aroma substance of the four kiwifruit cultivars.

Aroma phenotype of the four kiwifruit cultivars

The main aroma substances of acids and esters are palmitic acid, methyl palmitate, methyl stearate and the total content of the sweet aroma is 3105.57 μg L^{-1}, so the aroma of 'Qihong' can be described as a mixture of delicate fragrance and sweetness. The content of fruity esters in 'Xuxiang' is 1441.81 μg L^{-1}, so it has a mixture of delicate fragrance and fruity odour; the content of fruity esters in 'Hayward' is 1765.53 μg L^{-1}, it is also a mixture of delicate fragrance and fruity odour; the content of the sweet and fruity odour in SunGold is relatively low. Therefore, the aroma of this cultivar is delicately fragrant.

Morphological observation of fruits and seeds and nutrient analysis of five wild kiwifruits in Yunnan Province

Yujie Ma[1,a], Wei Chen[2], Shiyu Wang[1,b], Yingyun He[2], Zhongrong Hu[2], Yao Chen[2] and Kunming Li[2,c]

[1]Horticultural Academy of Yunnan Agricultural University, Kunming, Yunna, China; [2]Horticulture Crop Research Institute of Yunnan Academy of Agricultural Sciences, Kunming, Yunna, China.

Abstract

The fruit and seed morphology of five wild kiwifruits, i.e., *Actinidia chinensis*, *A. deliciosa*, *A. fulvicoma*, *A. fulvicoma* var. *lanata* and *A. eriantha,* were observed in this paper and the nutrients of the first four of these wild kiwifruits were analyzed and evaluated. The results showed that fruits of *A. chinensis* and *A. deliciosa* were large, with few hairs, good shape and beautiful appearance and their seeds were big and full. The fruit surface of *A. fulvicoma* was smooth and its fruit shape was long. The fruit surface of *A. fulvicoma* var. *lanata* and *A. eriantha* had dense hairs. The soluble solids, total sugar and total acid content of *A. deliciosa* were highest while the vitamin C (Vc) content of *A. fulvicoma* var. *lanata* was highest. The evaluation of comprehensive nutritional quality from best to poorest was *A. deliciosa* > *A. fulvicoma* var. *lanata* > *A. fulvicoma* > *A. chinensis*.

Keywords: kiwifruit, morphology, nutrients, evaluation of comprehensive nutritional quality

INTRODUCTION

Kiwifruit are deciduous vines of Actinidicaceae, dioecious perennial vines which carry fruit defined as berries. It is one of the most successful wild fruit plants to have been domesticated and cultivated in the 20th century (Warrington and Weston, 1990). There are 66 species in the genus *Actinidia*; except for *A. strigosa* Hook. & Thoms., *A. petelotii* Diels, *A. rufa* Planch. ex Miq. and *A. hypoleuca* Nakai, all 62 species are found in China, and the majority are restricted to China. China is the native center of kiwifruit resources (Cui, 1993; Huang et al., 2000; Jiang et al., 2004; Hopping, 1976). There is an abundant genetic diversity in the genus *Actinidia*, and there is great variability in morphological characters, nutrient contents, gender and chromosome ploidy (Huang et al., 2000). Therefore, exploiting and utilizing the wild resources of kiwifruit has a unique advantage in China.

Kiwifruit, containing a high amount of vitamin C, polysaccharide and many kinds of amino acids, mineral elements, etc. (Tang and Zhang, 1997; Wang et al., 2005; Kang and Zhang, 2008; Lu et al., 2005), is one of the important fruit resources all over the world in recent years. In view of the important value of kiwifruit, researchers have made many analyses of fruit quality, mainly focusing on the effects of different factors on fruit quality, such as harvest time (Tang et al., 2012), the NPK ratio (Jin et al., 2011), fruit parts (Jin et al., 2015), fertilizer practices (Chen et al., 2014) and production areas (Song et al., 2012). *Actinidia* species have been compared: Li et al. (1995) measured and studied the nutritional composition of fruits for 35 species of the genus *Actinidia*. Zhang et al. (2010) analyzed and compared nutrients of fruits of three kinds of wild kiwifruit, namely *A. polygama*, *A. kolomikta* and *A. arguta* in the Changbai mountains.

[a]E-mail first author: 1157739177@qq.com
[b]E-mail corresponding author: wsygfg@aliyun.com
[c]E-mail corresponding author: 15925209220@163.com

Yunnan is abundant in wild kiwifruit resources, with 31 species, 23 cultivars and 2 formae (Hu et al., 2003). To preliminarily grasp the morphology of fruits and seeds and the nutritional quality of wild kiwifruit in Yunnan area, the morphology of fruits and seeds of five wild kiwifruits in Yunnan, *A. chinensis*, *A. deliciosa*, *A. fulvicoma*, *A. fulvicoma* var. *lanata* and *A. eriantha*, were observed and measured, the nutrient composition of the first four of these wild kiwifruits were analyzed and evaluated in this paper. It will provide the theoretical basis for preservation, evaluation, exploitation and utilization of wild kiwifruit resources, and then provide reference for cultivation and breeding of kiwifruit in Yunnan.

MATERIALS AND METHODS

Materials

The experimental materials used in this paper were collected from the National Wild Fruit and Rootstock Germplasms Repository in Yunnan province. The ripe fruits of five wild kiwifruit were collected for observation and nutrient analysis.

Observation and measurement of the morphology of fruits and seeds

According to "Descriptors and Data Standard for Kiwifruit (*Actinidia* spp.)" (Hu et al., 2006), the morphology of fruits and seeds, including fruit shape, sepal persistence, color of fruit skin, form of fruit lenticels, shape of fruit-shoulder, shape of fruit apex, shape of fruit depression apex, form of fruit hair, flesh color, form of fruit core, transverse section shape of fruit core, seed shape, seed color, fruit weight, fruit vertical length, fruit greater equatorial diameter, fruit lesser equatorial diameter, fruit shape index and 1000-seed weight were observed and measured. Twenty fruit of each of the wild kiwifruit were analyzed. The fruit shape index was calculated.

$$\text{Fruit shape index} = \text{Fruit length}/\text{fruit greater equatorial diameter}$$

Analysis of nutrients

Ripe fruits, each kind of kiwifruit not less than 1 kg, were randomly selected as the test samples. The content of soluble solids, vitamin C (Vc), total sugar and total acid were measured by the Quality of Agricultural Products Supervising and Testing Center of Ministry of Agriculture (Kunming). The sugar:acid ratio was calculated.

$$\text{Sugar: acid ratio} = \text{Total sugar content} / \text{Total acid content}$$

Statistical analysis

Excel 2010 software was applied for statistical treatment. The algorithm of Duncan of SPSS19.0 was applied to analysis significance of differences in interspecific data.

RESULTS AND ANALYSIS

The morphology of fruits and seeds of five wild kiwifruit

As shown in Table 1, fruit of *A. chinensis* were short round and some were short cylindrical. The fruit did not have persistent sepals. Most fruit had rounded shoulders but some individuals had square shoulders. Most of the fruit apices (stylar end of the fruit) were concave, a few relatively flat, very few convex. The shape fruit depression apex was convex, but the extent differed. The fruit skin was light green or brown, with hair of varying abundance and obvious raised brown fruit spots. The flesh was green or yellow-green. The fruit core is white, middle size. Transverse section shapes of fruit core were oval, a few were round. Seeds were nearly round, oval, and oblong, with colored brown or coffee.

Fruit of *A. deliciosa* were short cylindrical, trapezoid, and obovoid with persistent sepals, a few did not have sepals. Fruit shoulder was round. The shape of the fruit apex was similar to those of *A. chinensis*. The shape fruit depression apex was deep-dull-convex.

The skin was brown or dark brown, having relatively sparse hard hairs and obvious raised brown spots. Flesh was yellowish green. The fruit core was big, white or yellowish white. Transverse section shapes were oval or oblong. Seeds were nearly round or oval, brown in color.

Table 1. The morphology of fruits and seeds of five wild kiwifruit.

The morphology of fruits and seeds	*A. chinensis*	*A. deliciosa*	*A. fulvicoma*	*A. fulvicoma* **var.** *lanata*	*A. eriantha*
Fruit shape	Short round short cylindrical	Short cylindrical, trapezoid, obovoid	Long cylindrical, oblong	Short round, long cylindrical	Short round, short three prism
Sepal persistence	None	Present or none	Present	Present	Present
Color of fruit spots	Light green, brown	Brown, dark brown	Green, light green	—	—
Form of fruit spots	Typically, brown, convex	Typically, brown, convex	Typically, small, brown, flat	—	—
Shape of fruit-shoulder	Round, square	Round	Round	Round	Round
Shape of fruit apex (Stylar end)	Concave, flat, convex	Concave, flat, convex	Flat, convex	Concave, flat	Concave
Shape of fruit depression apex	Convex	Deep, dull, convex	Shallow, sharp, convex	Deep, dull, convex	Shallow, dull, convex
Form of fruit hair	Present, variable density	Sparse, hard hair	None	Dense, short hair	Dense, milky, long hair
Flesh color	Green, yellow-green	Yellow-green	Green	Dark green	Dark green
Form of fruit core	Medium, white	Large, white, white yellow	Medium, white green	Medium, white yellow	Large, white yellow
Transverse section shape of fruit core	Oval, round	Oval, oblong	Round, oval	Oval	Oval, triangle
Seed shape	Nearly-round, oval, oblong	Nearly-round, oval	Oval	Oval	Oval, Oblong
Seed color	Coffee color, Light brown	Brown	Dark brown	Dark brown	Coffee color

Fruit of *A. fulvicoma* were long cylindrical or oblong, lacking sepals. Fruit shoulder was round. Shapes of fruit apex were flat or convex. The shape fruit depression apex was shallow-sharp-convex. The skin was green or light green, smooth with obvious, small, flat, brown spots. Flesh was green. The fruit core was whitish green, middle size. Transverse section shape was round or oval. Seeds were oval, brown.

A. fulvicoma var. *lanata* is a variety of *A. fulvicoma*. The fruits were short round or long cylindrical with persistent sepals. The fruit shoulder was round. The shape of fruit apex was concave or flat. The shape fruit depression apex was deep-dull-convex. The fruit skin had dense short hairs. The flesh was dark green. The fruit core was yellowish white, middle size. Transverse section shape was oval. Seeds were oval, dark brown.

Fruit of *A. eriantha* were short round, individually were short three prism with persistent sepals. The fruit shoulder was round. The shape of fruit apex was concave or flat. The shape fruit depression apex was deep-dull-convex. The fruit skin had thick white long hair. The flesh was dark green. The fruit core was big, yellowish white. Transverse section shapes were oval or triangular. Seeds were oval or oblong, coffee colored.

Table 2 shows that the fruit of *A. chinensis* were the heaviest, 30.74 g, and the second was *A. deliciosa*, 27.68 g. They were significantly heavier than fruit of the other taxa which were not significantly different. The smallest was *A. fulvicoma* var. *lanata* at 5.09 g. The vertical diameter (length) of *A. deliciosa* was the largest, 3.98 cm, the second was *A. chinensis* at 3.91 cm, and *A. eriantha* was the smallest at 2.12 cm. Variance analysis showed that the vertical diameters of *A. deliciosa* and *A. chinensis* were very significantly higher than others, and there were significant differences between *A. eriantha* and *A. fulvicoma*.

The greater fruit equatorial diameter of *A. chinensis* was the largest, 3.76 cm, the second was *A. deliciosa*, 3.71 cm, and the smallest was *A. fulvicoma var. lanata*, 2.10 cm. The lesser fruit equatorial diameter of *A. chinensis* was the largest, 3.58 cm, the second was *A. deliciosa*, 3.42 cm, and the smallest was *A. fulvicoma var. lanata*, 2.03 cm. The result of variance analysis of fruit vertical diameter and equatorial diameter were consistent with the fruit weights. The fruit shape index of *A. fulvicoma* was the largest, 1.33, that of *A. eriantha*'s was the smallest, 0.87, and those of *A. deliciosa* and *A. chinensis* were close to 1, at 1.04 and 1.07, respectively. The 1000-seed weight of *A. deliciosa* was the heaviest, 1.60 g, then *A. chinensis* at 1.33 g. Seed of *A. fulvicoma* var. *lanata* were the lightest at 0.32 g. The result of variance analysis of the 1000-seed weight was consistent with fruit weights. The results showed that changes of fruit weight, fruit equatorial diameters of the five kiwifruits were consistent. The result of variance analysis too. *A. deliciosa* fruit weight was less than that of *A. chinensis*, the vertical diameter and 1000-seed weight were larger than *A. chinensis*, but the difference was not significant. The morphology of fruits and seeds of *A. fulvicoma* were better than those of *A. fulvicoma* var. *lanata*, and the differences were not significant, except for the fruit shape index.

Table 2. The morphology of fruits and seeds of five wild kiwifruit.

The morphology of fruits and seeds	*A. chinensis*	*A. deliciosa*	*A. fulvicoma*	*A. fulvicoma* **var.** *lanata*	*A. eriantha*
Fruit weight (g)	30.74±8.29bB	27.68±7.89bB	8.70±1.82aA	5.09±0.33aA	7.42±0.53aA
Fruit vertical diameter (cm)	3.91±0.41cC	3.98±0.71cC	2.99±0.26bAB	2.50±0.18abA	2.12±0.20aA
Fruit geater equatorial diameter	3.76±0.28bB	3.71±0.38bB	2.24±0.14aA	2.10±0.10aA	2.47±0.18aA
Fruit lesser equatorial diameter (cm)	3.58±0.25bB	3.42±0.36bB	2.17±0.14aA	2.03±0.19aA	2.28±0.14aA
Fruit shape index	1.04±0.05bB	1.07±0.15bB	1.33±0.04dC	1.19±0.08cBC	0.87±0.06aA
1000-seed weight (g)	1.33±0.27bB	1.60±0.20bB	0.59±0.13aA	0.32±0.02aA	0.56±0.05aA

Note: The different lowercase letters are significantly different at P<0.05 and different capital letters are very significantly different at P<0.01.

Analysis of content of nutrients of four wild kiwifruit

As shown in Table 3, the *A. chinensis* soluble solids content (SSC) was 11.92%, the vitamin C (Vc) content was 112.43 mg 100 g^{-1}, the total sugar and total acid content were lowest, respectively 6.93% and 2.56%, and the sugar: acid ratio was 2.5. The *A. deliciosa* SSC, total sugar and total acid content were highest, respectively 14.88%, 7.97% and 3.04%, but the sugar: acid ratio was the lowest, 2.23, Vc content was close to that of *A. chinensis*, 111.68 mg 100 g^{-1}. The *A. fulvicoma* SSC was 12.25%, Vc content was the lowest, only 13.3 mg 100 g^{-1}, the total sugar and total acid contents were respectively 7.38% and 2.7%, the sugar: acid ratio was the highest, 2.73. The *A. fulvicoma* var. *lanata* SSC was the lowest, 11.44%, the content of Vc was the highest, reaching to 426.7 mg 100 g^{-1}, which was almost 4 times that in *A. chinensis* and *A. deliciosa*, and 32 times that in *A. fulvicoma*, the content of total sugar was less than in *A. fulvicoma*, 7.1%, the content of total acid was 3.04%, more than in *A. fulvicoma*, the sugar: acid ratio was 2.34.

Table 3. The content of nutrients of four wild kiwifruit.

Species	Soluble solids content (%)	Vc (mg 100 g^{-1})	Total sugar (%)	Total acid (%)	Sugar:acid ratio
A. chinensis	11.92	112.43	6.39	2.56	2.50
A. deliciosa	14.88	111.68	7.97	3.58	2.23
A. fulvicoma	12.25	13.30	7.38	2.70	2.73
A. fulvicoma var. *lanata*	11.44	426.70	7.10	3.04	2.34

Evaluation of nutritional quality of four wild kiwifruits

According to the method of comprehensive index of quality (Yuan et al., 2016; Li et al., 2009; Bai and Zhu, 2008), the content of nutrients was equidistantly divided into four levels, with the average value as the benchmark. Hierarchical distance = (maximum minimum) / levels. The grading standards are shown in Table 4. Summing the indexes of all nutritional qualities gave the comprehensive index. The larger the comprehensive index, the better the fruit nutritional quality. The results are shown in Table 5.

Table 4. The graded standard of nutritional quality of four wild kiwifruits.

Level	Soluble solids content (%)	Vc content (mg 100 g^{-1})	Total sugar (%)	Total acid (%)
1	X < 11.76	X < 62.68	X < 6.82	X < 2.72
2	11.76 ≤ X < 12.62	62.68 ≤ X < 166.03	6.82 ≤ X < 7.21	2.72 ≤ X < 2.97
3	12.62 ≤ X < 13.48	166.03 ≤ X < 269.38	7.21 ≤ X < 7.61	2.97 ≤ X < 3.23
4	13.48 ≤ X	269.38 ≤ X	7.61 ≤ X	3.23 ≤ X

Note: X is the measured content of the nutrients.

Table 5. The evaluation of nutritional quality of four wild kiwifruit.

Nutritional quality	*A. chinensis*	*A. deliciosa*	*A. fulvicoma*	*A. fulvicoma* **var.** *lanata*
Soluble solids content (%)	2	4	2	1
Vc content (mg 100 g^{-1})	2	2	1	4
Total sugar (%)	1	4	3	2
Total acid (%)	1	4	1	3
Comprehensive evaluation index	6	14	7	10

As shown in Tables 4 and 5, in the four evaluated kiwifruits, *A. chinensis* levels of SSC and vitamin C were 2, levels of total sugar and total acid were 1, and the comprehensive index was the lowest, 6. *A. deliciosa* levels of nutritional qualities were 4, except Vc content's was 2, and the comprehensive index was the highest, 14. *A. fulvicoma* level of total sugar content was 3, the SSC was 2, Vc and total acids were 1, and the comprehensive index was 7. *A. fulvicoma* var. *lanata* level of Vc content was 4, the total acid content was 3, the total sugar content was 2, the SSC was 1, and the comprehensive index was 10. The result shows that the comprehensive nutritional quality of four kiwifruits from highest to lowest was *A. deliciosa* > *A. fulvicoma var. lanata* > *A. fulvicoma* > *A. chinensis.*

DISCUSSION

Vitamin C is a necessary organic compound to maintain a healthy body, it can't synthesized and stored in the human body, and needs to be ingested regularly. The content of vitamin C in fruits and vegetables is rich, which is the main source of people's daily intake of vitamin C, but vitamin C content varies with fruits and vegetables, among which the content of vitamin C in kiwifruits is higher than other species. The vitamin C content of *A. fulvicoma* measured in this paper was lower than the range of 30-148 mg 100 g^{-1}, reported by Huang et al. (2000). *A. chinensis* Vc content was higher than the main kiwifruit cultivars 'E Mihoutao 2', 'E Mihoutao 3', 'Cuiyu' and 'Ganmi 5', reported by Gong et al. (2005). The vitamin C content of *A. deliciosa* was much higher than the New Zealand cultivars 'Gracie' and 'Hayward' (Gong et al., 2005). The vitamin C content of *A. fulvicoma* var. *lanata* was up to 426.7 mg 100 g^{-1}, which was nearly four times as much as in *A. chinensis* and *A. deliciosa*, and 32 times that in *A. fulvicoma*. Thus the advantage of high Vc content can be used to select and breed new cultivars by hybridizing with other kiwifruits.

The sugar: acid ratio is an important indicator of the fruit flavor. It can make the fruit have moderate sweet and sour only in a certain range, but the optimum sugar: acid ratio is different for every fruit. Li et al. (1995) found that the optimal sugar: acid ratio of

kiwifruits was 5:7. The sugar: acid ratio in this paper were all less than the optimal sugar: acid ratios. After analysis we found that, although its total sugar contents were higher than that of the 35 kiwifruits measured by Li et al. (1995), the total acid contents were excessive. The total acid content of *A. chinensis* that was the lowest in this paper, was higher than *A. callosa* var. *henry* Maxim. which was the highest measured by Li et al. (1995). The highest content of total acid was in *A. deliciosa*, reaching 3.58%. The wild kiwifruits were so sour and astringent that they were hard to eat. Therefore, to develop these wild kiwifruits into fresh and palatable cultivars, the primary solution is to reduce the acid content of fruit.

The contents of vitamin C and total sugar in the paper were consistent with the relationship of nutrient content and the color of flesh (Li et al., 1995). About the difference of nutrients in species, Zhang et al. (2010) believed that differences of individuals that are offspring of seed propagation of the wild kiwifruit or differences of growth environments lead to the difference of the vitamin C content of fruit. Song et al. (2012) analyzed the content of polysaccharide in kiwifruits in different habitats, and also thought that different growth environments had some effects on the content of polysaccharide in kiwifruit. The results in this paper showed that *A. eriantha* fruit shapes have short three prism, it has a fruit shape index of less than 1, the transverse section shape of fruit core is triangular, its seeds are full, and has not deformed fruit. In addition, the morphology of kiwifruits in the paper were slightly different from what Huang et al. (2008) reported. Pang and Jiang (1995) believed that the genetic diversity of groups lead to morphological differences, and the pressure of ecological environment make phenotypic diversities of groups. Therefore, kiwifruits in different habitats may have morphological differences due to their different ecological environments.

There are no unified methods to evaluate the comprehensive quality of fruits and vegetables. Methods of evaluation of nutritional quality in kiwifruit are the method of principal component analysis, the method of quality comprehensive index evaluation, and the method of reasonableness-satisfaction, etc. The latter two methods are often used in the evaluation of quality of kiwifruits in different species or cultivars.

Except for *A. deliciosa*, when developing and utilizing the five kiwifruits, we can consider to use *A. fulvicoma* var. *lanata*, and can also use fine characteristics of each kiwifruits for development and utilization, such as: *A. chinensis* fruit were large and beautiful, and the skin surface of *A. fulvicoma* was smooth. The ecological environment in Yunnan is diverse; wild kiwifruits resources are abundant, the genetic variation is large; which is convenient for the breeding and development of new cultivars. So studies in regard to kiwifruit are yet to be further and extensively investigated.

CONCLUSION

The morphology of fruits and seeds of kiwifruits

In the five kiwifruits, the shape of fruits were mostly short round, short cylindrical or long cylindrical, and some *A. deliciosa* genotypes had trapezoid and obovoid fruits, *A. fulvicoma* had oblong fruits, and *A. eriantha* had short three prism. The fruit had persistent sepals, except for *A. chinensis* and a few genotypes of *A. fulvicoma*. The fruit skin was brown and green of different shades. Fruit of *A. chinensis*, *A. deliciosa* and *A. fulvicoma* had obvious white fruit spots, but the spots on *A. fulvicoma* fruit were smaller and flatter. The fruit shoulders were round, except for a few genotypes of *A. chinensis* which had square shoulders. The fruit apex are concave, flat and convex. The shape of fruit depression apex were convex to different degrees. The fruit surface of *A. fulvicoma* was smooth, *A. chinensis* and *A. deliciosa* had sparse hairs, *A. fulvicoma* var. *lanata* and *A. eriantha* had a dense covering of hairs. The fruit flesh was green. The fruit core was white and had a medium size, except in *A. deliciosa* and *A. eriantha*, which it was large. The transverse section shapes of fruit cavity are round, oval and oblong, *A. eriantha* has a triangle shape. Seeds have near round, oval and oblong shapes, their color is brown or

coffee. The fruit weight, vertical diameter, equatorial greater and lesser diameters, suture diameter and 1000-seed weight of *A. chinensis* and *A. deliciosa* were significantly larger than the other three species. The fruit shape index of *A. fulvicoma* was the biggest, those of *A. chinensis* and *A. deliciosa* were close to 1.

Content and evaluation of nutrients of kiwifruits

Among the four species of kiwifruits form which nutrients were analyzed, the soluble solids, total sugar and total acid content of *A. deliciosa* were the highest, and the evaluation of nutritional quality was the best. The total sugar and total acid content of *A. chinensis* were the lowest, and the evaluation was the worst, the other two nutrients neither had an outstanding performance in the four species. Fruit of *A. fulvicoma* had the lowest Vc. Fruit of *A. fulvicoma* var. *lanata* had the highest Vc. The evaluation of comprehensive nutritional quality from best to poorest was: *A. deliciosa* > *A. fulvicoma* var. *lanata* > *A. fulvicoma* > *A. chinensis*.

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Field experiment and design of kiwifruit harvesting robot

Longtao Mu[1,a], Yadong Liu[1,2], Yongjie Cui[1,b], Lipeng Chen[1], Haozhou Liu[1], Longsheng Fu[1] and Yoshinori Gejima[3]

[1]College of Mechanical and Electronic Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China; [2]China Aviation Lithium Battery Research Institute Co., Ltd, Changzhou, Jiangsu 213200, China; [3]Agronomy department, Miyazaki University, Miyazaki 8892192, Japan.

Abstract

In order to develop a robot to harvest kiwifruit in the orchard, this paper's aim is to study the picking robot to automatically undertake the fruit information recognition, positioning, picking order planning, movement to kiwifruit picking area, nondestructive picking by bionic fingers, and unloading of fruit to the basket. The kiwifruit harvesting robot can perceive information to recognize the kiwifruit, basing on chromatic aberration method and k-means method, and refers the calyx as the subject, obtain fruit coordinates by image processing, and Cartesian coordinates point using Kinect sensor. Then the manipulator moves to coordinate position and transports end-effector to the position of the fruit for nondestructive harvest. The harvesting robot of the prototype with sensors can detect the best position for grabbing, use the pressure sensors to guarantee a nondestructive picking within damage and adjust springs for fruit size and shape tolerance, can integrated grab and separate adjacent or clustered kiwifruits, the robot arm can arrive to kiwifruit and then pick and unload individual fruit without damage. The verification tests in the orchards showed that the prototype harvesting robot can separate adjacent or clustered kiwifruit and grab individual fruit, then pick and unload the fruit in an average time of 10±1 s with a high recognition rate of 92.2% and low damage rate of less than 7.3%.

Keywords: kiwifruit, harvesting robot, nondestructive picking, end-effector

INTRODUCTION

Agricultural mechanization operations can improve the level of productivity and efficiency, indicate social development and are an important symbol of agriculture productivity (Bechar and Vigneault, 2016). "Much starker choices – and graver consequences – in" in our country means that agricultural mechanization is an important area for research. The development of robot technology is likely to transform agriculture in future years (McCoy, 2016). Harvesting of kiwifruit is time consuming and inefficient requiring much labor (Fu et al., 2015). Picking end-effector is one of the important parts of the picking robot, being the part in direct contact with the fruit (Mu et al., 2017). Innovative design based on the characteristics of fruit growth and form provides an efficient and reliable end-effector for harvesting robot.

A. Scarfe in New Zealand and Y.-J. Cui of China's Northwest Agriculture and Forestry University of Science and Technology have undertaken research on robots for harvesting kiwifruit. Scarfe's robot was of low efficiency (Scarfe et al., 2009; Scarfe, 2012). Cui developed a nondestructive end-effector, but the robot was slow and did not consider what happened to the fruit after harvest (Cui et al., 2014). Other types of fruit picking robots based on characteristics of robot's information perception have been developed

[a]E-mail: clever19880817@163.com

[b]E-mail: cuiyongjie@nwsuaf.edu.cn, Tel/Fax: +86-13720581232

for tomatoes, cucumbers, apples, strawberries and other fruit and vegetables (Carabin et al., 2016; Bonadies et al., 2016; Bechar and Vigneault, 2016; Yamamoto et al., 2014; Liu et al., 2016).

Therefore, we studied the end-effector specifically for picking kiwifruit and in particular bionic fingers and perception of information to avoid fruit damage. The robot unloads fruit and puts it into a basket.

MATERIALS AND METHODS

Kiwifruit picking robot structure

The picking robot structure is designed to automatically take the fruit information recognition (Cui et al., 2013), positioning, picking order planning, movement to kiwifruit picking area, nondestructive picking, and unloading fruit to the basket. It consists of five main parts, namely, machine vision, end-effector, coordinate manipulator, vehicle, and control system (Mu et al., 2017). Kiwifruit picking tests were conducted on the test harvesting robot with end-effector, manipulator, and information perception system, etc. The experiment emulated fruits in artificial shelf in farm machinery laboratory of Northwest Agriculture and Forestry University October, 2017. The non-destructive harvest integrated operation of "grabbing, picking and sliding" and field test in Meixian Kiwifruit Station from October, 2017, validated the efficiency and non-destructive harvest of the end-effector.

Sensor for kiwifruit picking robot

The robot can perceive information, obtain fruit coordinates by image processing, and Cartesian coordinates using Kinect sensor. Next, the three-dimensional mechanical manipulator moves to coordinate position and transports end-effector to the position of the fruit for nondestructive harvest (Mu et al., 2017). Analysis on methods and steps of the manual picking was conducted as well to develop a picking model based on angle control, and a nondestructive picking end-effector was trial-manufactured and tested as well. Using the optic fiber sensors installed on the fingers helps detect the optimum location and accurate grabbing of the end-effector, pressure sensors adjust clamping force, spring tolerate various shapes and sizes of fruits and all these coordinate an integration harvesting from grabbing, picking to unloading following trajectory model.

End-effector design of structural features

The harvesting robot structured stable control, picking efficiency and automatic unloading was designed considering the physical properties of 'Hayward' kiwifruits in cultivation on orchard support systems. The kiwifruit sample statistics reported the following physical parameters of 'Hayward' kiwifruit (Davidson and Mo, 2016): the minimum breaking resistance occurs at an angle of 60° between stalk and fruits axis, fruits average physical parameters: the width, 52.16 mm; thickness, 47.86 mm; length, 64.98 mm; stem length, 58.7 mm; rubber friction coefficient fluctuates between 0.38 and 0.51 and the maximum friction angle is 27°.

RESULTS

Structure of the harvest robot

The kiwifruit picking robot consists of five main parts, including machine vision, end-effector, coordinate manipulator, vehicle, and control system, as shown in Figures 1 and 2. The robot can perceive information, obtain fruit coordinates by image processing, and Cartesian coordinates point using Kinect sensor; then the manipulator moves to coordinate the position and transports end-effector to the position of the fruit for nondestructive harvest. Analysis on methods and steps of the manual picking was conducted as well to put forward a picking model based on angle control; and the picking

robot was trial-manufactured and tested as well. Kiwifruit position is detected by optic fiber sensors, clamping force detected by pressure sensors, shape and size of bionic picking fingers thereby set and an integration harvesting prototype following trajectory model from grabbing, picking to unloading designed and evaluated in the lab and orchard.

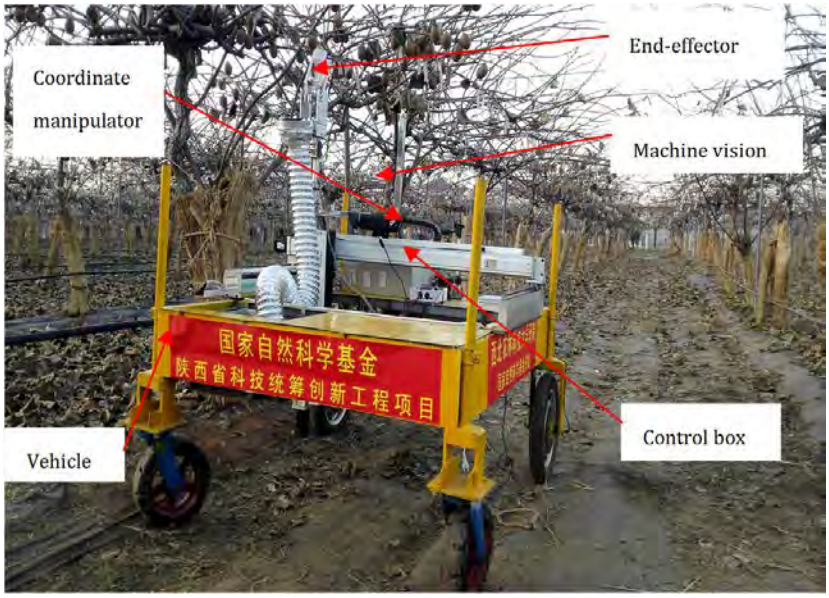

Figure 1. Kiwifruit harvesting robot structure.

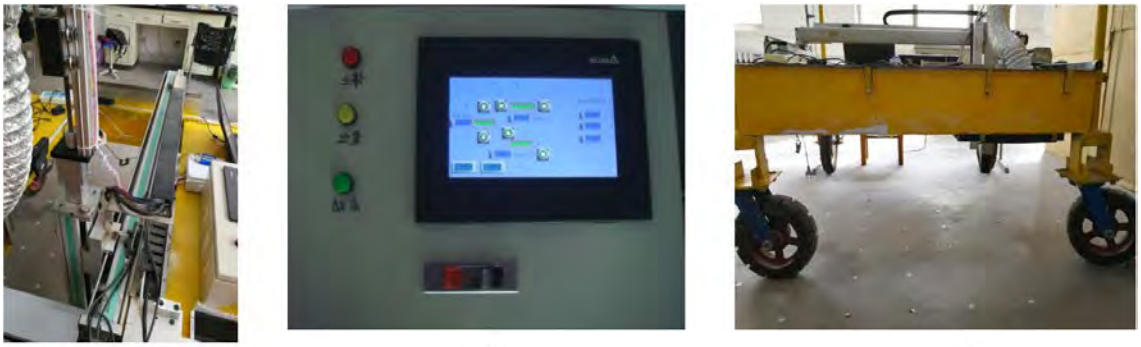

Figure 2. (a) Coordinate manipulator, which can move towards three dimensions, (b) control box, which includes all control system of the harvesting robot, (c) vehicle, which consisted of two universal and two electric wheels.

Nondestructive picking end-effector

To separate kiwifruit and to realize nondestructive picking and unloading of clustered kiwifruit in cultivation on support structures, the physical and mechanical properties of kiwifruit (Table 1) were taken into account. The first end-effector approached a fruit from below and enveloped and grabbed the fruit from two sides, and then rotated up to separate the fruit from the stem. The first and, second end-effectors with a "grabbing-picking-sliding" harvesting method and automatic picking and unloading connecting rod linkage following its trajectory model is presented based on the research on manual picking methods, fruit growth and fruit physical properties, shown in Figures 3 and 4.

Table 1. Kiwifruit physical parameters.

	Minimum	Maximum	Range	Average
Weight (g)	81.40	128.70	47.30	97.41
Width (mm)	45.85	57.13	11.28	52.16
Length (mm)	58.91	74.41	15.50	64.98
Thickness (mm)	42.70	51.84	9.14	47.86
Geometric average (mm)	51.29	59.89	8.60	54.51
Static friction coefficient	0.38	0.51	0.014	0.44

Figure 3. The first end-effector for harvesting robot.

Figure 4. The second end-effector for harvesting robot.

Different mechanical structures of end-effector were suggested considering the growth characteristics of clustered kiwifruit in cultivation on support structures their harvesting of upward enveloping, adjacent fruits separating from the bottom, and "grabbing-picking- sliding" integration.

Machine vision harvesting experiment

The robot can perceive information and obtain fruit coordinates by image processing. Images of the fruits were taken in late October during the harvesting season on the most common cultivar 'Hayward' at the Meixian Kiwifruit Experimental Station at the Northwest A&F University. The example of the lab experimental set-up is shown in Figure 5, while Figure 6 shows an example of multiple kiwifruit in the images. Analysis on methods and steps of the manual picking was conducted as well as putting forward a picking model based on angle control. In order to develop a robot to harvest kiwifruit at night and overcome the problems of the complex background and fruit overlapping by the conventional fruit image capturing method during daytime, a machine vision system was proposed in this study.

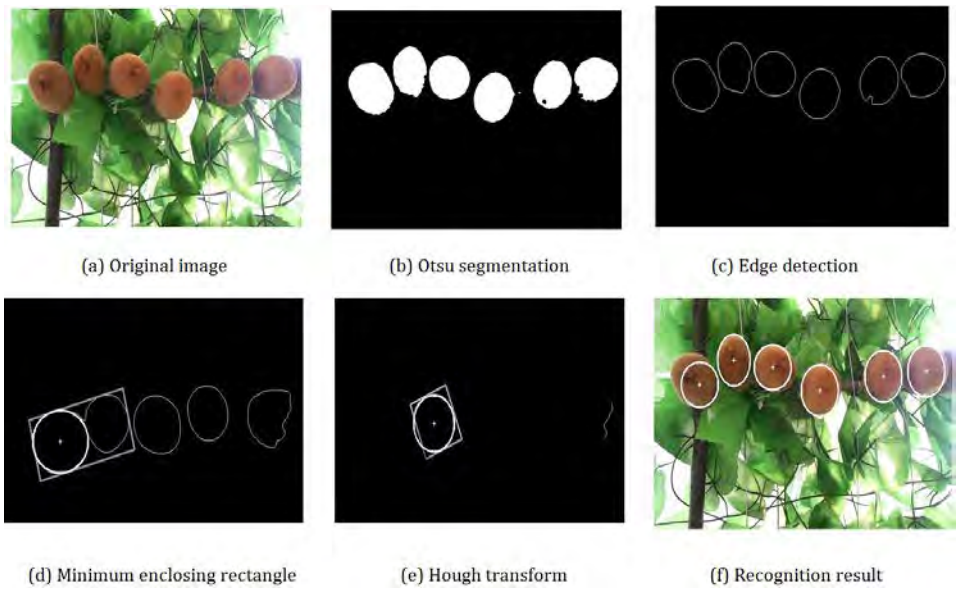

Figure 5. Original image and segmentation with different methods.

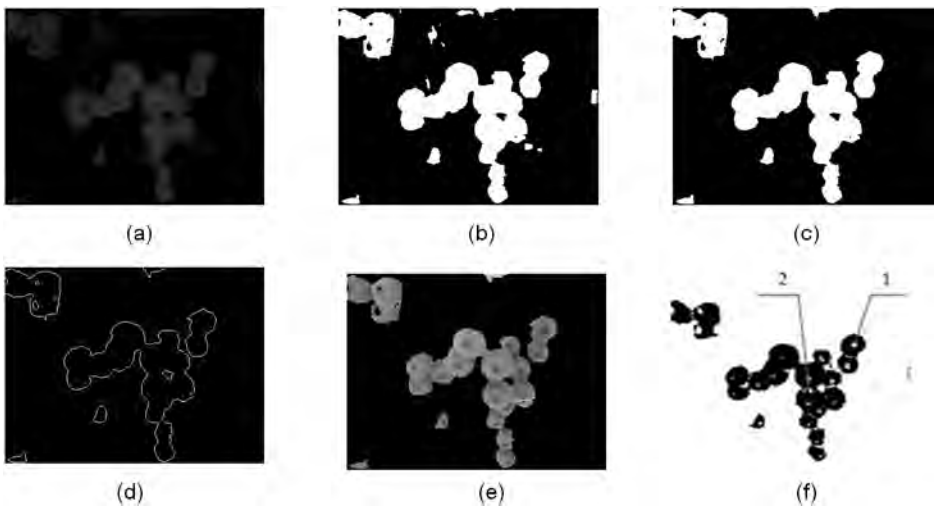

Figure 6. Recognition process, (a) R-G component image, (b) threshold segmentation image, (c) removal of interference noise , (d) prewitt detection, (e) recognition of multi-objective kiwifruits, (f) identifying the calyx of kiwifruit: 1. calyx, 2. noise.

Harvesting experiment

In the experiment with 90 kiwifruit samples picked in the Meixian Kiwifruit Experimental Station of the Northwest Agriculture and Forestry University (34°07'39" N, 107°59'50" E, and 648 m in altitude), the successful picking rate reached 92.2% on average, and the average damage rate was 7.3% (Tables 2 and 3).

Table 2. Rate of successful kiwifruit samples picking by harvesting robot.

Time	Morning	Afternoon	Night
Total	30	30	30
No. of successful picking	28	26	29
Number of failures	2	4	1
Rate of success	93.3%	86.7%	96.7%

Table 3. Damage rate of kiwifruit picked by harvesting robot.

Time	Morning	Afternoon	Night
Total	30	30	30
Number of injury	3	2	2
Damage rate	10%	6%	6%

Factors affecting successful or nondestructive harvesting include: fruit maturity, fruit stalk length, clamp moment, bending angle and scope, fruiting range, tree branches and the support structure, ground potholes affecting the coordinates, fruit drop causing fruit injury, etc. Causes for harvest failure could be the failure to separate adjacent fruits, failure to grab the fruit properly, finger slipping, or fruit injury in sliding.

DISCUSSION

The test also showed that picking robots end-effector failed to work in picking point recognition, grabbing, bending picking and other processes, which on one hand meant that the algorithm in these processes remains to be improved, also verified the practical value of the end-effector hardware design assisted by testing platform in testing and improving the robot vision, control algorithm, and the robot fault-tolerant structure design.

Comparative analysis indicated that factors determining the end-effector picking accuracy include: finger position offset, assembly clearance error, fruit drop, surface roughness, etc. When fingers crowded to separate adjacent fruits, the other kiwifruit coordinates were changed, which disabled the end-effector from accurately reaching the picking position, then the end-effector may slide or fail to grab the fruits when it went on to picking the next fruit. To solve this, the width of the finger is optimized to have a 10 mm tolerance which can envelope kiwifruits of different sizes, shapes, even of skewed growth. Some fruit were harvested with stem remaining attached because of the uneven pulling force of the fingers.

CONCLUSION

The kiwifruit picking robot verification tests validated that the end-effector can nondestructively harvest the orchard kiwifruit with a higher efficiency contributed by the integrated grabbing-picking-sliding harvest.

Future studies should focus on mechanical operation and control system improvement for more efficient and accurate operation. Multi-target recognition and multi- manipulator coordination could be applied in the control system to improve the picking efficiency of robots.

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Genome-wide analysis of WRKY transcription factor in kiwifruit

A'ling Zhang, Yanfei Liu, Zhande Liu[a] and Zhiyao Ma

Horticultural College, Northwest A&F University , Yangling, Shaanxi Province, China.

Abstract

WRKY transcription factors participate in diverse physiological and developmental processes in plants. In this study, 94 kiwifruit WRKY genes (*AcWRKY*) were identified in the latest kiwifruit genome sequence and renamed on the basis of their respective chromosome distribution. With the exception of chromosome 9, 82 *AcWRKY* genes could be mapped on to kiwifruit 28 chromosomes and 12 not on any chromosome. Furthermore, approximately 81% (76 out of 94) *AcWRKY* genes participated in gene duplication events, including 18% (14 out of 76) tandemly duplicated genes and 82% (62 out of 76) segmental duplicated genes. A multiple sequence alignment analysis using 94 AcWRKY protein sequences, together with those from *Arabidopsis thaliana*, indicated that all the 94 AcWRKY proteins were found to have the highly conserved sequence WRKYGQK. Based on the number of WRKY domains and the features of the specific zinc-finger motifs were classified into three main groups (I–III), with the second group further divided into five subgroups (IIa-IIe). The motifs were predicted using the program MEME, finally 25 distinct motifs were identified and motifs 1, 2, 3, 5, 8 were characterized as WRKY domains, which were broadly distributed in the AcWRKY protein sequences. In addition, intron-exon structure analysis suggested that the number of exons ranged from 1 to 14 within the 94 AcWRKY genes, and 93 genes had 1 to 13 introns, while only one gene had no intron.

RESULTS

Table 1. The WRKY gene family in kiwifruit.

Name	ID	G	Chr	Location	Name	ID	G	Chr	Location
AcWRKY1	Achn229941	II d	1	9993108-9996016	AcWRKY48	Achn022171	II d	17	3569090-3573539
AcWRKY2	Achn175001	I	2	5551913-5566282	AcWRKY49	Achn155261	I	17	5824456-5828958
AcWRKY3	Achn258601	III	2	5874200-5876108	AcWRKY50	Achn053001	I	17	7145804-7153201
AcWRKY4	Achn287861	I	3	4805144-4808719	AcWRKY51	Achn132991	II c	17	11591666-11594732
AcWRKY5	Achn287671	III	3	5217134-5219357	AcWRKY52	Achn011781	II e	18	7008675-7010959
AcWRKY6	Achn188191	II e	3	7790645-7793787	AcWRKY53	Achn182561	II e	19	827190-828406
AcWRKY7	Achn062591	III	3	10898318-10900689	AcWRKY54	Achn306131	I	19	3017472-3020059
AcWRKY8	Achn062881	III	3	10906971-10911609	AcWRKY55	Achn013921	II e	20	223063-226105
AcWRKY9	Achn159361	II d	4	9181465-9182642	AcWRKY56	Achn014021	II d	20	365337-367671
AcWRKY10	Achn229111	II c	5	5927518-5929152	AcWRKY57	Achn236811	II c	20	9681415-9683240
AcWRKY11	Achn374841	II c	5	7597682-7599223	AcWRKY58	Achn299611	II d	21	10180344-10181530
AcWRKY12	Achn235341	II c	5	13188460-13190041	AcWRKY59	Achn390831	II c	22	2982453-2985897
AcWRKY13	Achn182751	I	6	4624671-4633256	AcWRKY60	Achn351801	I	22	6072800-6075686
AcWRKY14	Achn028681	II c	6	4753601-4756729	AcWRKY61	Achn059561	II c	22	11180044-11181955

Multiple sequence alignment of 94 AcWRKY domains using DNAMAN. *Red boxes* and *arrows* indicated the WRKYGQK motif and the zinc-finger motif sequences were highlighted by *red triangles* and *boxes*.

[a]E-mail: dezhanliu@vip.sina.com

Table 1 Continued. The WRKY gene family in kiwifruit.

Name	ID	G	Chr	Location	Name	ID	G	Chr	Location
AcWRKY15	Achn230981	II c	6	6265303-6284139	AcWRKY62	Achn114471	II a	23	2042573-2046866
AcWRKY16	Achn103931	II c	6	7197206-7221447	AcWRKY63	Achn068771	I	23	9973405-9980518
AcWRKY17	Achn125121	II e	6	10303613-10314782	AcWRKY64	Achn191331	I	23	16731271-16746475
AcWRKY18	Achn000371	II c	7	12969930-12983983	AcWRKY65	Achn359541	II d	23	18748380-18750809
AcWRKY19	Achn150821	II	8	1038920-1041554	AcWRKY66	Achn320511	II c	23	19798200-19499541
AcWRKY20	Achn040601	II c	8	17570203-17587920	AcWRKY67	Achn103451	II c	24	823935825779
AcWRKY21	Achn066811	I	8	18771303-18782996	AcWRKY68	Achn148371	III	24	13198619-13200899
AcWRKY22	Achn143171	II c	10	11822759-11828617	AcWRKY69	Achn147941	III	24	13207972-13210368
AcWRKY24	Achn336561	II d	11	1631188-1632982	AcWRKY71	Achn132821	II e	25	10208166-10209274
AcWRKY25	Achn263441	II c	11	8771315-8772871	AcWRKY72	Achn329191	II a	26	6881287-6883386
AcWRKY26	Achn134501	II a	11	9594030-9601500	AcWRKY73	Achn217531	II b	26	9613263-9617180
AcWRKY27	Achn275661	II e	11	16156448-16158683	AcWRKY74	Achn271431	I	27	50354-56201
AcWRKY28	Achn316621	II b	12	725014-727913	AcWRKY75	Achn353831	II c	27	9798517-9799672
AcWRKY29	Achn071331	I	12	2070667-2083930	AcWRKY76	Achn234481	II b	27	10136959-10141863
AcWRKY30	Achn229421	II c	13	200752-202271	AcWRKY77	Achn360351	II d	28	56089-60632
AcWRKY31	Achn017781	II d	13	1855945-1857888	AcWRKY78	Achn377931	II c	28	908957-923138
AcWRKY32	Achn280261	II b	13	4737467-4740338	AcWRKY79	Achn196241	II c	29	3072913-3074608
AcWRKY33	Achn214251	I	13	7110514-7112130	AcWRKY80	Achn355811	I	29	6785236
AcWRKY34	Achn350681	II c	13	13892772-13911357	AcWRKY81	Achn015341	I	29	12452066-12487983
AcWRKY35	Achn144931	I	13	16186032-16191557	AcWRKY82	Achn152191	II b	29	13004033-13009727
AcWRKY36	Achn278571	II e	14	6373751-6375239	AcWRKY83	Achn165131	II e	0	5876140-5879162
AcWRKY37	Achn184451	III	15	529723-538857	AcWRKY84	Achn079441	I	0	11993114-11998009
AcWRKY38	Achn005621	II e	15	1450449-1457802	AcWRKY85	Achn282451	II d	0	14451626-14453353
AcWRKY39	Achn005631	II c	15	1468282-1468827	AcWRKY86	Achn289221	I	0	36309732-36314430
AcWRKY40	Achn314301	I	15	3212637-3230412	AcWRKY87	Achn245731	II e	0	59904170-59909164
AcWRKY41	Achn109391	II c	15	8149197-8170353	AcWRKY88	Achn369221	II c	0	89844517-87848662
AcWRKY42	Achn109071	I	15	8668617-8676826	AcWRKY89	Achn370671	II c	0	95137967-95141659
AcWRKY43	Achn026311	II b	15	18051640-18057171	AcWRKY90	Achn353281	II c	0	9621775396222600
AcWRKY44	Achn365871	II b	16	4458644-4462429	AcWRKY91	Achn010691	II c	0	113725488-113729382
AcWRKY45	Achn309921	II a	16	7931565-7933705	AcWRKY92	Achn057931	III	0	131920717-131925826
AcWRKY46	Achn064731	II c	17	664306-666657	AcWRKY93	Achn063361	III	0	137164537-137169472
AcWRKY47	Achn064651	II d	17	760152-763928	AcWRKY94	Achn075461	II d	0	139097701-139102150

Multiple sequence alignment of 94 AcWRKY domains using DNAMAN. *Red boxes* and *arrows* indicated the WRKYGQK motif and the zinc-finger motif sequences were highlighted by *red triangles* and *boxes*.

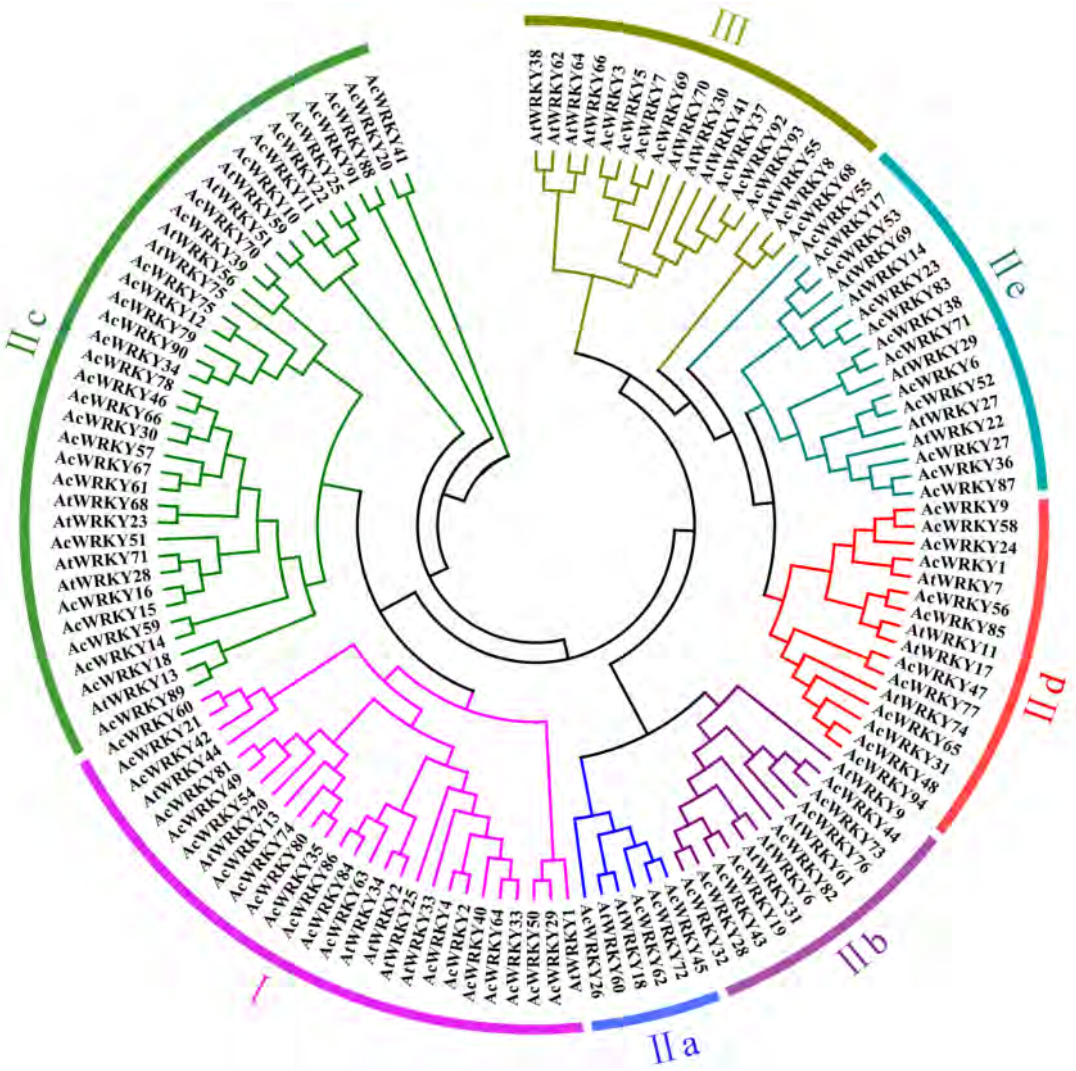

Figure 1. Phylogenetic tree of WRKY proteins from kiwifruit, *Arabidopsis*.

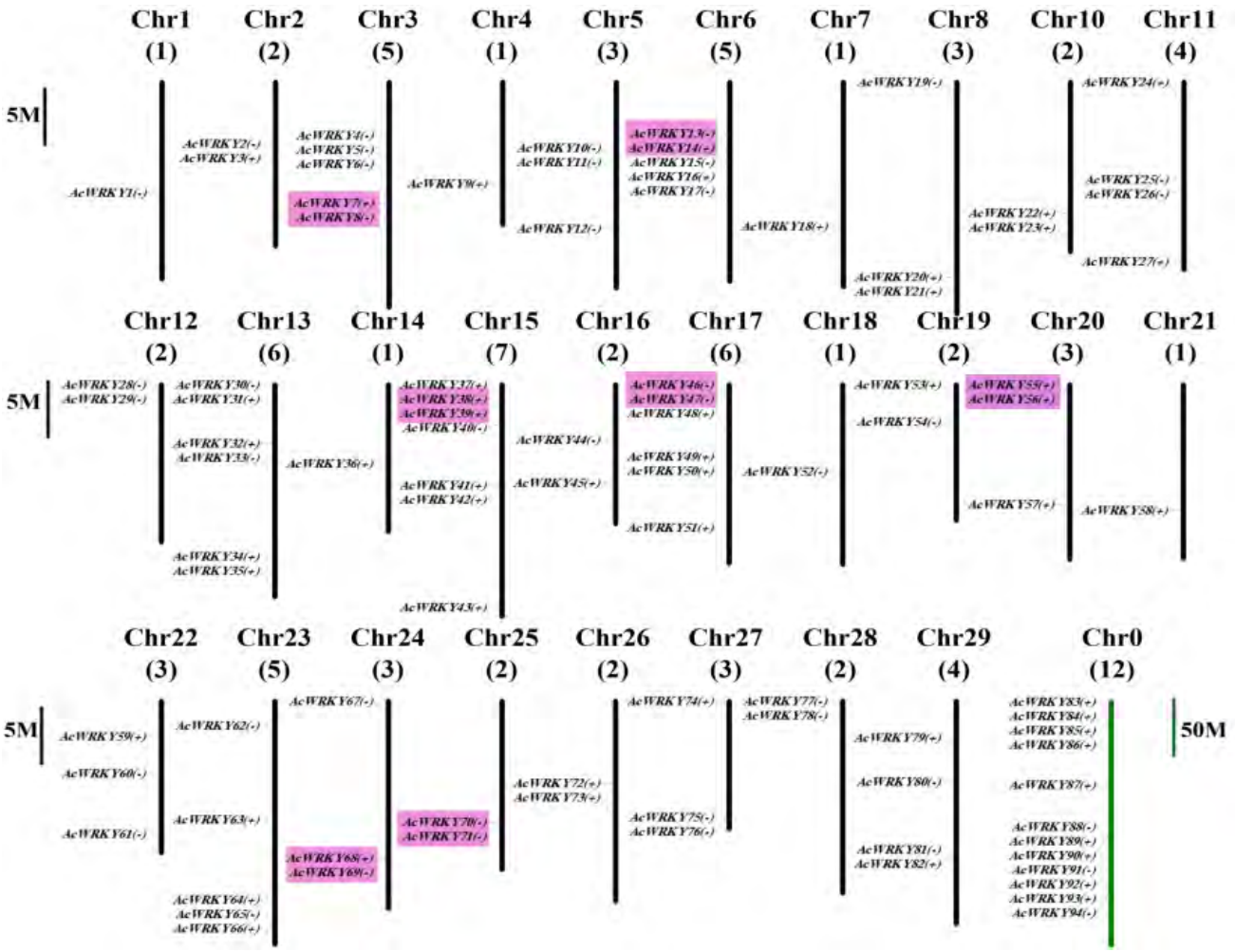

Figure 2. Chromosomal locations and predicted cluster for *AcWRKY* genes. The *positive (+)* and *negative (–) signs* following each gene represent forward and reverse orientation of the respective gene. Tandemly duplicated gene clusters are indicated in *pink.*

Figure 3. Twenty-five predicted motifs were identified by the MEME.

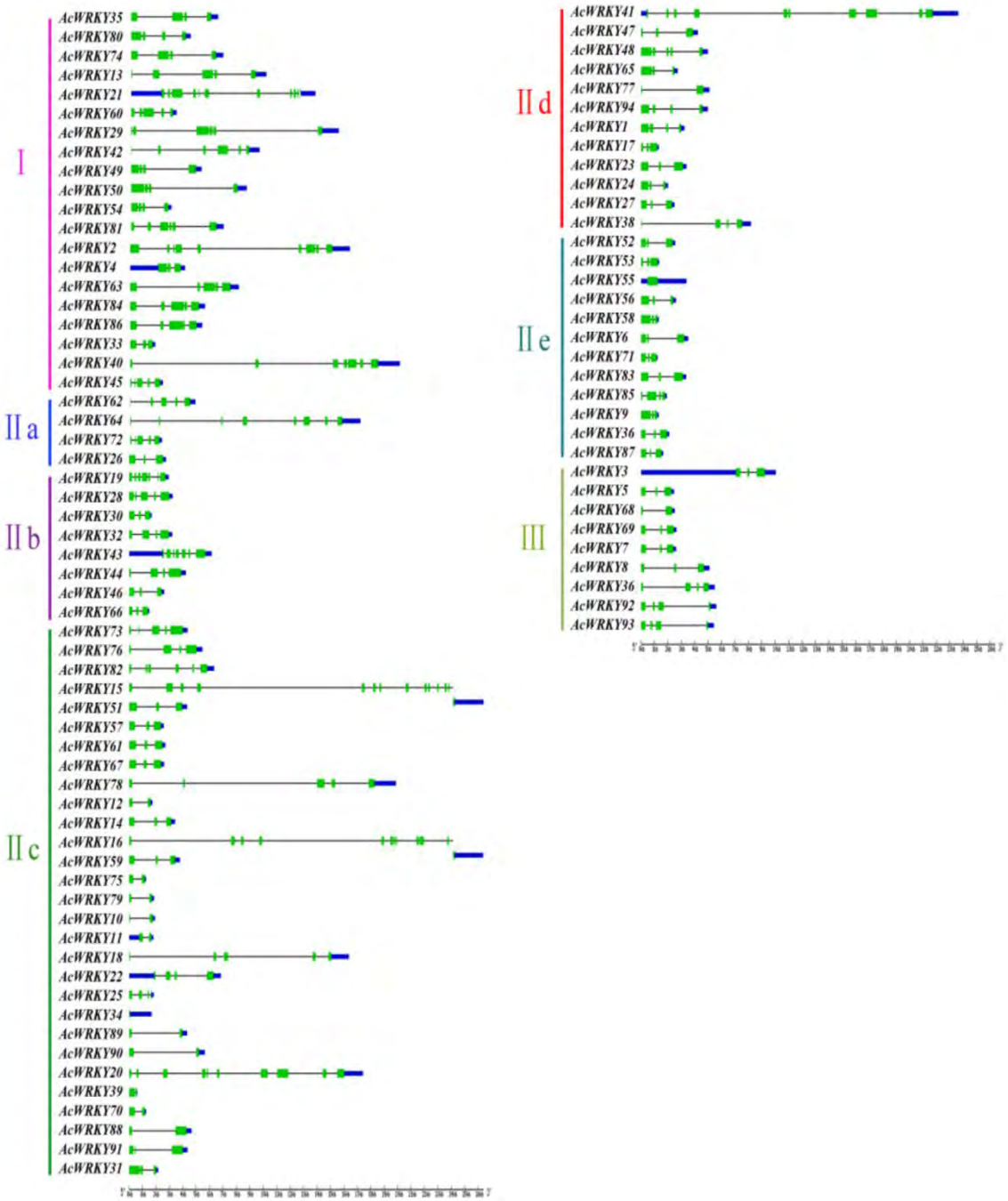

Figure 4. Exon-intron structure of *AcWRKY* genes. Exons and introns are indicated by *green boxes* and *single lines*, respectively.

CONCLUSION

The information generated about the structure of AcWRKY proteins will shed light on their functional analysis. The comparative and phylogenetic analyses of WRKY members of important model plants will provide insight into the identification and comprehensive functional characterization of the WRKY gene family from kiwifruit.

Identification and biocontrol of *Pseudomonas syringae* pv. *actinidiae* in Shaanxi Province, China

Zhaobin Jing[1,2,3,a], Zhande Liu[3], Chunchao Yao[3] and Yu Ma[4]

[1]Weinan Vocational and Technical College, Weinan 714026, Shaanxi, China; [2]Weinan Fruit Industry Institute of Shaanxi, Weinan 714026, Shaanxi, China; [3]College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China; [4]Shaanxi Microbiology Institute, Xi'an, 710043, Shaanxi, China.

Abstract

The aim of this study was to isolate and identify *Pseudomonas syringae* pv. *actinidiae* (Psa) and to develop the biocontrol agents for Psa. Psa was isolated from different cultivars in Shaanxi province by the conventional tissue method and 16S-23S rDNA ITS methods, and the pathogenicity also was measured. Results showed that: 1) 45 Psa strains were obtained from different cultivars; 2) four strong strains were selected based on the results of leaf pathogenicity; 3) the biocontrol agents of kiwifruit canker disease were obtained, and the biocontrol agents had meaningful control efficacy against Psa.

Keywords: Psa, pathogen, molecular identification, pathogenicity, biocontrol

INTRODUCTION

Kiwifruit canker is one of the most damaging bacterial diseases, and caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) (Butler et al., 2013). In recent years, a number of studies have reported on the bacterial canker of kiwifruit, and these results mainly focused on the identification, pathogenesis, molecular mechanism, and technology systems for the control of the pathogenic bacteria (Takikawa et al., 1989; Koh and Lee, 1992; Ferrante and Scortichini, 2010; Vanneste et al., 2011, 2012; Young et al., 2012). Nonetheless, the relationships among pathogenicity of the strains and geographic origin and cultivar are largely unknown.

In this study, we studied the isolation of the Psa strains from different cultivars and different geographic origins, and reported a biocontrol agent against kiwifruit bacterial canker.

MATERIALS AND METHODS

Materials

The *A. chinensis* cultivars 'Hongyang' and 'Huayou' and *A. deliciosa* 'Hayward' were selected from the northern area of Qinling, Shaanxi Province in China which includes Meixian County, Zhouzhi County, and Yangling District. The pathogen of Psa was isolated from infected trunks of the three cultivars.

Methods

1. Isolation and purification of Psa strains.

The pathogen of Psa was cultured on BPA medium (beef extract 3 g L^{-1}, peptone 7.5 g L^{-1}, sugar 10 g L^{-1}, yeast extract 1 g L^{-1}, agar 17 g L^{-1}, pH 7.0) for 72 h at 25°C. Then a single colony of each strain was isolated by the slant and plate culture dish on the BPA medium for the following experiment.

[a]E-mail: jingzhaobin@nwsuaf.edu.cn; 353813638@qq.com

2. Extraction of genomic DNA.

For pure isolates, cultures were grown overnight in LB medium (tryptone 10 g L^{-1}, yeast extract 5 g L^{-1}, NaCl 10 g L^{-1}, pH 7.0) at 26°C. DNA were extracted using TIANamp Bacteria DNA kit (TIANGEN Biotech Co., Ltd., Beijing, China). The concentration and quality of DNA samples were quantified by a Nanodrop 2000 spectrophotometer (Gene Company Limited, Hong Kong) and electrophoresis agarose gel and then adjusted to a final concentration of 40 ng μL^{-1} with TE buffer and stored at -20°C.

3. Molecular detection.

PsaF1/R2 PsaF1 (5'-TTTTGCTTTGCACACCCGATTTT-3'), PsaR2 (5'-CACGCACCCTTC AATCAGGATG-3') were synthesised (Rees-George et al., 2010). Amplification was performed in a total volume 25 µL, DNA (40 ng μL^{-1}) 2 µL, Taq DNA polymerase (5 U μL^{-1}) 0.15 µL, dNTPs (2.5 mmol L^{-1}) 1.4 µL, primer (10 µmol L^{-1}) 1.5 µL, 2 × Reaction Mix 12.5 µL, ddH_2O 7.85 µL (TIANGEN: Golden Easy PCR System, Beijing, China). The following conditions were used for amplifications; 1 cycle at 95°C for 2 min; 30 cycles at 94°C for 15 s, 55°C for 30 s, 72°C for 1 min 30 s, and a final extension cycle at 72°C for 5 min. The amplification products were detected on 2.0% TAE agarose gels. Then the target DNA fragments were obtained using TIANgel Purification kit (TIANGEN Biotech Co., Ltd., Beijing, China). The sequence of the target DNA fragment was sequenced by the Aoke Biotech (Yangling, China). The nucleotide sequences were analyzed on the NCBI database by Blast program. The sequences were submitted to the GenBank database (KT731213~KT731232).

4. Pathogenicity measurement of Psa strains.

'Hongyang' cultivar was used for pathogenicity measurement of Psa strains. We collected healthy leaves in June and stored at 4°C for in vitro inoculation. Before inoculation, leaves were disinfected using 70% alcohol solution and then rinsed three times in sterile distilled water. Detached leaves were inoculated by spraying bacterial suspension in vitro. The spore concentration was 1×10^8 CFU mL^{-1}. Six leaves were used for each measurement strain. Then the leaves were placed in a wide-mouthed bottle in a growth chamber under controlled conditions (18°C, 16 h, light; 16°C, 8 h, dark; 85% relative humidity) (Figure 1). The rate of infection of leaves was recorded on each day. The pathogenicity grade was analyzed using software ImageJ 1.50b (National Institutes of Health, USA). The rate of infection (%) = infection spot area (cm^2)/total leaf area (cm^2). The pathogenicity grade was classified according to the rate of infection: 0 (weak), 0.1% < rate of infection ≤ 10%; 1 class (relatively weak), 10% < rate of infection ≤ 35%; 2 class (relatively strong), 35% < rate of infection ≤ 70%, 3 class (strong), rate of infection > 70%.

Figure 1. The field symptoms for different kiwifruit cultivars.1: 'Huayou' from Zhouzhi, 2: 'Hayward' from Zhouzhi, 3: 'Huayou' from Yangling, 4: 'Hongyang' from Meixian.

RESULTS AND DISCUSSION

Disease symptoms

The disease symptoms of the different cultivars ('Hongyang', 'Huayou' and 'Hayward') and different geographical origins were similar, i.e., shallow brown to brown or red brown lesions on the infected branches. However, the infection intensity is different (Figure 1).

Isolation and purification of Psa, morphological characteristics of bacterial colony

A total of 45 Psa strains was isolated from the three different cultivars. The characteristics of bacterial colony from different cultivars and different geographical origin showed diversification (Figure 2).

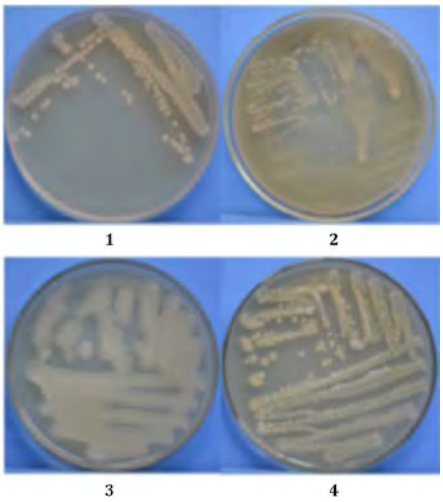

Figure 2. Morphological characteristics of *Psa* for different cultivars; 1: ' Hongyang' from Meixcian, 2: 'Hayward' from Zhouzhi, 3: 'Huayou' from Zhouzhi, 4: 'Huayou' from Yangling.

rDNA-ITS sequence analysis of Psa

Forty-five Psa strains were detected using PsaF1/R2 primer; the results showed that twenty strains could amplified 280bp products (Figure 3). Based on the analysis of Blast on NCBI, we found that these twenty strains have a high similarity with CP011972.1 strain.

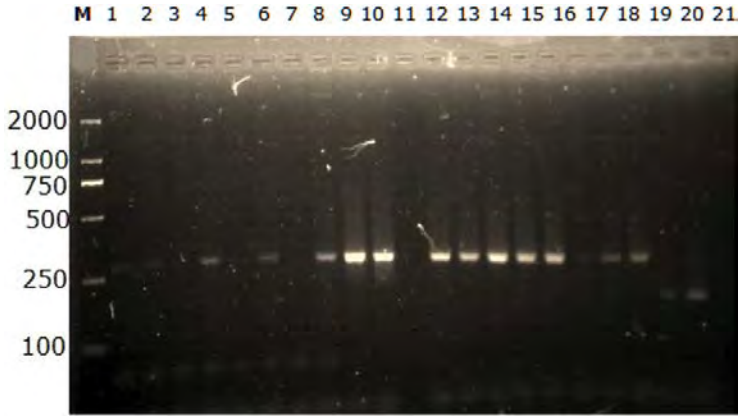

Figure 3. Electrophoresis of PCR reactions of 16S-23S rDNA ITS for part strains (M: DL2000 DNA marker, 1-21: different strains).

Pathogenicity analysis

A total of fifteen strains was used for pathogenicity measurements. The leaves of 'Hongyang' cultivar showed different intensity infection spots (Figure 4). The pathogenicity grade was calculated using software ImageJ. Based on these results, we could conclude that there were no relationships between pathogenicity of the strains and geographic origin and cultivars.

Figure 4. Pathology character for pathogenicity identification of Psa from fifteen different geographic origins and cultivars using 'Hongyang'.

Biocontrol of canker disease with different agents

The results of the antagonistic test in the laboratory (Figures 5 and 6) and the control experiment in the fields show that the biocontrol agents and products are able to inhibit this disease spreading effectively.

Figure 5. To develop the biocontrol agents and products for kiwifruit canker disease.

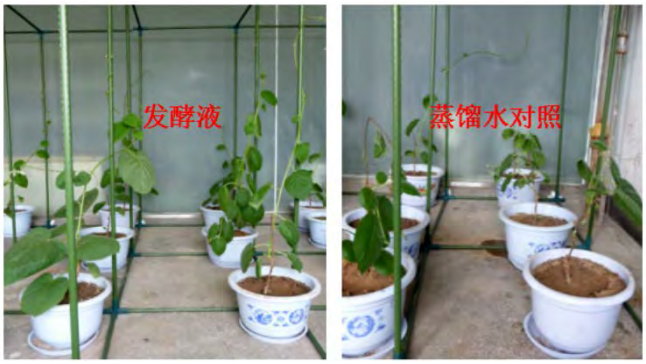

Figure 6. The control experiment in kiwifruit.

CONCLUSIONS

The following conclusions can be drawn from the study:

- A total of 20 Psa strains was obtained by isolation, purification and molecular identification. The colonial morphology showed a large difference for different geographic origins and cultivars.
- There were no relationships among pathogenicity of the strains and geographic origin and cultivar.
- The quantitative and accurate methods of leaf pathogenicity was established based on the software ImageJ 1.50b, and four strong strains were selected.
- Biocontrol agents had meaningful control efficacy against Psa.

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Rapid detection of *Pseudomonas syringae* pv. *actinidiae* by loop-mediated isothermal amplification

Lei Ding[1], Wen Yang[1], Yuanle Zhang[1], Zhande Liu[2], Qiaochun Wang[2], Lei Zhao[1,a] and Yunfeng Wu[1,b]

[1]College of Plant Protection, Northwest A&F University, Yangling, 712100, China; [2]College of Horticulture, Northwest A&F University, Yangling, 712100, China.

Abstract

Kiwifruit is an important and native fruit in China. Shaanxi province is the largest kiwifruit producer in both cultivation area and yield in China. Kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* has been a serious threat for the global kiwifruit industry and is considered to be the most devastating. This report describes a rapid loop-mediated isothermal amplification (LAMP) assay for the detection of *Pseudomonas syringae* pv. *actinidiae*. The primers were designed using 16S-23S rDNA Internal transcribed spacer (ITS) regions. LAMP amplification was optimized and its sensitivity was approximately 100-fold higher than that of the conventional PCR assay in detecting the presence of *Pseudomonas syringae* pv. *actinidiae*. Thirty-three bacterial strains were selected for specificity tests, and the results of the amplification were negative except for *P. syringae* pv. *tomato* and *P. syringae* pv. *theae*. Because pv. *tomato* and pv. *theae* are unlikely to be found on kiwifruit, the method is considered sufficiently specific for screening bacterial strains isolated from kiwifruit tissue. It appears that this LAMP method is fast (1 h), cost-effective and adaptable and has potential usefulness for *Pseudomonas syringae* pv. *actinidiae* identification, detection, and its control strategies.

Keywords: *Pseudomonas syringae* pv. *actinidiae*, LAMP, detection, kiwifruit, crop protection

INTRODUCTION

Kiwifruit is an important and native fruit in China, with the world's biggest cultivation area at approximately 250,000 ha. Shaanxi Province is the largest kiwifruit producer in both cultivation area and yield in China. Up to now, more than 10 types of economically important diseases have been reported to occur in kiwifruit, amongst which kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* has been a serious threat for global kiwifruit industry and is considered to be the most devastating (Koh et al., 1994, 2003). It has caused severe economic losses of kiwifruit in China (Shen et al., 2009), Italy (Ferrante and Scortichini, 2010), New Zealand (Vanneste, 2012), Japan (Takikawa et al., 1989), South Korea (Koh et al., 1994), France (Vanneste et al., 2011), Portugal (Renzi et al., 2012), and Spain (Abelleira et al., 2011). Symptoms of the disease consist of brown discolouration of the buds, dark brown spots surrounded by yellow haloes on leaves, cankers with reddish exudates on twigs, leaders and trunks, and collapse of fruit (Balestra et al., 2009). Since kiwifruit bacterial canker has been included in quarantine objectives for forest plants in China, rapid, efficient and sensitive detection of its causal agent *Pseudomonas syringae* pv. *actinidiae* is required. Several methods based on polymerase chain reaction (PCR) and BOX-PCR are the most conventional methods to detect the DNA of *Pseudomonas syringae* pv. *actinidiae* (Balestra et al., 2013; Koh and Nou,

[a]E-mail: zhaolei5105@nwsuaf.edu.cn

[b]E-mail: wuyf@nwsuaf.edu.cn

2002; Rees-George et al., 2010; Vanneste et al., 2010). Recently, a nucleotide-based assay called loop-mediated isothermal amplification (LAMP) has been developed for RNA or DNA amplification under isothermal conditions at 50-65°C (Notomi et al., 2000). By using two or three primer pairs, the target DNA is amplified and DNA stem-loop structures with several inverted repeats of the target DNA are produced (Notomi et al., 2000). The results can be directly visualized or separated by gel-electrophoresis (Nagamine et al., 2002; Notomi et al., 2000). Similar to real-time PCR, LAMP has shown high sensitivity. Moreover, LAMP requires no special equipment, and a simple temperature-controlled water bath usually suffices (Nagamine et al., 2002).

In this study, *Pseudomonas syringae* pv. *actinidiae* was firstly and successfully detected by using the LAMP method, which could result in more rapid, highly sensitive, and efficient diagnosis for monitoring *Pseudomonas syringae* pv. *actinidiae*.

MATERIALS AND METHODS

Isolation and purification

Fragments of infected tissues were aseptically removed from petiole and trunk. Then isolation and purification of *Pseudomonas syringae* pv. *actinidiae*, *Terribacillus* sp., *Enterobacter* sp. and *Erwinia* were carried out using LB medium at 25-28°C. Fifteen *Pseudomonas syringae* pv. *actinidiae* strains were collected and isolated from different regions of Shaanxi Province in 2011. All the pure cultures were analysed by PCR using primers PsaF1/PsaR2 (Rees-George et al., 2010) and 27F/1492R, and confirmed by DNA sequencing. *Pseudomonas syringae* pv. *actinidiae* (Psa-V), *Pseudomonas syringae* pv. *actinidiae* (Psa-LV), *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *theae*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae* pv. *papulans*, *Pseudomonas cichorii*, *Pseudomonas corrugata*, *Pseudomonas fluorescens*, *Pseudomonas marginalis*, *Pseudomonas viridiflava* and *Pseudomonas* sp. strains were provided by the International Collection of Microorganisms from Plants, Landcare Research Manaaki Whenua, New Zealand. Detailed information about the bacterial strains is listed in Table 1.

Table 1. Bacterial strains used in this study for specificity of LAMP detection for *Pseudomonas syringae* pv. *actinidiae*.

Species	Culture number	Host	Country of origin
Pseudomonas syringae pv. *actinidiae*[a]	-	*Actinidia chinensis*	China
Pseudomonas syringae pv. *actinidiae* (Psa-V)	-	*Actinidia chinensis*	New Zealand
Pseudomonas syringae pv. *actinidiae* (Psa-LV)	-	*Actinidia chinensis*	New Zealand
Pseudomonas syringae pv. *syringae*	ICMP 3676	*Prunus avium*	New Zealand
Pseudomonas syringae pv. *syringae*	ICMP 4610	*Solanum lycopersicum*	New Zealand
Pseudomonas syringae pv. *syringae*	ICMP 5823	*Cucumis sativus*	New Zealand
Pseudomonas syringae pv. *theae*	ICMP3923	*Camellia sinensis*	Japan
Pseudomonas syringae pv. *tomato*	ICMP 4608	*Solanum lycopersicum*	New Zealand
Pseudomonas syringae pv. *papulans*	ICMP 4043	*Malus domestica*	USA
Pseudomonas syringae pv. *papulans*	ICMP 4055	*Malus domestica*	Canada
Pseudomonas cichorii	ICMP 5707	*Cichorium endivia*	Germany
Pseudomonas corrugata	ICMP 8898	*Solanum lycopersicum*	New Zealand
Pseudomonas fluorescens	-	-	China
Pseudomonas marginalis	ICMP 8127	*Allium cepa*	New Zealand
Pseudomonas viridiflava	ICMP 11126	*Brassica chinensis*	China
Pseudomonas sp.	ICMP 3272	*Actinidia deliciosa*	New Zealand
Terribacillus sp.	-	*Actinidia chinensis*	China
Enterobacter sp.	-	*Actinidia chinensis*	China
Erwinia.	-	*Actinidia chinensis*	China

[a]Fifteen *Pseudomonas syringae* pv. *actinidiae* strains isolated from different regions of Shaanxi, China.

DNA extraction

Cultures for DNA extraction were grown overnight in LB at 28°C on a rotating shaker at a speed of 200 rpm. DNA was extracted by using the DNeasy Tissue Kit (Qiagen, USA). DNA quality and concentration were detected by agarose gel-electrophoresis and spectrophotometric analysis (DU Series 500UV-Vis, Beckman, CA, USA). The DNA samples were stored at -80°C until used.

Primer design

The internal transcribed spacer (ITS) region of *Pseudomonas syringae* pv. *actinidiae* (AY342165) was used as reference sequences for primer design. LAMP primers were designed using Primer Explorer V4 software from Eiken Chemical Co. Ltd., Japan with default settings (http://primerexplorer.jp/e/v4_manual/index.html). A set of six primers were designed: inner primers FIP and BIP, outer primers F3 and B3 and loop primers LF and LB. Primer sequences are listed in Table 2. The primers for PCR were designed according to Rees-George et al. (2010). All primers were PAGE purified and synthesized by Sanggon (Shanghai, China).

Table 2. The nucleotide sequences of the primers used in the LAMP assay.

Primer name	Type	Sequence 5'-3'
F3	Forward outer	CCGATTTTGGGTCTGTAGCT
B3	Backward outer	GTAAGTGGTGGAGCCAAGC
FIP	Forward inner	CGCGCTCTGACCAACTTCACCACCCCTGATAAGGGTGAGG
BIP	Backward inner	TACGACACCCGGATACGGGGATCGAACCGCTGACCTCC
LF	Loop forward	GGCAGATTCGAACTGCCGA
LB	Loop backward	CCATAGCTCAGCTGGGAGA

Optimization of LAMP amplification

LAMP of *Pseudomonas syringae* pv. *actinidiae* was carried out in 25 µL total volume containing 1.6 µM each inner primer (FIP and BIP), 0.2 µM each outer primer (F3 and B3), 0.8 µM each loop primer (LF and LB), 1 mM of the dNTP mix (Promega, Madison, WI, USA), 8 mM of $MgSO_4$, 1×ThermoPol buffer (20 mM of Tris–HCl, 10 mM of KCl, 10 mM of $(NH4)_2SO_4$, 0.1% Triton X-100), 10 U of *Bacillus stearothermophilus* (*Bst*) DNA polymerase (large fragment; New England Biolabs Inc., Beverly, MA, USA) and 1 µL of the target DNA. To optimize the LAMP reaction, different primers, reaction temperatures, Mg^{2+} concentrations, and dNTPs concentrations were tested. The mixture was incubated at 61-65°C for 60 min followed by 5 min at 80°C using Loopamp real-time turbidimeter (LA-230; Eiken Chemical Co., Ltd., Tochigi, Japan). Two different methods were used to detect LAMP products. For direct visual inspection, the reaction product became white turbid for the positive reaction, or remained translucent and clear for the negative reaction (Figure 1). For monitoring turbidity, the real-time LAMP products were monitored through spectrophotometric analysis by recording the optical density at 400 nm every 6 s (LA-230; Eiken Chemical Co., Ltd., Tochigi, Japan). All the experiments were repeated three times.

Sensitivity of LAMP and PCR

To determine the sensitivity of primers in LAMP and PCR detection of *Pseudomonas syringae* pv. *actinidiae*, pure genomic DNA was serially diluted tenfold (from 8 ng μL^{-1} to 8 fg μL^{-1}) and amplified using the two amplifications which were set-up sequentially. The sensitivity of LAMP was determined under optimal reaction conditions and amplification products were analyzed by real-time monitoring through spectrophotometric analysis. The PCR assay for *Pseudomonas syringae* pv. *actinidiae* was described previously using PsaF1/PsaR2 as primers in this study (Rees-George et al., 2010) and the amplification product was analyzed by gel-electrophoresis followed by sequence confirmation.

Figure 1. Visual detection of *Pseudomonas syringae* pv. *actinidiae* by LAMP. An obvious white precipitate could be observed with the naked eye under the black ground in the positive samples. The negative control remained clear and translucent.

Specificity of LAMP

To test the specificity of LAMP primers for *Pseudomonas syringae* pv*. actinidiae*, 33 bacterial strains were used, including *Pseudomonas syringae* pv. *actinidiae* (fifteen, all from China), *Pseudomonas syringae* pv. *actinidiae* (Psa-V), *Pseudomonas syringae* pv. *actinidiae* (Psa-LV), *Pseudomonas syringae* pv. *syringae* (three), *Pseudomonas syringae* pv. *theae*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae* pv*. papulans* (two)*, Pseudomonas cichorii*, *Pseudomonas corrugata*, *Pseudomonas fluorescens*, *Pseudomonas marginalis*, *Pseudomonas viridiflava*, *Pseudomonas* sp*.*, *Terribacillus* sp*.*, *Enterobacter* sp*.* and *Erwinia*, with distilled water as the negative control. Details of the bacterial strains are shown in Table 1.

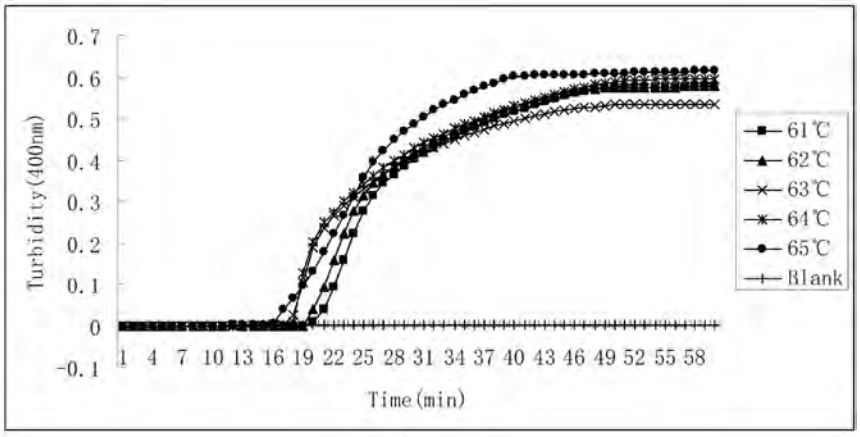
Detection of kiwifruit field samples

The clinical samples collected from the field were detected using both PCR and LAMP assay. Two kinds of templates were used. One was DNA extracted from plant samples with symptoms and the other was bacterial suspension isolated from plant samples, which was directly subjected to LAMP and PCR without DNA extraction. DNA samples from healthy leaves were used as a negative control. The LAMP amplification products were firstly analyzed by direct visual inspection of the reaction tube under white light and both for LAMP and PCR confirmed by agarose gel-electrophoresis analysis.

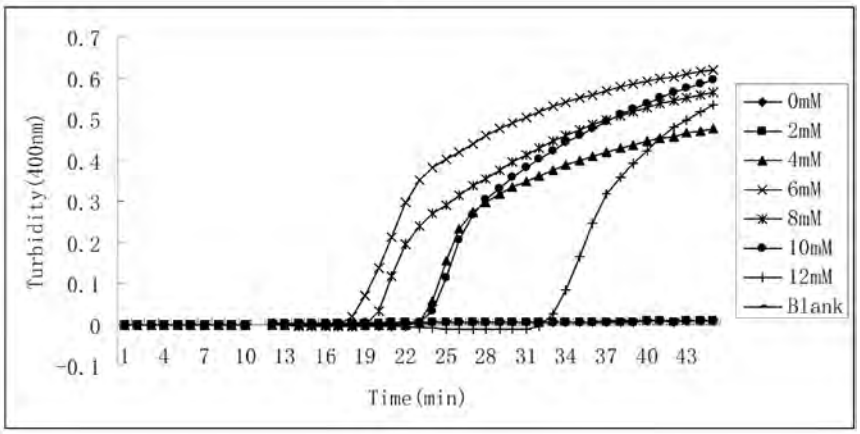
RESULTS AND DISCUSSION

Optimization of LAMP amplification

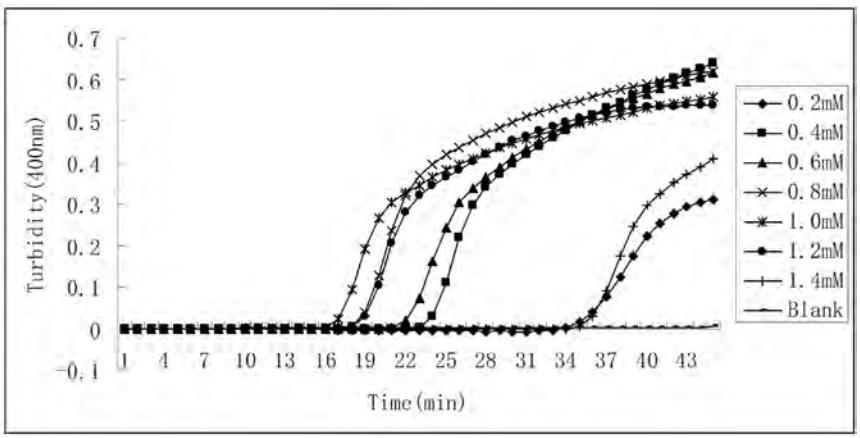
The optimal temperature of LAMP for the designed primers was determined with the DNA used as a template. We observed different temperatures from 61-65°C, at 1°C intervals. The result indicated that the suitable reaction temperature was 65°C (Figure 2A). Eventually, we chose 65°C as the reaction temperature. The influence of Mg^{2+} and dNTPs concentration on LAMP reaction was evaluated for the primers by examining the amplification time. The results exhibited that the optimal concentration of Mg^{2+} was 8 mM (Figure 2B), and dNTPs 1.0 mM (Figure 2C).



A



B

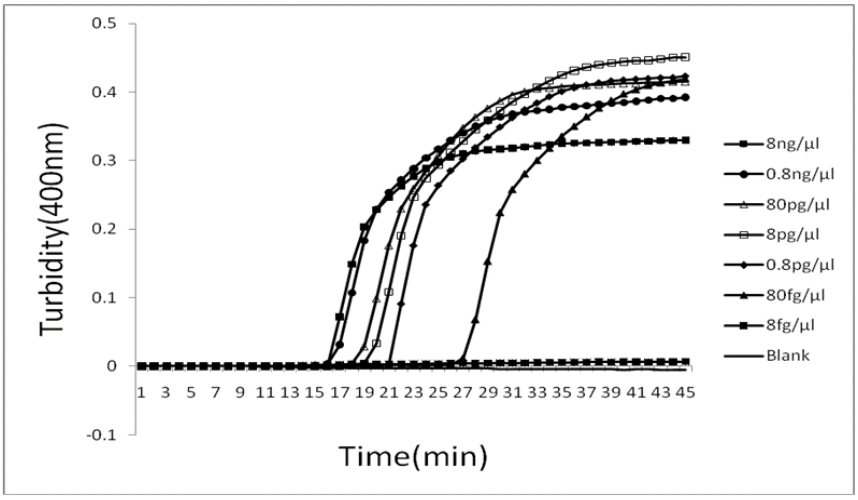


C

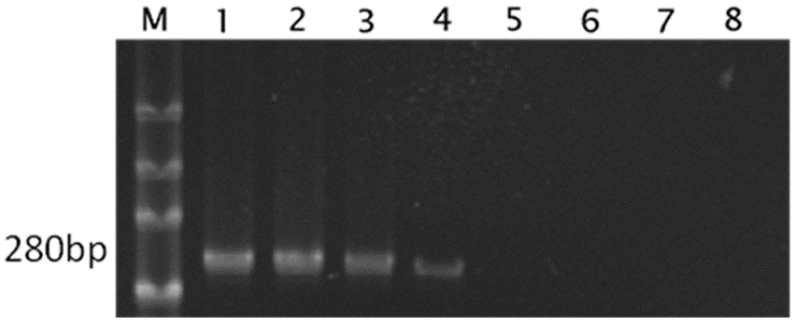
Figure 2. (A) Effect of temperature on the time kinetics of the LAMP reaction of *Pseudomonas syringae* pv. *actinidiae* as monitored by measurement of turbidity in a Loopamp real-time turbidimeter (LA-320C). (B) Effect of Mg^{2+} concentration on the time kinetics of the LAMP reaction of *Pseudomonas syringae* pv. *actinidiae* as monitored by measurement of turbidity in a Loopamp real-time turbidimeter (LA-320C). (C) Effect of dNTPs concentration on the time kinetics of the LAMP reaction of *Pseudomonas syringae* pv. *actinidiae* as monitored by measurement of turbidity in a Loopamp real-time turbidimeter (LA-320C).

Sensitivity of LAMP and PCR

The LAMP amplification was compared with the conventional PCR assay. As shown in Figure 3A, the reaction time increased with the decreased DNA diluted concentration. The reaction was preceded with 1 µL of tenfold serial dilutions of genomic DNA (8 ng μL^{-1}), and the minimal detection limit of the LAMP assay was 80 fg μL^{-1}. However, for PCR, the weak band could be at least observed at the concentration of 8 pg μL^{-1} (Figure 3B). In conclusion, the detection sensitivity of LAMP for *Pseudomonas syringae* pv. *actinidiae* was 100 times higher than that of the conventional PCR.



A



B

Figure 3. (A) Sensitivity of the LAMP assay for detection of *Pseudomonas syringae* pv. *actinidiae* DNA as monitored by measurement of turbidity in a Loopamp real-time turbidimeter (LA-320C). Serial 10-fold dilutions of DNA ranging from 8 ng μL^{-1} to 8 fg μL^{-1} were tested. (B) Sensitivity of the PCR assay for detection of *Pseudomonas syringae* pv. *actinidiae* DNA by agarose gel-electrophoresis analysis. Lanes 1-7: Serial 10-fold dilutions of DNA ranging from 8 ng μL^{-1} to 8 fg μL^{-1} were tested, 8: Blank.

Specificity of LAMP

To test the specificity of LAMP primers, we used 19 bacterial strains to compare, and distilled water as the negative control (Table 1 and Figure 4). All the genomic DNA was extracted from pure cultures. The results showed that *Pseudomonas syringae* pv. *actinidiae* (from China), *Pseudomonas syringae* pv. *actinidiae* (Psa-V), *Pseudomonas syringae* pv. *actinidiae* (Psa-LV), pv. *tomato* and *Pseudomonas syringae* pv. *theae* were tested positive when by the LAMP method (Figure 4). Because pv. *tomato* and *Pseudomonas syringae* pv.

theae are unlikely to be found on kiwifruit, the primers are recommended for screening bacterial strains isolated from kiwifruit tissue.

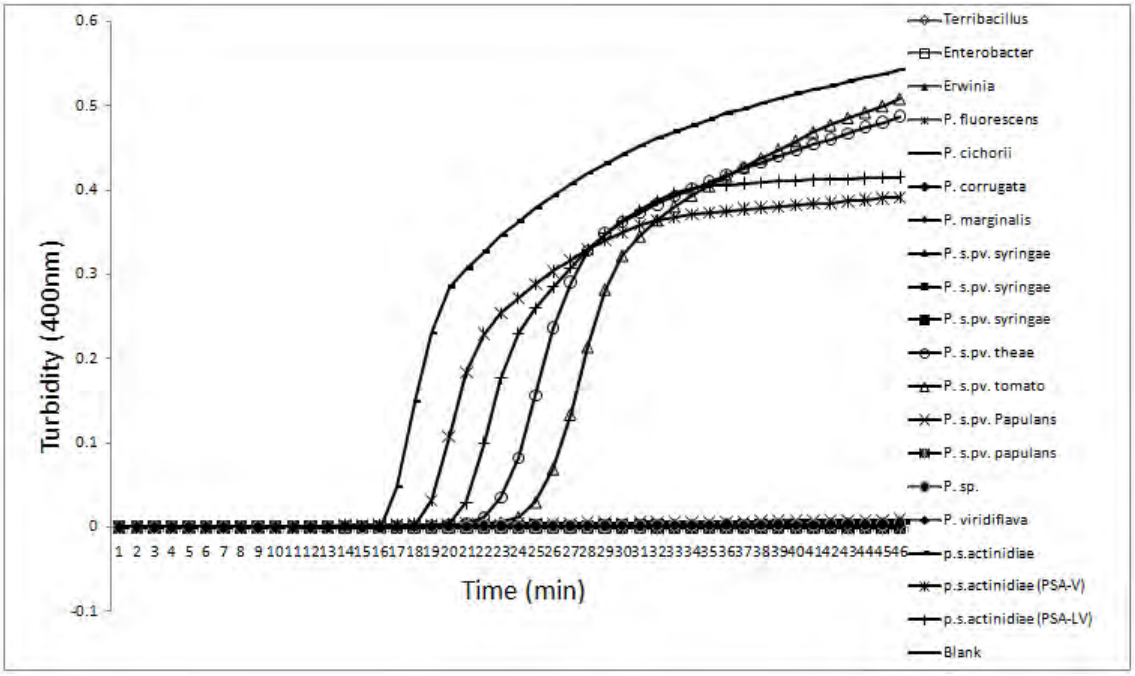


Figure 4. Specificity of LAMP for *Pseudomonas syringae* pv. *actinidiae* detection as monitored by measurement of turbidity in a Loopamp real-time turbidimeter (LA-320C). DNA templates of the LAMP amplification included 15 *Pseudomonas syringae* pv*. actinidiae* (from China), *Pseudomonas syringae* pv*. actinidiae* (Psa-V), *Pseudomonas syringae* pv. *actinidiae* (Psa-LV), *Terribacillus* sp., *Enterobacter* sp., *Erwinia*, *Pseudomonas fluorescens*, *Pseudomonas cichorii*, *Pseudomonas corrugata*, *Pseudomonas marginalis*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *theae*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae* pv. *papulans*, *Pseudomonas* sp., *Pseudomonas viridiflava* and distilled water, respectively. Detailed information is given in Table 1.

Detection of kiwifruit field samples

A total of 50 field samples collected from different areas was tested using both LAMP and PCR assays, respectively (data not shown). Eighteen of 50 samples were positive by LAMP, using both DNA and bacterial suspension templates. All of the 18 samples were positive when detected by PCR using primer PsaF1/PsaR2 and PsaF3/PsaR4 (Rees-George et al., 2010). The 18 PCR amplification products were analyzed by gel-electrophoresis followed by sequence confirmation. This indicates that bacterial suspension could be the template for LAMP. Meanwhile, it's obvious that LAMP assay takes less time than the PCR assay. All the results confirmed the ease of use of this LAMP assay for routine detection.

Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* is currently an emerging disease that causes major losses in China and many other countries. Vanneste et al. (2010) reported that production of a red exudate on kiwifruit vines is not a symptom that is specific to bacterial canker. Therefore, it is necessary to develop highly sensitive and reliable detection protocols for improving disease management. PCR methods have been developed and applied to the detection of *Pseudomonas syringae* pv. *actinidiae* in previous studies (Rees-George et al., 2010; Koh and Nou, 2002; Balestra et al.,

2013). But PCR detection methods need circulation amplification and gel imaging instruments, which are expensive for local laboratories and experimental stations. However, LAMP requires no special equipment except for a simple temperature-controlled water bath (Notomi et al., 2000). Furthermore, LAMP was faster than PCR. It takes at least three hours to get the PCR results, but less than one hour for LAMP. In addition, sensitivity of LAMP was approximately 100-fold higher than that of the conventional PCR method in detecting the presence of *Pseudomonas syringae* pv*. actinidiae.* Finally, Wang et al. (1993) developed a simple and rapid method for preparing plant samples as templates for PCR, and equally applied it to LAMP. In this study, bacterial suspension could also be the templates for LAMP, which makes the LAMP method more probable in field detection.

In this study, ITS regions were used to design LAMP primers. When it comes to specificity of LAMP, it was shown that LAMP was not able to distinguish *Pseudomonas syringae* pv. *actinidiae* from *Pseudomonas syringae* pv. *theae* or pv. *tomato*, Which is the same problem as faced by Rees-George et al. (2010) in PCR detection. It was because of their phylogenetically close relationship based on the core genome analysis (Sarkar and Guttman, 2004; Vanneste et al., 2009). However, the gelatin liquification test and utilization of trigonelline could be able to distinguish *Pseudomonas syringae* pv*. actinidiae* from *Pseudomonas syringae* pv. *theae* and pv. *tomato* (Scortichini et al., 2002). On the other hand, Vanneste et al. (2010) developed BOX-PCR detection methods for *Pseudomonas syringae* pv. *actinidiae*, which allowed differentiation between *Pseudomonas syringae* pv. *actinidiae* and *Pseudomonas syringae* pv. *theae*. Moreover, *Pseudomonas syringae* pv. *theae* generally was isolated from tea plants (Scortichini et al., 2002). So the LAMP primers are considered specific enough for routine detection. .

Compared with conventional PCR, this LAMP assay possesses the advantages of cost-effective instruments, speed (1 h) and convenient operation, and may be useful for *Pseudomonas syringae* pv. *actinidiae* identification, detection, and its control strategies.

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Nondestructive internal quality assessment of kiwifruit by Near-Infrared (NIR) spectroscopy

Jing Li[1,2,3,a], Tiao Ning[1,2,3], Ruili Zhang[2], Yu Zheng[1,2], Qingqing Li[4,5], Yongfa Yang[2], Jianying Chen[6] and Biao Zen[7,b]

[1]Agricultural College, Kunming University, Yunnan, Kunming 650214, China; [2]Engineering Research Center for Urban Modern Agriculture of Higher Education in Yunnan Province, Kunming University, Yunnan, Kunming 650214, China; [3]Yunnan Miaozai Technology Co. Ltd., Yunnan, Kunming 650204, China; [4]Key Laboratory for Forest Resources Conservation and Use in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming 650224, China; [5]Life Science College, Southwest Forestry University, Kunming 650224, China; [6]Yunnan Dianjing Agricultural Technology Service Co., Ltd Yunnan, Kunming 650000, China; [7]Transformation management office of scientific and technological achievements, Yunnan Academy of Agricultural Sciences, Yunnan, Kunming 650205, China.

Abstract

In this study, original near-infrared spectra were collected from 350 kiwifruit samples from 15 cultivars in Yunnan province. The samples were analyzed with three types of pretreatment approaches including second derivative, smoothing denoising by nine point moving average window, and multiplicative scatter correction. The partial least-square regression (PLS) method was employed to correct and predict six property data models of kiwifruit including soluble solids content, pH, total sugar, total acid, Vitamin C and protein content. Comparison of the coincidence degrees for the corresponding content models of kiwifruit showed Protein>Vitamin C>soluble solids content>total sugar>total acid>pH.

Keywords: Near-Infrared (NIR) spectroscopy, nondestructive determination, kiwifruit, soluble solid content (SSC), pH

INTRODUCTION

Kiwifruit has high nutritional value and is extensively consumed worldwide. When purchasing kiwifruit, consumers not only consider the external factors including size, hardness, color and surface but also the internal qualities including sweetness, acidity, vitamin and nutrient content. Besides, both the shelf-life and additional values could be improved using the appropriate classification method in picking and post-harvest processes. Therefore, fast, accurate and nondestructive detection of kiwifruit internal quality has become a priority for planting and processing enterprises, and consumers.

Along with the development of spectral analysis technique and its application in agricultural production, the optical fiber probe based spectral analysis has been gaining acceptance among the nondestructive methods for determining internal qualities of fruits (Bobelyn et al., 2010; Nicolaï, 2007). The pectic substances in the kiwifruit cells contain O-H and C-H chemical bonds that facilitate near-infrared (NIR) absorption, which led to the chemical basis for quantitative analysis using NIR spectroscopy (Osborne et al., 1998; Costa et al., 1999). In recent years, researchers in China and other countries have made many efforts for the detection of kiwifruit internal quality with the NIR characteristics. McGlone and Kawano (1998) presented the prediction model for hardness, dry matter, and soluble solids content (SSC) of kiwifruit using the NIR spectrum ranging from 800 to 1100 nm. Clark et al. (2004) predicted the storage deterioration of kiwifruit in harvest process using the NIR spectrum ranging from 300 to 1140 nm. Martinsen and Schaare (1998) compared the NIR spectra of kiwifruit core, seed, and pericarp using the NIR spectra ranging from 650 to

[a]E-mail: 5455o3991@qq.com
[b]E-mail: 1046412028@qq.com

1100 nm. Slaughter and Crisosto (1998) analyzed the spectral characteristics of mature and immature kiwifruits using NIR reflectance ranging from 700 to 1100 nm. To the best of our knowledge, few studies have reported NIR analysis of internal qualities of different varieties of kiwifruits in Yunnan Province.

In this study, original NIR spectra were collected from 350 kiwifruit samples from 15 cultivars in Yunnan Province, and then analyzed with three types of pretreatment approaches including second derivative, smoothing denoising by nine point moving average window, and multiplicative scatter correction. The partial least-square regression (PLS) method was employed to correct and predict six property data models of kiwifruit, including SSC, pH, total sugar, total acid, vitamin C and protein content. It was used to demonstrate the feasibility of measuring routine physical and chemical parameters using NIR spectroscopy.

MATERIALS AND METHODS

Sampling and experimental approaches

Kiwifruit samples from 15 cultivars were delivered from five production bases in Yunnan Province. A total of 360 kiwifruit samples were picked without strumae, deformity, mildew on surface, stabbing wound, rot, cold injury and softening. Of these, 300 samples were used as calibration set, 50 samples were used as forecast set, and 10 samples were reserved for backup.

The Antaris II type Fourier transform NIR spectrometer (Thermo Scientific, United States) was employed to collect the spectra. The PAL-1 type digital refractometer (ATAGO, Japan) was employed to measure the SSC content. The FE 20 type laboratory pH apparatus (Mettler-Toledo instruments) was employed to measure the pH value. Vitamin C was measured using GB5009.86-2016 as the standard. For determining the total sugar, proteins were removed from the samples, and the oligosaccharides were hydrolyzed to monosaccharides with hydrochloric acid by heating, and then its content was measured by direct titrimetric method using GB5009.86-2016 as the standard. Total acid was measured using GB/T12456-2008 as the standard. Protein content was measured using Dumatec 8000 type nitrogen determination system (Foss).

NIR spectra collection

The Antaris II Fourier transform NIR spectrometer was first activated for normal inspection and preheated over 30 min. Meanwhile, the RESULT-Integration software was employed to establish the corresponding workflow. The samples were prepared and sequentially numbered. Three NIR spectra were collected along kiwifruit equatorial range with an interval of 120° using the pedicel and stylar end as two poles. The model was established by averaging the three measured spectra. The spectra were collected using the integrating sphere diffuse reflection method, and the acquisition spectral range was from 4000 to 10000 nm with resolution of 8 cm^{-1} and scanning number of 64.

Treatment and analysis of spectral data

The TQ Analyst software was employed for the analysis and model building of NIR spectra collected from 300 kiwifruit samples. The models were established based on the PLS method combined with pretreatment approaches including multiplicative scatter correction, derivative processing, and differential filtering for smoothing. The correlation coefficient (R^2), root-mean-square error correction (RMSEC), and root-mean-square error prediction (RMSEP) were employed to comprehensively evaluate the correction and prediction effects of these models. The prediction effect of the model was positively related to R^2, and negatively related to RMSEP. In addition, NIR spectra collected from 50 kiwifruit samples were randomly selected to establish an independent check set without model building, which were used to determine the difference between the measurement results and database.

RESULTS AND DISCUSSION

Selection of spectral range

The full band information was employed for model building to avoid the loss of effective information and achieve better data integrity. The spectral ranges of SSC, vitamin C content, pH value, total sugar content, total acid content and protein content were 4043.07-9994.970, 4019.91-8994.11, 4031.23-9977.34, 4070.85-9768.35, 4027.28-9985.24, and 4035.74-9972.95 cm^{-1}, respectively. The measured NIR spectra are shown in Figure 1.

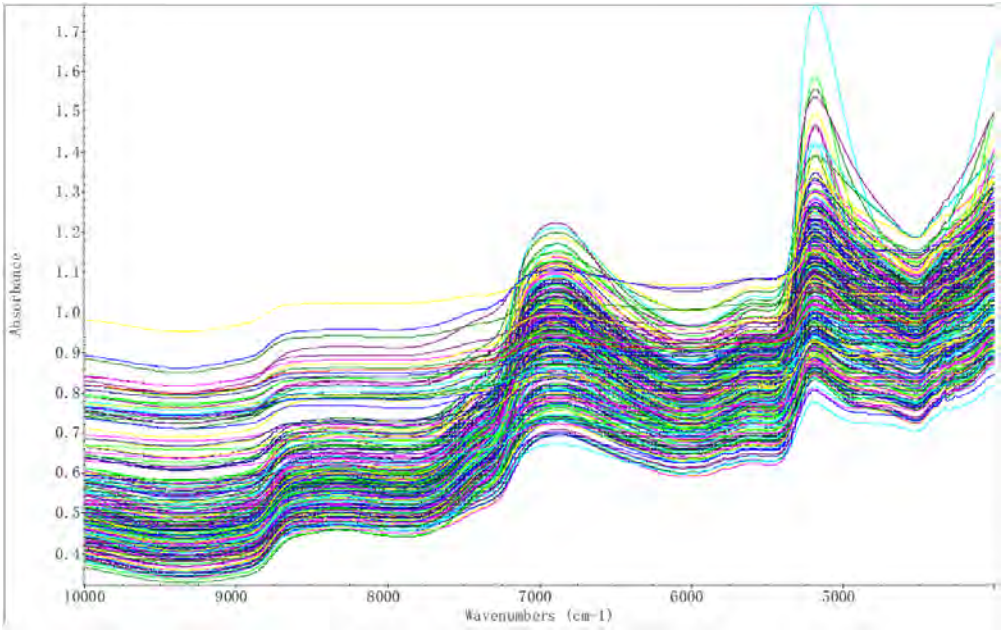

Figure 1. NIR spectra of kiwifruit samples.

Spectral pretreatment

The RMSEC, Root Mean Square Error of Cross Validation (RMSECV), main factors and R^2 were determined by PLS modeling method and employed as reference for analysis of different combinations of pretreatment conditions to achieve the optimal combination. The results are shown in Table 1.

Table 1. Analysis of results with different combinations of pretreatment conditions.

Parameters	Combination of pretreatment conditions[1]	Factors	Correction set		Verification set	
SSC	MSC+2nd+Ndf(5,3)	9	0.9525	0.801	0.7744	1.97
pH	MSC+2nd+Ndf(7,3)	8	0.9039	0.120	0.8216	0.160
Vc	MSC+2nd+SGf(11,3)	4	0.9532	28.60	0.9256	73.3
Total sugar	MSC+2nd+SGf(5,6)	6	0.9456	0.415	0.5856	0.951
Total acid	MSC+2nd+SGf(5,3)	6	0.9449	3.360	0.7953	1.27
Protein	MSC+2nd+SGf(5,5)	6	0.9941	0.00774	0.5375	0.0778

[1]MSC: multiplicative scatter correction; 2nd: second Derivative; Ndf: Norris Differential Filter; SGf: Savitzky Golay Filter.

Selection of optimal factor

The interaction analysis approach was commonly employed for determining the optimal factor to avoid excessive noise induced by "under-fitting", "inadequate use of information" or "over-fitting". This is because RMSECV would decrease along with the main factors in the interaction analysis process. The main factor was optimal, while the RMSECV was minimal. The prediction error was minimal as the optimal main factor was employed for model building and unknown sample prediction.

Optimization and verification of the model

This model was established to analyze the NIR spectra collected from kiwifruit samples. The NIR spectra of samples with extraordinary and similar characteristics were excluded to achieve even distribution in the principal component space. The RMSEC, RMSECV, factors, and R^2 were employed as reference parameters. Comprehensive judgment was achieved using Mahalanobis distance, leverage values, biochemical residuals to optimize the model step-by-step for achieving the best condition. The data related to the established models are shown in Table 2. The scatter diagrams of chemical measurement and model prediction results are shown in Figures 2-7.

As shown in Table 2 and Figures 2-7, six types of nutrient contents were highly related to the NIR spectra of kiwifruit in this study, and the correlation coefficient was >0.90. This indicated that the prediction of corresponding component contents in kiwifruit using the model had high accuracy and practical value. Among them, the protein content had highest relativity.

Table 2. The parameters related to the established models.

Parameters	Number of samples in calibration set	R^2	Number of main factors	RMSEC%	Prediction range (%)
SSC	300	0.9525	9	0.801	8-20
pH	300	0.9039	8	0.120	1-7
Vc	300	0.9532	4	28.6	30-500
Total sugar	216	0.9456	6	0.415	1-15
Total acid	209	0.9449	6	3.36	15-80
Protein	166	0.9941	6	0.00774	0-5

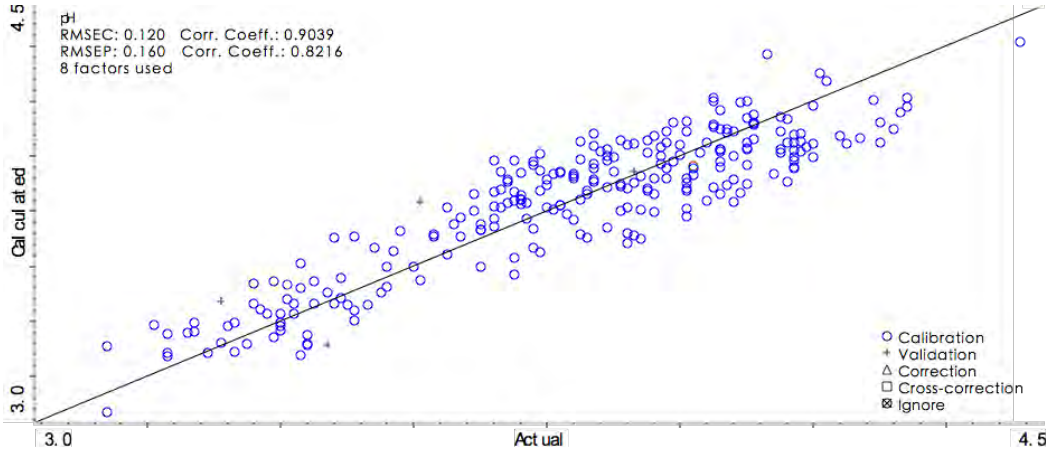

Figure 2. The scatter diagrams of chemical measurement results and model prediction results for pH values.

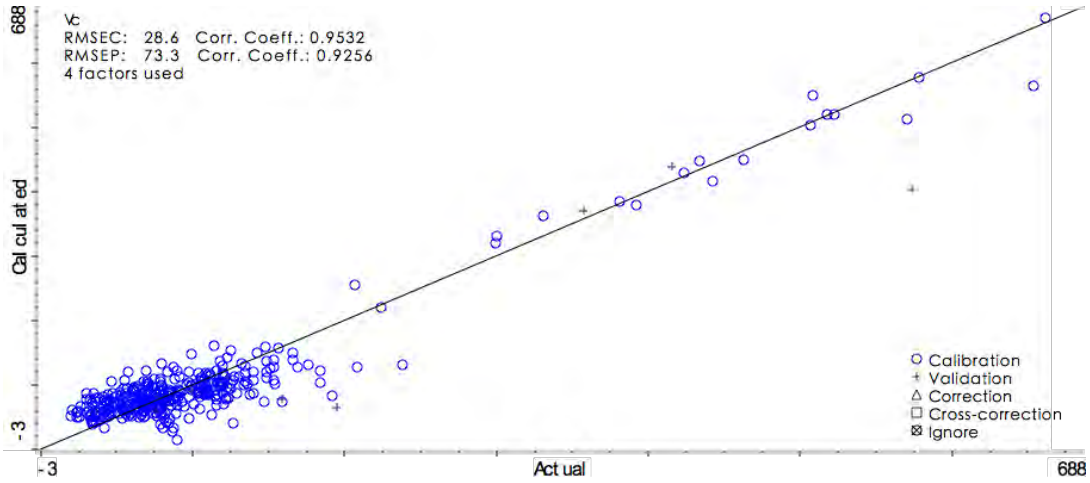

Figure 3. The scatter diagrams of chemical measurement results and model prediction results for vitamin C.

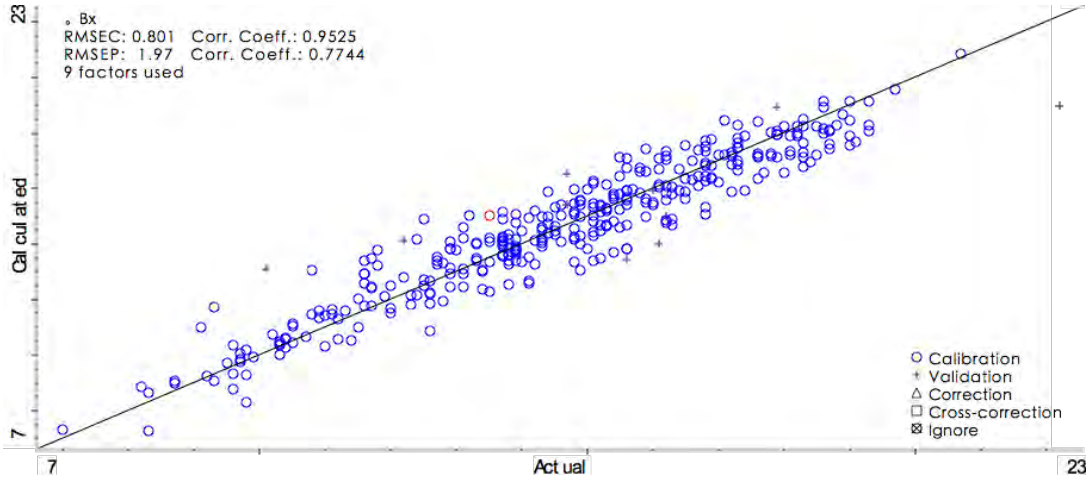

Figure 4. The scatter diagrams of chemical measurement results and model prediction results for degrees Brix.

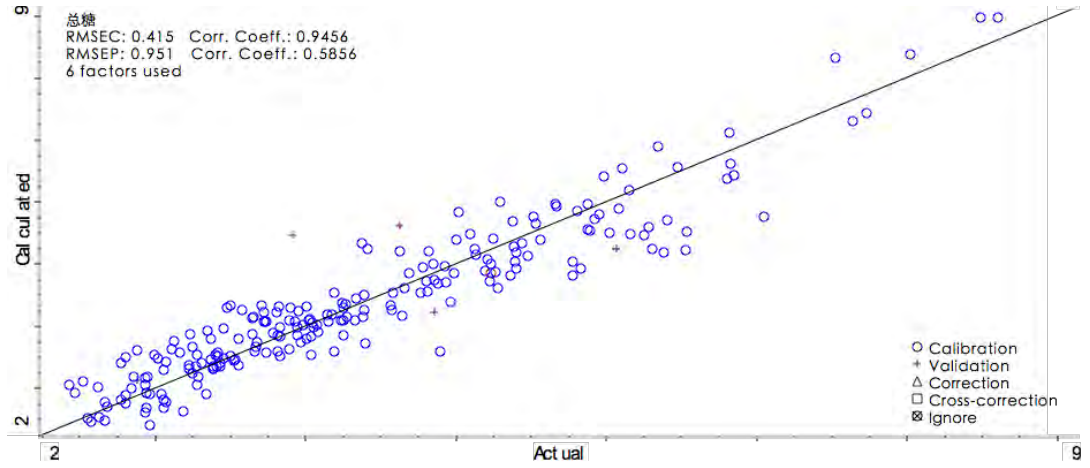

Figure 5. The scatter diagrams of chemical measurement results and model prediction results for total sugar.

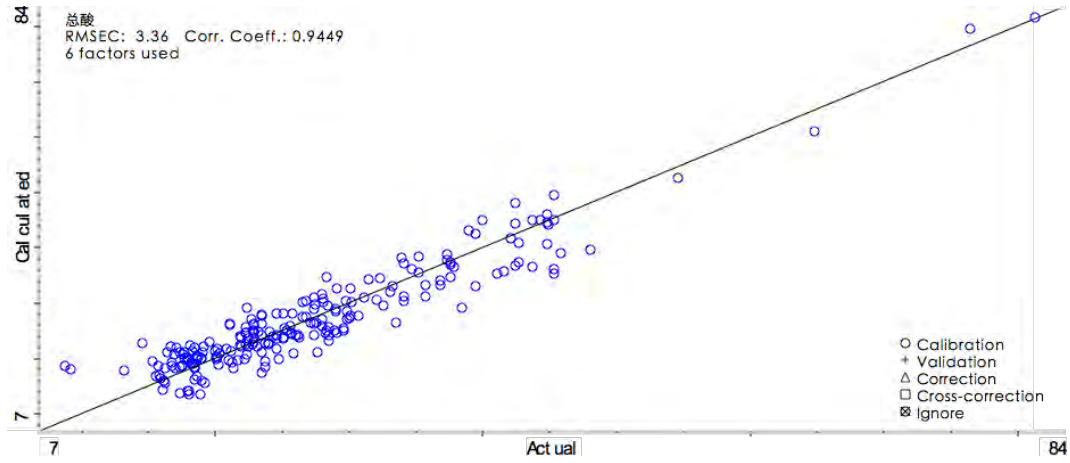

Figure 6. The scatter diagrams of chemical measurement results and model prediction results for total acid.

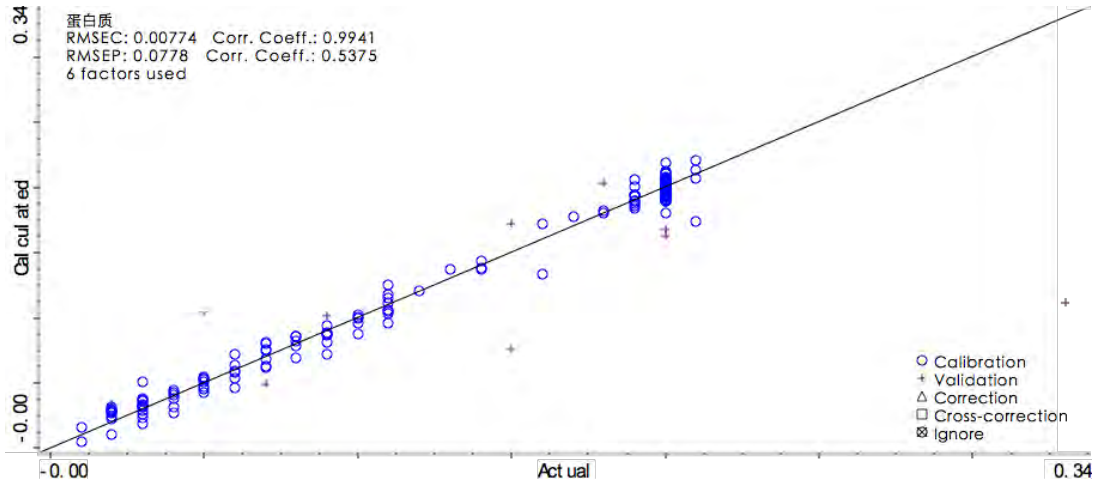

Figure 7. The scatter diagrams of chemical measurement results and model prediction results for proteins.

Comparison of prediction reliability

Fifty spectral data were randomly selected from the 350 spectral data to establish the validation set to test the reliability and stability of the correction model. This set was treated as an independent check set without model building, and used to determine the difference between the measurement results and database. The validation results are shown in Table 3.

The R^2 values of SSC, pH value, vitamin C content, total sugar content, total acid content, and protein content were 0.9525, 0.9039, 0.9532, 0.9456, 0.9449, and 0.9941, while the corresponding averaged relative error (%) were 0.119, 0.065, 0.270, 0.339, 0.180, and 0.246, respectively. These data indicated that the NIR prediction value had minor difference with the chemical measurement results. Therefore, the model had practical value, and could be used for predicting six types of nutrient contents in the kiwifruit samples.

Table 3. Validation results.

Parameter	Averaged relative error (%)
Brix degree	0.119
pH	0.065
Vitamin C	0.27
Total sugar	0.339
Total acid	0.18
Protein	0.246

CONCLUSION

The internal quality of kiwifruit varies with time. In this study, the Fourier transform NIR spectrometer was employed for nondestructive analysis and evaluation of kiwifruit, and correlation models between the NIR spectra and kiwifruit compositions including SSC, pH value, vitamin C content, total sugar content, total acid content and protein content were established to analyze the related data. The results showed that the modeling spectral range for SSC, pH value, vitamin C content, total sugar content, total acid content, and protein content were 4043.07-9994.970, 4019.91-8994.11, 4031.23-9977.34, 4070.85-9768.35, 4027.28-9985.24, and 4035.74-9972.95 nm, respectively. The number of their main factors was 9, 8, 4, 6, 6, and 6, respectively. The R2 values of their calibration set were 0.9525, 0.9039, 0.9532, 0.9456, 0.9449, and 0.9941, respectively. Their RMSEC values were 0.801, 0.120, 28.6, 0.415, 3.36, and 0.00774%, respectively. Their RMSECV values were 0.119, 0.065, 0.27, 0.339, 0.18, and 0.246%, respectively. The coincidence degrees for the corresponding content models of kiwifruit were: protein>vitamin C>soluble solids content>total sugar>total acid>pH.

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Kiwifruit quality: management in the supply chain

J. Burdon

The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Victoria Street West, Auckland 1142, New Zealand.

Abstract

The management of kiwifruit quality in the supply chain starts in the orchard. Growing a cultivar in the right environment, applying the best vine management and careful use of plant growth regulators all play roles in producing fruit with the potential for good quality. Making sure that the potential at harvest is delivered further down the supply chain depends on understanding the fruit. The physiological state of the fruit at harvest determines the suitability for specific marketing periods, therefore fruit should be harvested to meet a marketing plan. The temperature management applied should match any chilling risk and also the storage period requirement. If fruit are being harvested and sold early, an ethylene ripening system ensures the supply of good quality uniform fruit through retail and to the consumer. However, if attempting to prolong storage, ethylene should be avoided and measures taken to prevent exposure within the coolstore. Finally, the storage life of the fruit may be extended by controlled atmosphere storage and/or the use of SmartFresh™. Commercially, robust quality assurance procedures ensure that the at-harvest potential is translated into a high quality fruit for the consumer.

Keywords: kiwifruit, *Actinidia*, storage, quality, softening, disorder, chilling, environment

BACKGROUND

Kiwifruit belong to the genus *Actinidia* with the first international shipments of fruit from New Zealand occurring in the 1950s. The global industry has been built on the *Actinidia chinensis* var. *deliciosa* cultivar 'Hayward', originally from New Zealand, but now grown in all the major kiwifruit-exporting countries including New Zealand, Italy and Chile, as well as the home of kiwifruit – China (Belrose Inc., 2016). Part of the commercial success of the 'Hayward' cultivar is that the fruit can be stored for a long time, as well as being of good size and flavour.

Kiwifruit are harvested in a mature but unripe state, allowing the fruit to withstand the rigours of commercial handling and storage. By the time the fruit reach the consumer, they should have ripened to a juicy, melting texture. This ripening largely occurs during storage at low temperature. The commercial challenge is therefore to ensure that the fruit store appropriately and ripen to provide good eating quality without any defects.

Whilst the global industry was built on the 'Hayward' cultivar, there are currently new cultivars available for commercial production with different attributes that distinguish them from 'Hayward' fruit. There is no guarantee that these new cultivars will behave the same as 'Hayward' in their physiological development and postharvest management, thereby adding complexity to the general understanding of kiwifruit as trade diversifies into multiple cultivars (Burdon, 2018).

QUALITY

The concept of quality may differ depending on position in the supply chain, or may be reduced to a generalised phrase such as "fitness for purpose". However, quality can be thought about in a couple of ways. First there is the inherent quality of a particular cultivar: the fruit flavour, size, shape, colour etc. These are expected when buying a particular cultivar. Secondly, there are risks to quality which can occur through the production and postharvest supply chain.

During production the fruit environment and vine management will interact to affect the fruit development, including the composition and maturation of the fruit (Patterson and Currie, 2011; see also paper by Patterson and Currie in this issue). Too hot, too cold, too much or too little water, all affect vine productivity and therefore also the fruit. Likewise having too many fruit on the vine may limit the final fruit size or sweetness. Finally, the use of plant growth regulators, including cytokinins for increasing fruit size, can have serious detrimental effects on the fruit quality and composition. Common defects caused by the over-use of compounds such as forchlorfenuron (CPPU, a cytokinin plant growth regulator) to increase fruit size include the occurrence of cavities in the fruit core and reduced dry matter in the fruit.

The commonest forms of quality assessment for kiwifruit are based on fruit firmness measurements and visual inspection for freedom from defects. A considerable emphasis is placed on the consumer as the final purchaser of the fruit. However, the consumer can purchase only those fruit that a retailer chooses to stock. It is therefore critical to maintain the confidence of major retailers, since it is they who allow the consumer the opportunity to purchase a particular brand of fruit. Retailer confidence is maintained through a robust knowledge-based quality assurance programme to ensure that good quality fruit are supplied – and if there is a problem it can be managed quickly and with a minimum of fuss for the retailer. A knowledge of the fruit is essential for the identification of critical control points and the establishment of measurable and documented monitoring and response procedures.

HARVEST

The postharvest behaviour of fruit is dependent on the physiological state of the fruit at harvest. The current ability to understand the physiological state of the fruit is largely based on simple, easy-to-measure compositional aspects of the fruit. These simple-to-measure fruit attributes are somewhat blunt tools in determining the physiological state of the fruit (Burdon, 2015). Recent research has moved from looking at the fruit composition to the way in which the fruit responds to stimuli such as harvesting or response to ethylene or temperature. The responses to stimuli can indicate changes in the fruit physiological state of significance to postharvest behaviour, but not signalled by the compositional measures currently used to develop harvest indices.

Consideration of kiwifruit being ready for harvest tends to start once the seeds in the fruit have darkened fully. Thereafter two important factors are whether the fruit will have an acceptable taste when ripe, and whether they will withstand prolonged low temperature storage. The best storage potential, as judged by lack of chill sensitivity, tends to coincide with the cessation of fruit growth and carbohydrate accumulation. Thereafter the fruit physiology changes into ripening mode, with starch to sugar conversion and softening, which may reduce the potential storage life, yet reduces the risk of chilling damage.

For 'Hayward' kiwifruit grown in New Zealand, there has been a continual evolution and refining of harvest indices. In the 1950s, there was no harvest index and, perhaps not surprisingly, there were very mixed out-turns. This led in the late 1960s to the introduction of a start date of 1 May for harvesting fruit for export. While this improved out-turns, there were still some instances of poor quality on arrival. In 1980, a minimum harvest soluble solids content (SSC) of 6.2 °Brix was established for fruit for export. This index was based on the fact that once fruit had reached an SSC of 6.2 °Brix, the rate of soluble solids accumulation had increased associated with low night temperatures and a conversion of starch to soluble sugars (Burdon, 2015). In the late 1990s, harvesting was focused to meet specific marketing periods, whereby fruit not destined for long storage could be harvested earlier. Currently, the harvest window for 'Hayward' fruit grown in New Zealand is 2-3 months depending on the target sales period.

GRADING AND PACKING

Fruit brought into the packhouse in bins or crates can be dealt with in one of two ways. The first is simply to load the bins/crates into coolstores and store the fruit ungraded and unpacked. This may be in air or controlled atmospheres. Alternatively, the fruit may be graded on arrival. With most of the poor quality fruit removed and the remainder sized, the graded fruit may then go back into bins or be packed and palletised for storage. The major difference between storage of packed or unpacked fruit is the risk of water loss from the unpacked fruit. In addition, if unpacked fruit remain in the bins or crates until they are too soft, they may be damaged either through compression loads in the bin, or during subsequent handling. Irrespective of whether packed or not, the main challenge in storage is to maintain fruit firmness and avoid disorders.

Traditionally, the grading out of poor quality fruit has depended on having a trained workforce. However, it is now possible to have many external quality defects identified by vision systems, and the removal of surface defects such as misshapen fruit, blemishes, punctures and mould has been automated, limiting the need for human intervention. In addition, it is becoming more common for near infra-red systems to determine the internal quality of fruit – largely the dry matter or SSC of the fruit. An at-harvest dry matter measurement can predict the ripe fruit SSC (rSSC), which has a strong influence on consumer liking of the fruit.

The act of grading may cause damage to the fruit. This may arise from fruit- contact with equipment or from fruit-to-fruit contact. The potential for specific types of damage may depend on the cultivar or whether the fruit is firm or soft. An obvious difference between 'Hayward' and the *A. chinensis* var. *chinensis* cultivar 'Hort16A' is the presence of a beak at the stylar end of the 'Hort16A' fruit. This beak may cause damage when contacting other fruit. Impacts on 'Hort16A' tend to leave very distinctive round dark marks on the fruit; often the point of impact can be seen in the centre. This sort of dark discoloration is not seen in 'Hayward'. If fruit are too soft when graded, there is a risk of fruit being damaged and juice being spread onto other fruit. This juice provides a substrate for opportunistic fungi such as *Alternaria* to grow on in storage, creating a commercially unacceptable dark 'storage stain'.

FIRMNESS-BASED SUPPLY CHAIN DECISIONS

Firmness thresholds based on simple penetrometer measurements (Abbott and Massie, 1995) can be established so that fruit are not handled when too soft. Different thresholds can be set for holding fruit in a bin, for dumping onto the grader, for passing fruit across the grader and then for packing into single layer trays or bulk packaging. A final firmness threshold can be applied before fruit leave the packhouse/coolstore to ensure that the fruit have sufficient life to reach their destination market. To achieve these thresholds, a knowledge of the fruit softening pattern in storage is essential. Kiwifruit softening is not linear; it has three distinct phases (MacRae and Redgwell, 1996): an initial slow phase (Phase 1) followed by a fast phase (Phase 2) and a final slow phase (Phase 3; see Figure 1). However, if fruit are harvested late, the fruit softening on the vine will have progressed beyond the initial slow phase and only the second and third phases will be seen in storage. The softening pattern, and therefore potential for damage, is cultivar dependent. While all cultivars have a similar sigmoidal softening pattern, the relative timings and rates of softening differ, thereby affecting firmness-based decisions.

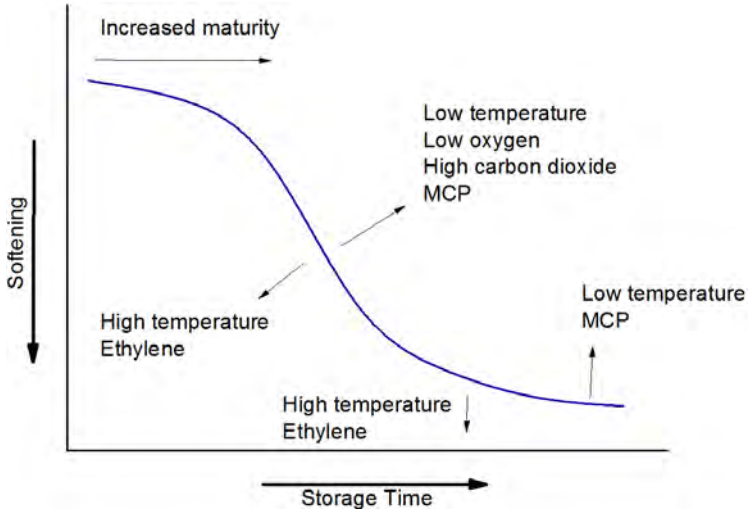

Figure 1. A generalised softening curve showing the way in which manipulation of kiwifruit physiology can be used to affect softening in storage. 1-methylcyclopropene (MCP) is the active ingredient in SmartFresh™.

SUPPLY WINDOW

Maximising the marketing period can be achieved by maximising both the harvest period and the storage life. The division of fruit into early and late supply is significant, since each sector requires specific harvest and supply chain conditions (Burdon and Lallu, 2011).

Early supply usually depends on harvesting fruit once they have the capacity to be ripened with ethylene to an acceptable taste. The use of ethylene provides fruit that are ripening, although fully ready-to-eat fruit are too fragile for many supply chains. The ethylene treatment also creates uniformity within and among pallets of fruit which may be from different orchards. The use of ethylene also prevents the occurrence of hard cores, which may be a risk in early harvested fruit. Finally, while the correct use of ethylene is beneficial for logistics, and can result in good eating-quality fruit, there can be problems. Used incorrectly, ethylene can accelerate fruit softening ahead of starch conversion, leaving a soft, ripe fruit without its full rSSC.

Late supply depends on the extension of the fruit storage life through technologies including refrigeration, controlled atmospheres (low oxygen and high carbon dioxide) and the management of ethylene: removal of exogenous ethylene from the store atmosphere or the blocking of ethylene action within the fruit by SmartFresh™. Managing softening through the manipulation of physiological responses is summarised in Figure 1.

For kiwifruit, and most fresh produce, temperature management is the primary method for extending storage life. Before cooling the harvested fruit, information is needed on the fruit maturity, and therefore the chilling risk, and the storage window required. This information will decide what cooling profile is required and, just as importantly, whether or not the requirement matches the cooling capacity available. Furthermore, whether there is any delay, or if there should be a delay, before cooling. A delay may be necessary for temperature management purposes or for botrytis stem-end rot control (termed curing; Lallu et al., 1997).

The cooling rate and storage temperature are both largely dictated by the chilling susceptibility of the fruit. The initial cooling rate may be achieved by pre-cooling or by passive room cooling. In both cases these should be knowledge-based decisions. If pre-cooling, then what air temperature will be used initially and what temperature will fruit reach before removing from the pre-cooler? Likewise, if using room cooling, is there sufficient cooling capacity for the heat load of the product in the room, and is the air flow going to be adequate?

It should be noted that when fruit are held unpacked in a room, water loss occurs; the longer the room is left not full, the greater the water loss from those fruit that are in the room. The final point is knowing the storage temperature required. To some degree this is dependent on the inherent risk of chilling for a specific cultivar and the storage period required. In general terms, the fruit storage temperature may be gradually decreased as the fruit ripen in the store.

In addition to refrigeration, controlled atmospheres (CA) can prolong the storage life of kiwifruit. The storage of kiwifruit in CA stores has been a commercial reality for many years. The procedure is necessary only where prolonged storage periods are required. While in the early days of CA storage the fruit tended to provide the atmospheric modification, today equipment is available for the initial establishment of a low oxygen atmosphere, achieved using a nitrogen generator/oxygen scrubber, usually of a carbon molecular sieve or membrane design. As the fruit are still respiring, even at low temperature and low oxygen, any excess carbon dioxide produced has to be removed, usually by an activated carbon dioxide scrubber or simple bags of lime.

Ethylene management is critical to the long-term storage of kiwifruit. The fruit are highly sensitive to ethylene, softening in response to even low dose exposure at low temperatures. It is therefore essential to avoid any exogenous ethylene: by avoiding sources of ethylene, by removing ethylene from the store atmosphere by ventilation, by absorption by chemical means such as potassium permanganate pellets, or through scrubbing with a platinum catalyst scrubber or treating with ozone to break down the ethylene molecule. More recently, the ethylene action blocker SmartFresh has become available. The active ingredient 1-methylcyclopropene (1-MCP) blocks the active site that would normally be filled by ethylene, preventing the fruit from responding to ethylene. In this way, SmartFresh treatment protects the fruit from exogenous sources of ethylene as well as blocking any ethylene produced within the fruit.

DISORDERS

Fruit disorders may be categorised as physical, physiological or pathological (Burdon and Lallu, 2011). Physical disorders arise from rough handling or trying to handle fruit after it has become too soft. Particular cultivars will have specific attributes which may make them more or less susceptible to certain forms of damage. 'Hort16A' fruit were well known for their distinctive stylar-end beak and the potential for it to cause damage during harvesting and grading. Much of the risk of physical damage can be managed through careful handling procedures and understanding the potential risks as the fruit soften.

A range of physiological disorders have been described, including tissue breakdown, chilling injury, physiological pitting, lenticel marks, soft patches, hard cores and white core inclusions (Burdon, 2018). Some of these have been sporadic or reported only in occasional scientific papers. Physiological pitting and lenticel marks were prevalent in the early commercialisation of 'Hort16A', but disappeared as vines became more established. Likewise, physiological pitting in 'Hayward' in New Zealand has disappeared recently, possibly a result of changes to vine management, or to changing environmental conditions.

The one physiological problem which appears common to all cultivars is chilling injury. Initially described as tissue breakdown, it was sometimes confused with senescence. With kiwifruit being stored commercially at, or close to, 0°C, the problem of chilling is sometimes overlooked. Yet chilling susceptibility is fundamental to determining the postharvest procedures for the kiwifruit supply chain. A chilling disorder termed low temperature breakdown (LTB) was originally described for 'Hayward' fruit and categorised by a granular appearance in the outer pericarp and water-soaked tissues in the outer or inner pericarp (Lallu, 1997). As more cultivars have been commercialised, further symptoms have been reported, including discoloration of the stylar end of the fruit in 'Hort16A' and the *A. chinensis* var. *chinensis* cultivar 'Zesy002'. Also, in 'Zesy002', severely disordered fruit may have an outer pericarp with a dry corky appearance. As

more cultivars are commercialised it is likely that a wider range of symptoms may be observed. Unlike many disorders, LTB can be managed. While susceptibility is dependent on fruit maturity at harvest, the expression of the symptoms depends on the subsequent temperature management and storage duration, i.e., fruit that are susceptible at harvest can be managed to minimise postharvest expression of the disorder. In particular, early harvested fruit should not be stored for too long.

SUMMARY

An understanding of the biology and postharvest responses of the fruit assists in the development of postharvest management and quality assurance procedures. These procedures define the operations of packhouse and coolstore operaters, and retailers, such that the consumer receives fruit of good quality. The procedures will be specific for both cultivar and marketing requirements (early or late supply). The physiological state of the fruit at harvest is the starting point after which the acceleration or slowing of fruit ripening allows for the orderly marketing of good quality fruit over a prolonged period.

ACKNOWLEDGEMENT

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Sugars and organic acids changes in flesh (outer and inner cortex) of kiwifruit during development and postharvest

Yanfei Liu[1], Bin Zhou[1], Xin Chen[2], Hongzhi Meng[3], Zhande Liu[1,a] and Xiaolin Ren[1,b]

[1]College of Horticulture, Northwest A&F University, Yangling, 712100, Shaanxi, China; [2]Shaanxi Fruit Industry Group, Yangling, 712100, Shaanxi, China; [3]Zhuolu County Forestry Bureau, Zhuolu, 075600, Hebei, China.

Abstract

'Qihong' is a new kiwifruit cultivar with red flesh in the inner pericarp, is of high quality and has a relatively long storage life. Previous studies on the soluble sugars and organic acids in kiwifruit have concentrated on their content during fruit maturation. The detailed changes in sugars and organic acids from development to postharvest of kiwifruit have seldom been investigated. Glucose, fructose, and sucrose were the major sugars in the inner and outer cortexes of three kiwifruit cultivars, 'Hayward', 'Hongyang' and 'Qihong'. However the concentrations and proportions of the individual sugars were distinctive for each cultivar. Quinic and citric acids were the main organic acids of each cultivar during development to postharvest, but their levels followed opposite trends: quinic acid decreased slightly during fruit growth and development in general, while citric acid increased. The ratio, sugar: organic acids, in the outer cortex of 'Qihong' was significantly high, but the correlative parameter value of sweetness index was highest in 'Hongyang'.

Keywords: kiwifruit, outer cortex, inner cortex, sugars, organic acids

INTRODUCTION

Kiwifruit is an important export crop mainly produced in China, Italy, New Zealand, and Chile (Mack et al., 2017). At present, kiwifruit (*Actinidia*) is not only commonly consumed in western countries (Henare et al., 2012) but it has also become a new commercial fruit crop in China (Ma et al., 2017). According to Li et al. (2007), the genus *Actinidia* comprises around 75 distinct taxa exhibiting a wide range of flesh colors. Not all taxa have commercial importance: *A. deliciosa* and *A. chinensis* are the main species (Garcia et al., 2012). The nutritional components of kiwifruit include: abundant vitamin C, amino acids, balanced mineral composition, high dietary fiber and various other healthful metabolites (Chen et al., 2015). Free sugars and organic acids are main nutrients and they play crucial roles in maintaining fruit quality and determining nutritive value (Wu et al., 2012). In particular, the balance of soluble sugars and organic acids is important in influencing the flavor of kiwifruit flesh (Nishiyama et al., 2008).

A number of studies have reported on the content and concentration of soluble sugars and organic acids in kiwifruit (MacRae et al., 1989; Nishiyama et al., 2008; Kim et al., 2012; Richardson et al., 2011). Sugars in kiwifruit are mainly sucrose, fructose, and glucose, and the organic acids include quinic, citric, malic, and ascorbic acids (Nishiyama et al., 2008). Many factors can affect the concentrations of sugars and organic acids. Genotype (species and variety) are the most important. Using high-performance liquid chromatography Nishiyama et al. (2008)determined the soluble sugars and organic acid contents in the fruit of various *Actinidia* genotypes at the eating-ripe stage. In addition, environmental and horticultural conditions can also influence the concentrations. The starch, quinic and citric acid concentrations varied at maturity in fruit of the same cultivar from two orchards (MacRae et al., 1989). High temperatures during growth reduced fruit

[a]E-mail:dezhanliu@vip.sina.com

[b]E-mail: rxlyy601@163.com

carbohydrate and vitamin C in kiwifruit but raised the relative sucrose concentrations (Richardson et al., 2004). Cold storage and ozone treatment had an effect on soluble sugars and organic acids in *A. deliciosa* (Barboni et al., 2010). These authors showed that during storage at 0°C, concentrations of non-volatile organic acids were maintained but organic acids decreased sharply after 25 weeks during storage in an ozone chamber. Furthermore, the concentrations of fructose, glucose, and quinic acid were higher in ethylene-induced ripened kiwifruit than in kiwifruit that had ripened naturally postharvest (Lim et al., 2017).

In conclusion, most previous studies concentrated on the content of sugars and organic acids in fruit at harvest. Besides these studies, there are only a few studies of changes in sugars and organic acids before maturation. Changes in sugars and organic acids during fruit development are important (Glew et al., 2003). Such changes in sugars and organic acids have been analyzed in different fruit such as citrus (Albertini et al., 2006; Chen et al., 2009), loquat (Chen et al., 2009), medlar (Glew et al., 2003), peach (Wu et al., 2012), and American cranberry (Wang et al., 2017). Only Kim et al. (2012) have investigated the changes of sugar and organic acid in hardy kiwifruit. Until now, there have been few studies of changes in sugars and organic acids in the various fruit tissues of different cultivars of kiwifruit.

In recent years, flesh color has become an important basis for consumer preferences (Jaeger and Harker, 2005; Li et al., 2012). 'Qihong' (QH) is a new kiwifruit cultivar selected from 'Hongyang' (HY). It has high quality (total sugar 12.56%, TA 1.14% and vitamin C 0.972 mg g^{-1}) and a relatively long storage life (Yu et al., 2015). Anthocyanins accumulate mainly in the inner cortex (pericarp), creating an attractive, red, star-shape in the center and it has a sweeter taste than HY. HY is one of the main commercial red-fleshed kiwifruit now grown in China. 'Hayward' (HWD), which has deep green fruit flesh, is widely cultivated around the world.

The objective of this work was to compare the changes in sugars and organic acids in different tissues in the three kiwifruit cultivars, QH, HY, and HWD, during fruit development to postharvest stages. Our results demonstrate differences in the ratios and concentrations of sugar and organic acid contents in the outer and inner cortex of the three kiwifruit cultivars.

MATERIALS AND METHODS

Fruit

'Hongyang' (acid-free cultivar) and 'Hayward' (acidic cultivar) are commercial cultivars in China, and 'Qihong' is a new kiwifruit cultivar with good characteristics selected from 'Hongyang'. Fruit were harvested from each of the three cultivars from similar-aged vines in Kiwifruit Farm in Meixian, Shanxi, China. Sampling dates for each cultivar are reported as days after pollination (DAP). Fruit were collected at seven preharvest stages: 25, 45, 65, 85, 105, 125, and 145 DAP. At each sampling time, 20 fruit were collected at random from three vines of each cultivar. By 145 DAP, the fruits of all three cultivars had reached commercial harvest maturity. Fruits were then harvested and stored at room temperature (±22°C). A total of 20 fruit of each genotype was examined after 10 days storage (155 DAP) and a further 20 at full ripeness on 170 DAP ('Hongyang' and 'Qihong') or on 190 DAP ('Hayward').

At each stage, 10 fruit were used to measure fresh weight and dry matter content. A 2 mm-thick transversal slice from the equator of each fruit was used to measure dry matter content. The fruit slices after their fresh weights (FW) had been determined were dried at 105°C in a vacuum oven (DZF-6050, Shanghai, China) to constant weight (DW). The dry matter content is FW/DW × 100%. These analyses were carried out in triplicate. 10 fruit were carefully separated into outer and inner cortexes (seeds were removed) and the separated tissues immediately frozen in liquid nitrogen after peeling (removing the

skin to approximately 1 mm depth). Outer and inner cortexes were lyophilized, powdered and stored at -80°C for analysis.

Determination of sugars and organic acids

For quantification of sugars and organic acids, 100 mg of each powdered sample was homogenized with 15 mL 80% ethanol. The mixture was incubated at 50°C with ultrasonic sound for 40 min. After cooling, the homogenate was centrifuged at 8,000× g for 10 min. The supernatant was transferred into a 5 mL centrifuge tube. The residue was re-extracted by incubation for 20 min. After centrifugation, the second supernatant was added to the first. After shaking, 1 mL of the combined supernatants was dried in a rotatory evaporator at 70°C and then dissolved in 1 mL ddH2O. After filtration through a 0.22 μm, 13 mm diameter syringe filter, the filtrate was collected for sugar and organic acid analysis. Three replicates were performed for each sample

Sugars were separated by HPLC, using a NH2 bound silica column at 40°C. Acetonitrile: water (80:20, v/v) was the mobile phase at a flow rate of 1.0 mL min^{-1} for elution. Peaks were detected with a refractometer index detector. Organic acids were determined using a Perkin Elmer Series 200 Model HPLC apparatus with a UV absorbance detector set at 214 nm. Isocratic elution was carried out with a methanol: 0.02 mol L^{-1} aqueous potassium dihydrogen phosphate solution (pH 2.8) 3:97 (v/v) as the mobile phase. The flow rate was 0.5 mL min^{-1} and the column temperature was maintained at 40°C.

Commercial standard solutions of each sugar and organic acid by appropriate dilutions were used to prepared calibration curves. Sugars and acids were identified and quantified by the retention time and the peak area in the chromatograms compared to the standard. Concentrations were expressed as mg g^{-1} fresh weight (FW).

Data analyses

Estimation of the kiwifruit taste parameter, Sweetness index (SI), for the different cultivars analyzed was calculated as described for strawberries (Keutgen and Pawelzik, 2007). Briefly, the contribution of each major sugar was calculated, considering that fructose and sucrose are 2.3 and 1.35 times sweeter, respectively, than glucose. Accordingly, SI = 1.0 [glucose] + 1.35 [sucrose] + 2.3 [fructose].

RESULTS

Changes in soluble sugar concentrations in the outer cortex of three kiwifruit cultivars during development and ripening

The outer cortex of kiwifruit contained three major soluble sugars: glucose, fructose, and sucrose (Figure 1). During development and postharvest, the trends in soluble sugar concentrations in the outer cortex of the three kiwifruit cultivars were approximately similar, but the concentration of any sugar was different. The concentrations of the three sugars remained low before maturity in all three cultivars, while they increased sharply after harvest. QH contained the highest concentration of sucrose (91.19 mg g^{-1} FW) compared with HY (59.96 mg g^{-1} FW) and HWD (14.82 mg g^{-1} FW) at postharvest. Interestingly we found the concentration of sucrose was higher than hexose, especially after S7 and finally reached the highest level (91.19 mg g^{-1} FW) in QH (Figure 1A). The concentrations of glucose and fructose were low and the concentrations and trends in concentration were basically the same and each highest concentration amounted to approximately only 30% of the sucrose. Throughout development and at the postharvest stages in HY (Figure 1B), the three sugars were almost identical whether in concentration or trend. However, in the outer cortex of HWD (Figure 1C), the concentration of sucrose always remained low and the concentration of fructose and glucose were respectively 2.84 and 2.95 times that of sucrose at eating-ripeness.

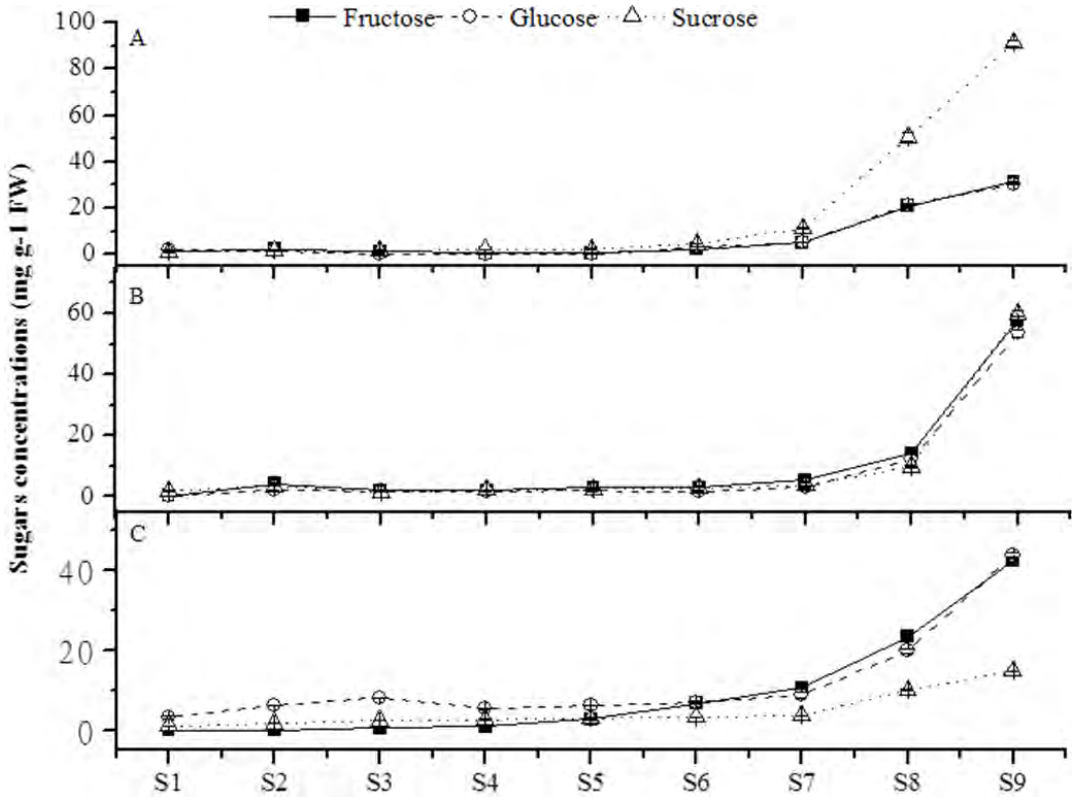

Figure 1. Changes in the concentrations of soluble sugars in the outer cortex of three kiwifruit cultivars during development and postharvest. Results represent means ± SE of three replicates. (A) 'Qihong', (B) 'Hongyang' and (C) 'Hayward'. Stages S1 – S7 correspond to 25, 45, 65, 85, 105, 125, and 145 days after pollination (DAP); S8 after storage at room temperature for 10 days after harvest (155 DAP), S9 when the fruit were eating ripe (170 DAP for 'Hongyang' and 'Qihong', 190 DAP for 'Hayward').

Changes in soluble sugar concentrations in the inner cortex of three kiwifruit cultivars during development and ripening

We found that the trends in soluble sugar concentrations in the inner cortex of the three kiwifruit cultivars were similar to those in the outer cortex (Figure 2). However, the total soluble sugar concentration in the inner cortex of HWD (Figure 2C) was higher than in the outer cortex, while the reverse was true for QH and HY. In the inner cortex of QH (Figure 2A), the total concentration of soluble sugar increased from 6.99 to 125.72 mg g^{-1} FW, although the concentration was higher at the earlier stage of development and then decreased followed by an rapid increase again. Sucrose of inner cortex also accounted for the largest proportion of sugar, but its concentration (68.14 mg g^{-1} FW) was low compared with outer cortex at the eating-ripe stage. The concentrations of the three sugar in inner cortex of HY were similar to those in the outer cortex (Figure 2B), but the glucose concentration was slightly higher than fructose during postharvest stages. During development and ripening, the hexose was the major sugar while sucrose increased quickly and then the concentration exceeded that of the hexose at final eating-ripeness. The concentrations of glucose, fructose, and sucrose in the inner cortex of HWD (Figure 2C) increased with some fluctuations and reached the highest levels (59.18, 51.82 and 18.79 mg g^{-1} FW) at eating-ripeness.

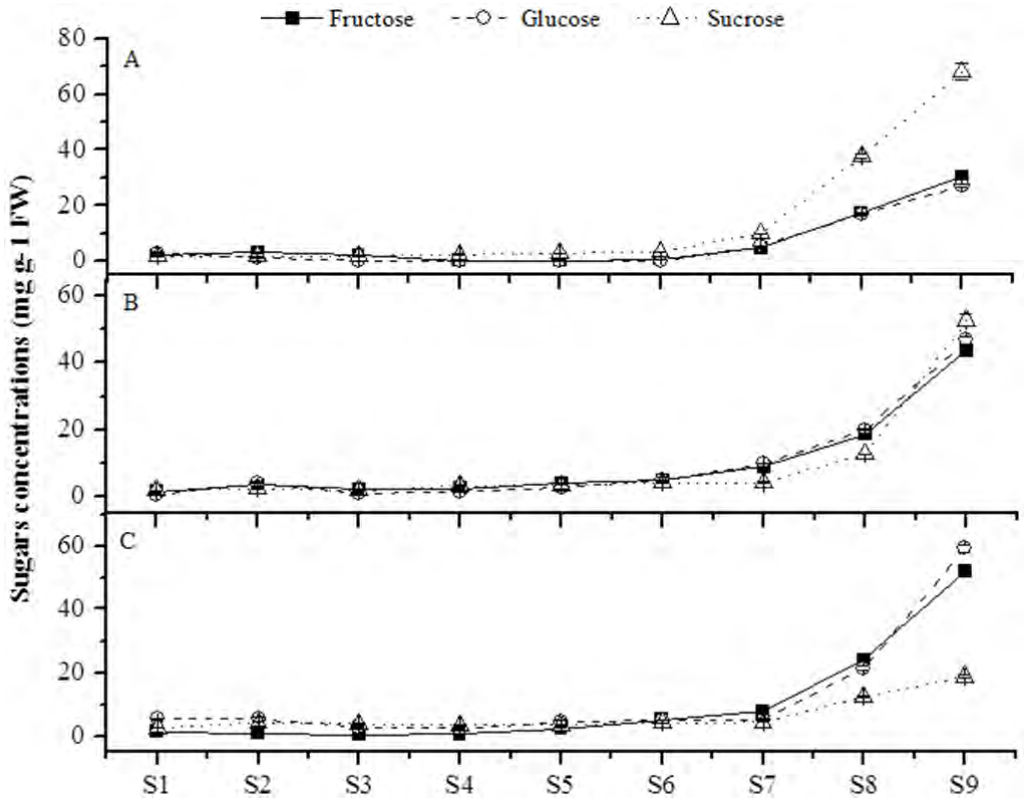

Figure 2. Changes in the concentrations of soluble sugars in the inner cortex of three kiwifruit cultivars during development and postharvest. Results represent means ± SE of three replicates. (A) 'Qihong' (B) 'Hongyang' and (C) 'Hayward'. Stages S1 – S7 correspond to 25, 45, 65, 85, 105, 125, and 145 days after pollination (DAP); S8 after storage at room temperature for 10 days after harvest (155 DAP), S9 when the fruit were eating ripe (170 DAP for 'Hongyang' and 'Qihong', 190 DAP for 'Hayward').

Changes in organic acid concentrations in the outer cortex of three kiwifruit cultivars during development and ripening

Concentrations of the five main organic acids (quinic, citric, malic, tartaric and ascorbic acid) in the outer cortex of three kiwifruit cultivars were measured by HPLC (Figure 3). Throughout development and the postharvest stages, the trends in organic acid concentrations differed in the three cultivars. In the outer cortex of QH (Figure 3A), the total concentration of organic acids decreased slightly from S3-S6, but overall generally increased and reached a peak concentration (38.42 mg g^{-1} FW) at eating-ripeness. In HY (Figure 3B), the total organic acid concentration tended to increase and then at the postharvest stages sharply increased, reaching a peak of 53.97 mg g^{-1} FW. We found that the organic acid concentration of HWD initially decreased and then, after fluctuating, reached a peak of 33.41 mg g^{-1} FW, while in the postharvest stages, it increased slightly (Figure 3C).

Quinic and citric acids were the most abundant organic acids while malic, tartaric and ascorbic acids were present at a low level in each kiwifruit cultivar. During early fruit development, quinic acid concentrations increased very slightly in the outer cortex of QH fruit but in the other two cultivars decreased (Figure 3). In the postharvest stages, quinic acid concentration of QH increased slightly at first and then decreased, the other acids varied inconsistently. Although the citric acid concentration was initially low in all three cultivars it steadily increased and eventually exceeded the concentration of quinic

acid. Of the three organic acids present in only low concentrations, there was apparently more malic acid than tartaric and ascorbic acids in the outer cortex of three cultivars. Trends in tartaric and ascorbic acid concentrations were similar in QH and HY (Figure 3A-B) but in HWD the concentration of ascorbic acid was higher than that of tartaric acid and initially it accounted for the largest proportion of the three organic acids (Figure 3C).

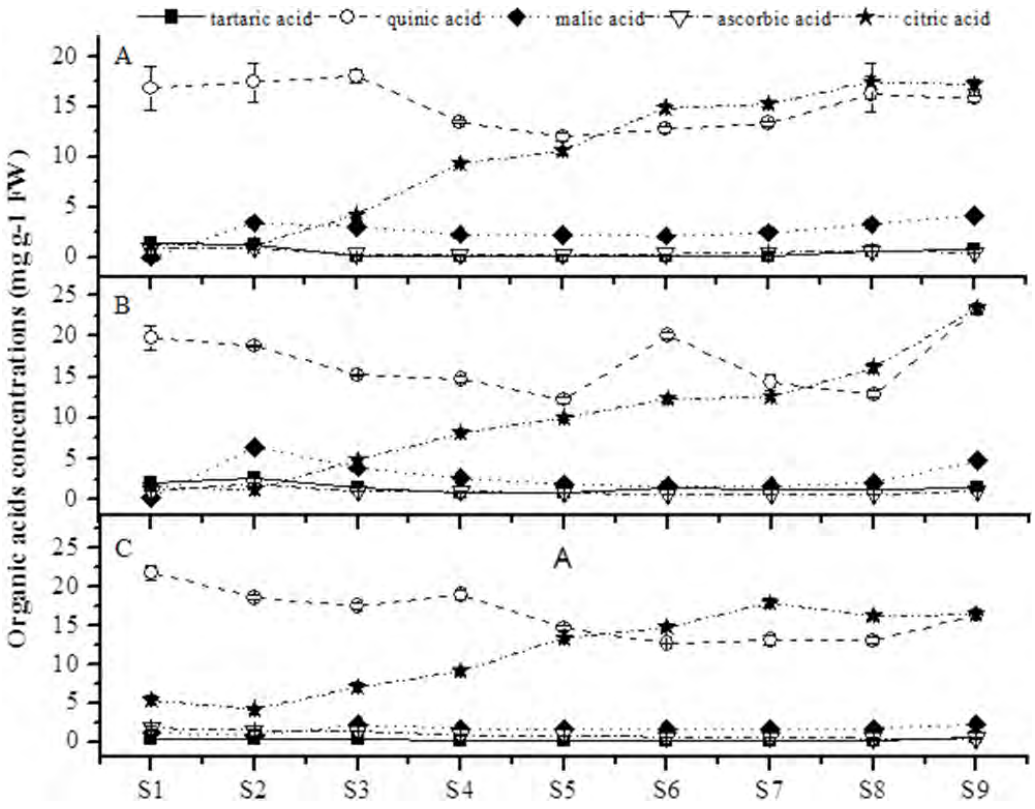

Figure 3. Changes in the concentrations of organic acids in the outer cortex of three kiwifruit cultivars during development and postharvest. Results represent means ± SE of three replicates. (A) 'Qihong', (B) 'Hongyang' and (C) 'Hayward'. Stages S1-S7 correspond to 25, 45, 65, 85, 105, 125, and 145 days after pollination (DAP); S8 after storage at room temperature for 10 days after harvest (155 DAP), S9 when the fruit were eating ripe (170 DAP for 'Hongyang' and 'Qihong', 190 DAP for 'Hayward').

Changes in organic acid concentrations in the inner cortex of three kiwifruit cultivars during development and ripening

The concentrations and trends in organic acid concentrations in the inner cortex of the three kiwifruit cultivars were very different from those in the outer cortex (Figure 4). In the inner cortex of QH, the total organic acid concentration was clearly higher than that in the outer cortex and the trends in individual acid were similar while the citric acid was the major content comparing with that in outer cortex, after S4. While the concentration of quinic acid fell only slightly after S3, with a peak value of 18.05 mg g^{-1} FW at S2. The three other acids showed similar trends and concentrations (Figure 4A).

Quinic and citric acid concentrations decreased in the postharvest stages in HY fruit and increased in HWD fruit (Figure 4B-C). Of the other three organic acids, malic acid predominated in all cultivars examined, and in QH and HY the changes in both the inner and outer cortexes were similar. However, in HWD fruit, the concentration of malic acid increased sharply and reached a peak of 4.43 mg g^{-1} FW, 2.03 times that in the outer cortex.

Tartaric acid in HY substantially increased from 1.09 to 4.57 mg g^{-1} FW; its average concentration was higher than that in QH and HWD and also than in the outer cortex of HY. The concentration of ascorbic acid was lower at eating-ripeness than that at early stages.

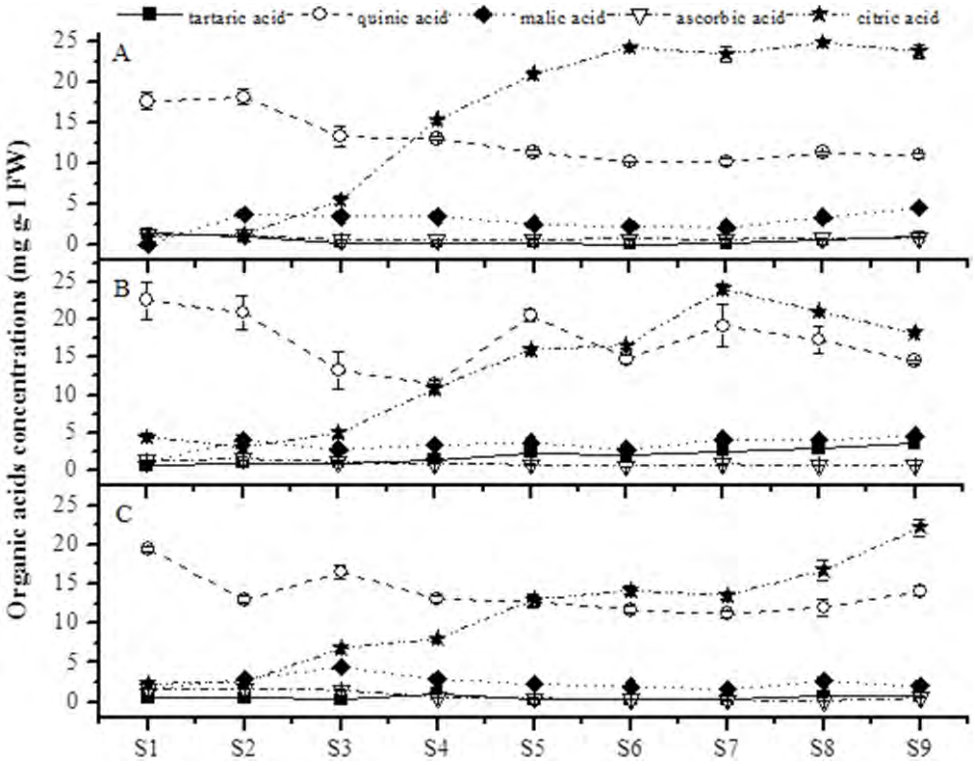

Figure 4. Changes in the concentrations of organic acids in the inner cortex of three kiwifruit cultivars during development and postharvest. Results represent means ± SE of three replicates. (A) 'Qihong' (B) 'Hongyang' and (C) 'Hayward'. Stages S1 – S7 correspond to 25, 45, 65, 85, 105, 125, and 145 days after pollination (DAP); S8 after storage at room temperature for 10 days after harvest (155 DAP), S9 when the fruit were eating ripe (170 DAP for 'Hongyang' and 'Qihong', 190 DAP for 'Hayward').

Changes in sugar: organic acids and sweetness index (SI) in the outer and inner cortex of three kiwifruit cultivars during ripening

The ratio sugars: organic acids and the sweetness index in the three cultivars generally increased with fruit ripening (Figures 5 and 6). But before commercial harvest, the ratios increased only slowly or even decreased slightly. Both the sugars: organic acids ratio and the sweetness index increased sharply after harvest.

We found that the ratio sugars: organic acids was higher in the outer cortex than in the inner cortex of QH at eating ripeness while the reverse was true of HY and HWD (Figure 5). The ratio sugars: organic acids in the outer cortex of QH (3.98) was higher than the ratios in the inner and outer cortexes of HY and HWD.

The sweetness index of the outer cortex of HY was generally lower before harvest than in the outer cortexes of QH and HWD but increased sharply at eating-ripeness and reached the highest value (267.54) (Figure 6). The SI in the outer cortex of QH and HY was higher than inner cortex at eating-ripeness, while in HWD it was the reverse.

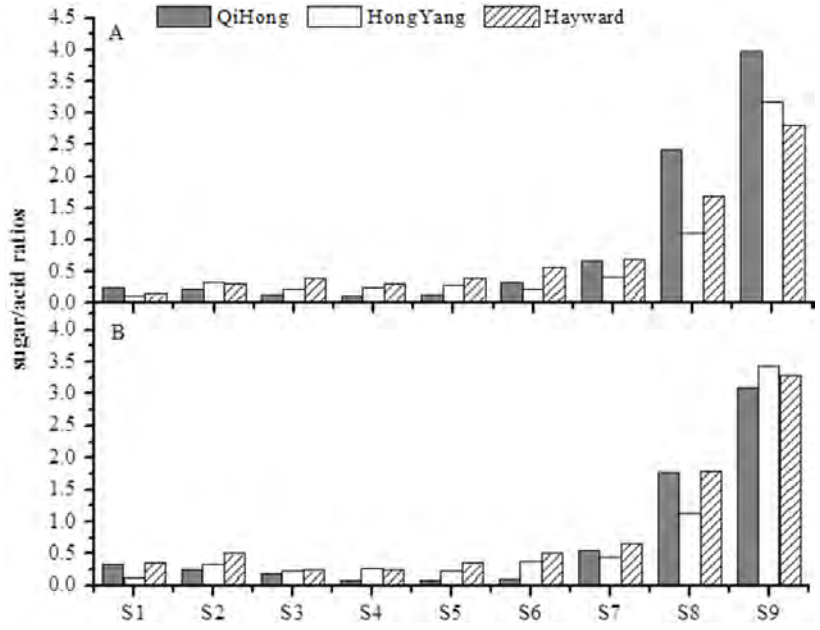

Figure 5. Sugar/organic acid in three kiwifruit at nine stages of ripening and senescence. (A) Outer cortex and (B) Inner cortex. Stages S1 – S7 correspond to 25, 45, 65, 85, 105, 125, and 145 days after pollination (DAP); S8 after storage at room temperature for 10 days after harvest (155 DAP), S9 when the fruit were eating ripe (170 DAP for 'Hongyang' and 'Qihong', 190 DAP for 'Hayward').

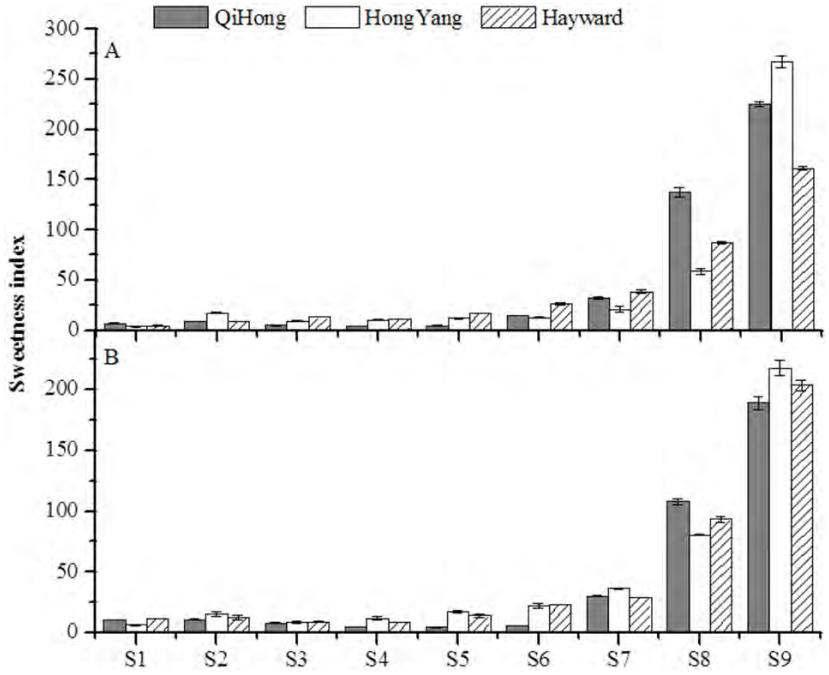

Figure 6. Sweetness index in three kiwifruit at ninestages of ripening and senescence. Results represent means ± SE of three replicates. (A) Outer cortex and (B) Inner cortex. Stages S1 – S7 correspond to 25, 45, 65, 85, 105, 125, and 145 days after pollination (DAP); S8 after storage at room temperature for 10 days after harvest (155 DAP), S9 when the fruit were eating ripe (170 DAP for 'Hongyang' and 'Qihong', 190 DAP for 'Hayward').

DISCUSSION

In previous studies, the changing concentrations of sugars and organic acids in various tissues of kiwifruit during development and postharvest were not systemically reported. And then our results presented several discrepancies with them.

The three kiwifruit cultivars all contained three major sugars: sucrose, fructose and glucose, which was similar to other fruits such as wolfberry (Zhao et al., 2015), pumelo (Sun et al., 2012), medlar (Glew et al., 2003), bayberry (Jiang et al., 2013), strawberry (Basson et al., 2010) and grape (Wu et al., 2011). However, Kim et al. (2012) and Chen et al. (2017) reported that in kiwifruit myo-inositol could also be detected, and that in hardy kiwifruit myo-inositol predominated. Although Nardozza et al. (2013) identified 11 different sugars and 3 sugar-alcohols using GC-TOF-MS with xylose, D-galactose and maltose as standards, we did not detect them in both the outer and inner cortexes of the three cultivars (data not shown). There could be many reasons for the differences observed in sugar composition. For instance, they could be due to the detection method used or the genetic background of the plants studied. Interestingly, we found that the concentration of each detected sugar was higher in the inner cortex of QH and HY than in the outer cortex, but the reverse was true of HWD. These differences may result in variations in enzyme activity (fructokinase, hexokinase, sucrose synthase, sucrose-phosphate synthase) of different tissue among the cultivars (Boldingh et al., 2000; Richardson et al., 2004). From this study, we could conclude that different cultivars had different carbohydrate compositions. Other research results presented that it was possibly because of variation in genetic backgrounds and other factors (such as geographical location, climatic conditions and nutritional status) (MacRae et al., 1989; Nardozza et al., 2010; Moscatello et al., 2011; Chen et al., 2017).

As for fluctuations in the three sugars, whether in QH, HY or in HWD, the concentration was similar with each other and remained low until close to maturity, when it sharply increased. But there were some discrepancies with the results of other researchers. Moscatello et al. (2011) reported that in HWD fruit glucose increased rapidly from 15 to 45 days after flowering (DAF). Similar results were reported by Chen et al. (2017). They found a relatively high level of glucose in 'Qinmei' from 15 DAF and that sucrose concentrations fluctuated considerably during fruit development. Nevertheless, our results are consistent with some other studies. Kim et al. (2012) and Nardozza et al. (2013) drew similar conclusions to ours through research on hardy kiwifruit and 10 other kiwifruit genotypes. At the start of the ripening phase, the soluble sugars began to increase owing probably to starch degradation (Moscatello et al., 2011). As in other fruits (Zhao et al., 2015; Albertini et al., 2006; Chen et al., 2009) the concentration and changing trends of total organic acid were different in various cultivars as well as fruit tissues. The reasons for this phenomenon might be genotype and tissue specificity.

Five organic acids were detected, with quinic, citric and malic acids being the major acids as previously reported for kiwifruit (Richardson et al., 2011; MacRae et al., 1989; Nishiyama et al., 2008; Kim et al., 2012). As reported by Richardson et al. (2011), Kim et al. (2012) and Marsh et al. (2009), the quinic acid concentrations were always high in the three kiwifruit cultivars during fruit development and post-harvest. However the trends they observed were slightly different to those found in previous studies. In agreement with MacRae et al. (1989), we found that the concentration of quinic acid was on average higher in the inner cortex than the outer cortex. Nishiyama et al. (2007, 2008) reported that *A. arguta* fruits were sweeter than traditional kiwifruit: low quinic acid content was one reason for the perception. Our investigators and the orchard managers surveyed generally believed that the flavor of QH was sweeter than that of HY: this was consistent with our analyses. The variation in citric acid in the inner and outer cortex of QH and outer cortex of HY was similar to that in *A. chinensis* reported by Marsh et al. (2009). However the change in HWD differed from that found in previous studies of *A. chinensis* (Marsh et al., 2009; Nardozza et al., 2013). The accumulation of citric acid in inner cortex was more than in the outer cortex of three cultivars. Similar results were reported by MacRae et al.

(1989). They also considered that if the inner cortex accounted for a high proportion of the fruit mass, the perception of low sugar and high acidity might be promoted, especially of citric acid. Considering the above factors, HWD has a more acid taste than QH and HY.

At the times sampled, malic acid concentrations remained relatively low in the three cultivars. However the concentration of malic acid in QH and HY was higher than that in HWD. The concentrations and trends in malic acid are consistent with the results of other research (Kim et al., 2012; Richardson et al., 2011; Marsh et al., 2009). In contrast to quinic, citric and malic acid, tartaric acid in kiwifruit has received little attention. Barboni et al. (2010) reported that the concentration of tartaric acid in HWD was 0.4 g L^{-1} at harvest and that the tartaric acid decreased during post-harvest with treatments of cold (0°C) and ozone storage. In different kiwifruits the concentration of tartaric acid at harvest varied from 0.11 to 0.34 g 100 g^{-1} (Ma et al., 2017). In our study, the concentration of tartaric acid in HY was similar to that presented in previous reports, but was low in QH and HWD, only about 0.5 mg g^{-1}. In the fruit of grapes (dos Santos Lima et al., 2014) and loquats (Ergönül and Negiz, 2010), tartaric acid was a major acid.

Ascorbic acid is an important antioxidant. In our results the trends in ascorbic acid concentrations in QH and HY were similar with those in hardy kiwifruit (Kim et al., 2012): a relatively high level initially, then declining before rising again slowly. Kim et al. (2012) also reported that in different cultivars of hardy kiwifruit, the ascorbic acid levels were similar at the time of harvest and decreased during storage. On the other hand, in HWD it decreased. Ge et al. (2013) found that ascorbic acid increased rapidly in during the initial development HY and 'Jinkui' fruit.

The relative amounts of sugar and acid have also been shown to affect the hedonics of flavor intensity (MacRae et al., 1989). Our results of the ratios total sugars: total organic acids were similar to that in *A. eriantha* Benth. (Chen et al., 2015). However the previous study by MacRae et al. (1989) reported that in various tissues of kiwifruit, the trends in sugar: acid ratios differed. In our study the particular tissue affected the trend slightly, but there was no distinct differences in the value of ratio and the value in the outer cortex accorded with the taste of three cultivars in that QH was sweeter. However, the value of SI differed slightly with the flavor in the tested cultivars.

CONCLUSION

In our study, the contents and concentrations of sugars and organic acids in different fruit tissues of three kiwifruit cultivars were accurately measured during development and postharvest, and the trends in sugars and organic acid concentrations were observed. The results suggest that although QH is a bud mutation of HY, the patterns of sugar concentrations were different. In QH sucrose was the main sugar and when the fruit was eating ripe, the concentration of sucrose was three times those of glucose and fructose, while in HY there was a similar level of three sugars during all stages of development and ripening. Sucrose was lowest in 'Hayward'. Compared with sugars, organic acids showed more variation in different tissues. Our study will provide a new point from which to explore the mechanisms of sugars and organic acids in different kiwifruit cultivars.

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Cultivar, environment and integration of cultural practices will determine the future of the kiwifruit industry

Jinhu Wu

The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Auckland 1142, New Zealand.

Abstract

In October 2017 Northwest A&F University in Yangling together with Baoji City, the administrative centre of Meixian (Mei county), Shaanxi Province, China, which has about 20,000 ha of kiwifruit orchards, organised a world kiwifruit conference on the industry, and the sixth Shaanxi – Meixian kiwifruit industry development conference. Twenty kiwifruit experts from China, Italy, Japan, Korea, New Zealand, Spain and Turkey presented papers covering cultivars, breeding, fruit quality, molecular work, vine management practices, pest and disease management, harvesting and storage, and processing. The experts reviewed the kiwifruit industry past and present in the rest of the world and in China, then discussed the future. At the invitation of the organisers, this article reviews some of the most interesting topics discussed at the conference. The aim of the conference was to assist the advancement of the Shaanxi kiwifruit industry through increased kiwifruit yields and orchard incomes, by breeding better cultivars of high quality and by improved orchard management.

Keywords: *Actinidia*, breeding, climate, fungus disease, germplasm, orchard management, pest, plant density, physiology, postharvest

INTRODUCTION

Since the first exports of what were then known as Chinese gooseberries from New Zealand to the United Kingdom in 1952, the world kiwifruit industry has expanded greatly, even if kiwifruit still make up less than 0.25% of total world fruit production. The name Chinese gooseberry has now been universally replaced by kiwifruit. More countries are growing kiwifruit and the total area of kiwifruit orchards has increased rapidly.

A.R. Ferguson from New Zealand and G. Costa from Italy described the current status of the world kiwifruit industry: the countries in which kiwifruit are grown, production, cultivars, changes in vine management and the response to bacterial canker of kiwifruit (caused by *Pseudomonas syringae* pv. *actinidiae*, Psa). The appearance of this disease, in Europe in 2008 and New Zealand in 2010, has meant increased costs for growers.

J.-B. Fang (Chinese Academy of Agricultural Sciences) outlined the development of the Chinese kiwifruit industry. This has expanded greatly over the last twenty years to about 130,000 ha, nearly half of which are in Shaanxi province, mainly Mei and Zhouzhi Counties. They produced about 37.7% of national kiwifruit production.

Z.-D. Liu from Northwest A&F University told how his team works with local government and growers to help the kiwifruit industry in Meixian. He outlined the strategy for future Shaanxi kiwifruit development (more than 30,000 ha of green-fleshed kiwifruit are to be planted in south and east Shaanxi).

A. Atak from Turkey, P.P. Gallego from Spain, and Y.-S. Cho from Korea introduced their countries' kiwifruit industries and kiwifruit research. Kiwifruit production is expanding in Turkey, with about 43,000 t produced in 2016. Plantings are mainly in Yalova, Ordu and Rize, Istanbul. The Spanish kiwifruit industry, based entirely on *Actinidia chinensis* var. *deliciosa* 'Hayward', mainly supplies fruit to the local market. The Korean industry, like that of China, has developed quickly with new yellow- and red-fleshed

cultivars. However, many environmental factors adversely affect kiwifruit growing there.

During the conference there were many good suggestions from both academics and researchers on ways to improve the world kiwifruit industry, particularly the industry in China.

Shaanxi Province in China has become the largest kiwifruit planting and production area in the world. The orchards are mainly in Mei and Zhouzhi counties. The former is in Baoji city with 20,000 ha; the latter belongs to Xian city, with close to 28,000 ha. Other cities such as Xianyang, Hanzhong, and Weinan also have some small planted areas. The two counties account for nearly half the total area in kiwifruit in China; the total planting area is estimated to be more than 130,000 ha, and produced around 37.7% total production of kiwifruit in China (in 2017, estimated to be about 2.6 M t; Meixian produces 0.46 M t, Zhouzhi 0.52 M t), of mainly green-fleshed kiwifruit. Red-fleshed and yellow-fleshed kiwifruit are grown mainly in Sichuan (e.g., Cangxi, Pujiang), Guizhou (e.g., Lupanshui; Jiangkou, Tongren; Dafang, Bijie), Hubei (e.g., Jiangshi, Enshi; Chibi, Xianning; Yichang), Hunan (e.g., Xiangxi), Anhui, Jiangxi, Yunnan, and other provinces in southern China. Red- and yellow-fleshed kiwifruit fetch a high price and result in high incomes for orchardists, but the average production per ha is currently lower than that from green-fleshed kiwifruit. Most red- and yellow-fleshed kiwifruit orchards are still very young and not fully producing.

THE IMPORTANCE OF NEW CULTIVARS

The development of new kiwifruit cultivars is essential for the continued survival and expansion of the world kiwifruit industry. This has been confirmed in both New Zealand and China. 'Hayward' has a pleasant taste distinct from other fruits and a good storage life. It was a chance selection in New Zealand from descendants of the seed introduced from Yichang, China, in 1904. 'Hayward' is still the most widely planted kiwifruit in the world. This cultivar was largely responsible for the Chinese gooseberry, grown on a localised scale, becoming the kiwifruit, a widely accepted new type of fruit crop, valued for its nutritional qualities (Ferguson, 2016). However, the industry in New Zealand has had to overcome challenges. Nearly all the kiwifruit produced in New Zealand are exported and the long sea voyages incur large costs in labour and transportation. Furthermore, from the 1970s onward the New Zealand industry had increasing competition from 'Hayward' kiwifruit produced in other countries. One solution was a new type of kiwifruit. In 2000, Zespri, the single-desk New Zealand exporter of kiwifruit, launched a yellow-fleshed kiwifruit, *A. chinensis* var. *chinensis* 'Hort16A', into the international markets. This new cultivar had been developed by HortResearch, now part of The New Zealand Institute for Plant & Food Research Limited (PFR). The arrival of this new type of kiwifruit totally changed the world kiwifruit industry: it attracted new consumers and expanded the market demand. The promising future of the industry seemed assured until the arrival of Psa. The highly profitable 'Hort16A' was particularly susceptible. This was a disaster for growers. Fortunately, the PFR kiwifruit breeding programme had under evaluation a number of promising selections. One of these, 'Zesy002', proved to be much more tolerant of Psa. *A. chinensis* var. *chinensis* 'Zesy002', commonly known to growers as Gold3, is a tetraploid with large, yellow-fleshed fruit which store well. Replacement of 'Hort16A' by 'Zesy002' helped to guarantee the continued growth of the New Zealand kiwifruit industry.

Another good example of the importance of cultivar innovation is the red-fleshed kiwifruit *A. chinensis* var. *chinensis* 'Hongyang' originally selected in Cangxi, Sichuan from seedlings grown from seed collected in Funiu mountain, Henan province (Wu, 2015), in which they were prepared as rootstocks (pers. comm. S.-Q. Wu, Cangxi Kiwifruit Institute, Sichuan, China; Wu, 1992; Wu and Li, 1993). The advantages of 'Hongyang' fruit include a circle of red flesh around the core and a very sweet flavour, but there are also disadvantages, most notably small fruit size, unreliable red colouration, low productivity (delays in establishing a complete canopy) and pronounced susceptibility to Psa such that

vines in China generally die two to three years after they start fruiting. Nevertheless, the high market demand and the resulting good prices have stimulated cultivation by growers, even though vines are short lived. Now the returns from 'Hongyang' and the small amounts of yellow-fleshed kiwifruit are estimated to be around 70% of the total revenue of kiwifruit inside China. Farmers in many mountainous regions of China, e.g., in Sichuan, Guizhou, Yunnan, Hubei, Hunan, Henan, Anhui, Zhejiang, Jiangsu and Jiangxi, have been encouraged to plant 'Hongyang' in an attempt to alleviate local poverty (Fig. 1B). Plantings of 'Hongyang' have expanded rapidly, changing the national composition of Chinese kiwifruit orchards. There is considerable interest in finding a red-fleshed replacement for 'Hongyang'; examples include 'Donghong', 'Hongsheng', and 'Hongshi'. However, it is still a real challenge to find a disease-tolerant, colour-stable, large-fruited and easy managed red-fleshed cultivar.

BREEDING OF NEW CULTIVARS

PFR, in partnership with the New Zealand kiwifruit marketing company Zespri, has demonstrated what is possible in the breeding of new kiwifruit cultivars. Their success is due to exploiting germplasm resources with both traditional breeding and modern molecular methodologies, knowing what the consumers want. Z. Hanley from PFR reviewed the various methods used by the New Zealand kiwifruit breeders (e.g., polycrosses for allele preservation, the bringing together of gene pools of different ploidies with chromosome doubling as a bridge, interspecific crosses, genomic selection) but stressed that genetic engineering techniques have not been used for the development of commercial cultivars. China has the richest kiwifruit germplasm resources. Outside China, PFR has the world's most comprehensive kiwifruit germplasm collection. Hanley estimated that less than 10% of the PFR collection had so far been used in breeding programmes. Over a seven-year period, 135,000 hybrid seedlings have been produced from the cross breeding pipeline, with a sex marker used to eliminate 90% of the males. Around 405 superior selections from these crosses are being further tested in trials (Hall, 2014). Progeny testing is revealing the contributions of male parents to fruit size, dry matter conyent, flesh colour and Psa tolerance, all important economic traits.

As consumer satisfaction is vital for the survival of the kiwifruit industry, an important breeding target is meeting customer requirements for flavour, health and convenience. R.G. Atkinson of PFR described work on the basics of kiwifruit flavour. The new cultivars have to adapt readily to the climatic environment, be tolerant of or resistant to Psa, and produce large numbers of big fruit which respond well to storage and transport. Currently, the most successful new kiwifruit cultivar developed in New Zealand is 'Zesy002', the replacement for 'Hort16A'.

A.R. Ferguson outlined the origin of the various kiwifruit cultivars currently grown in different countries. The New Zealand experience was that new cultivars had to be suited to the markets after all commercial traits had been systematically trialled, and that extensive promotion was necessary. New cultivars with fruit that had distinctive novel traits could complement existing cultivars, resulting in market expansion. The development of the yellow-fleshed 'Hort16A' revolutionised international trade in kiwifruit and provided an enormous stimulus to the New Zealand industry. In China many cultivars have been grown and new selections are still being trialled; e.g., in this conference as described in the reports by M.-Z. Li of trials in Sichuan, China, and S.-B. Liu from Jishou University in western Hunan. The market will decide which of these new selections are successful.

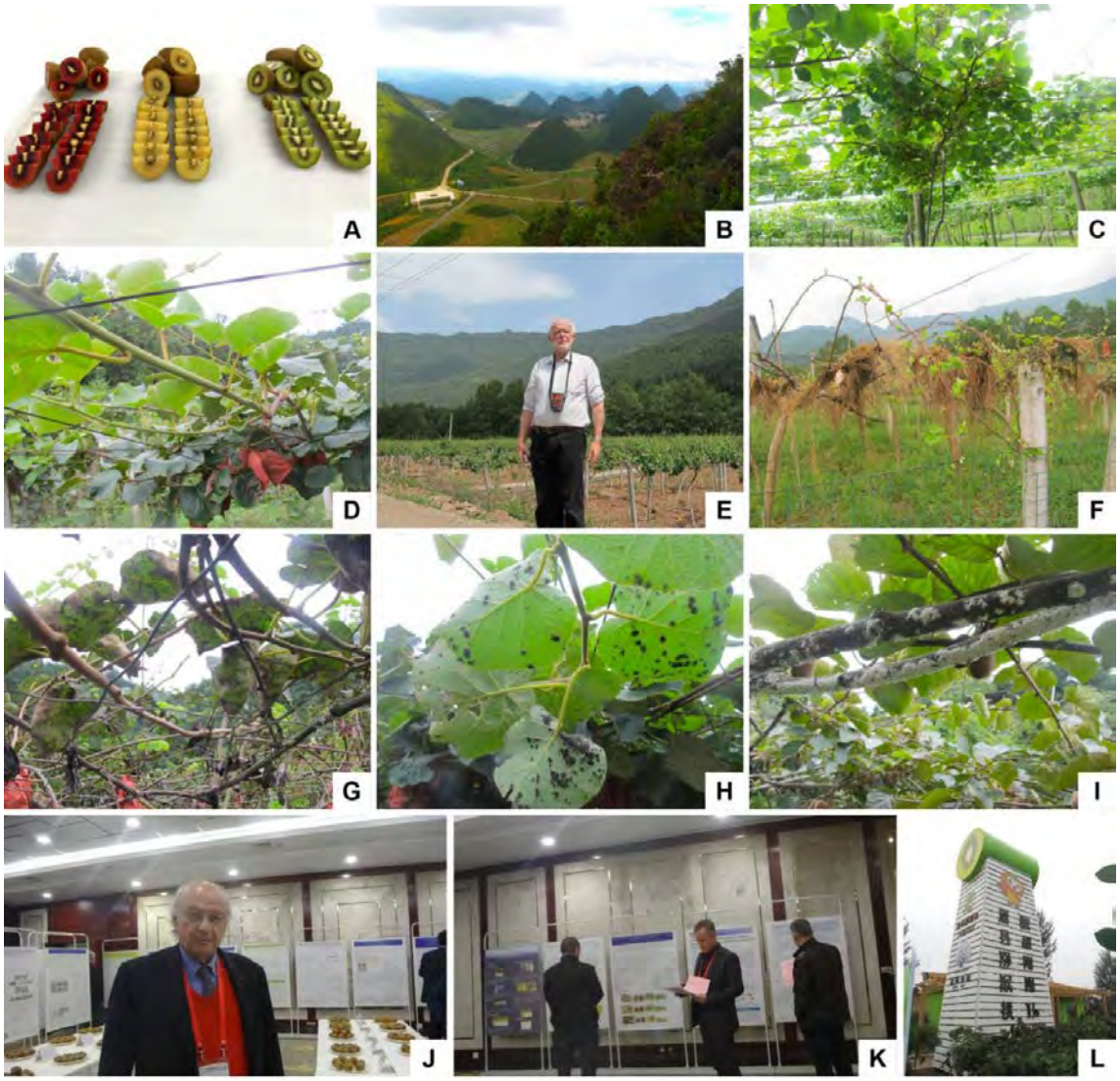

Figure 1. Current breeding for future kiwifruit cultivars by The New Zealand Institute for Plant & Food Research Ltd (A); New planting kiwifruit area in the mountains of south China (B); New planting system with strip males in a kiwifruit orchard (C); Many buds produce unnecessary shoots after incorrect canopy management in summer, and this affects fruit size and quality in the current year and also affects the fruiting canes in the subsequent season (D); Dr. A.R. Ferguson visited a kiwifruit orchard in 2013 alongside a stream in a mountainous area of Sichuan (E); The same kiwifruit orchard that Dr. Ferguson visited in 2013 after flooding in 2016 (F); Leaf fungal diseases mixed with other diseases together cause widespread early leaf drop in 'Hongyang' and other yellow-fleshed kiwifruit in south China (G, H); Widespread woolly scale infestation in kiwifruit orchards (I); Dr. G. Costa and Dr Z. Hanley lead the invited kiwifruit experts to evaluate all kiwifruit cultivar fruit and posters at the conference (J, K); A sign shows that kiwifruit produced in Meixian have good taste, as they have a good combination of sweetness and acidity (L).

THE ROLE OF CLIMATE

Climate can also be important in determining the success of kiwifruit orchards. The Bay of Plenty in New Zealand is the easiest place in the world to grow kiwifruit. The main

advantage there is the lack of climatic extremes: the rainfall is evenly distributed throughout the year (95-150 mm month^{-1}) and there are no temperature extremes during the growing season as there are in China and California, USA, sometimes up to 50°C (in Shaanxi, 2017), leading to damage to fruit and vines; or the low temperatures in mid-autumn (-20°C) or after budbreak in the spring (cold weather often returns) all of which can result in vine death in China. For example, on 13 November 2009, the cold weather with snow came early (over 8 days the average minimum temperature was -3.4°C, the extreme low temperature was -10.1°C). As a result all mature canes in more than 80% of fruiting vines of *Actinidia chinensis* var. *deliciosa* 'Qinmei' and 'Xuxian' at the Zhengzhou Fruit Institute, Henan, died and produced no new shoots the following spring; most young seedlings in the breeding programme also died (Qi et al., 2011).

In the Bay of Plenty, New Zealand, the annual rainfall can be more than 1500 mm, the warmest month is February, with an average temperature of 19.3°C, mean maximum temperature of 23.7°C; mean minimum temperature of 13.1°C; and the coldest month is July, with an average temperature of 9.5°C, mean maximum temperature of 14.1°C, and mean minimum temperature of 4.8°C. However, even in the Bay of Plenty and the Northland regions of New Zealand, climatic factors can limit kiwifruit growth or productivity (Cradock-Henry, 2017). The main limitation is inadequate winter chilling which can affect kiwifruit flower differentiation and decrease or delay budbreak. This can result in uneven or prolonged flowering. Lack of winter chilling can be overcome using hydrogen cyanamide (Hi-Cane®), however, this chemical has to be managed for potential environmental consequences. The relatively mild temperatures and high humidity are ideal for the growth of the Psa bacterium and this makes protection of the New Zealand vines against Psa more difficult than in some other countries which have higher summer temperatures or lower humidities. The damage caused by hail or wind can also be a problem in many kiwifruit-growing areas. G. Costa described the recent Italian experiences with growing kiwifruit under protective covers in an attempt to reduce the damage caused by hail or Psa.

The regions in China where kiwifruit are grown vary greatly in climatic conditions. Some regions, e.g., Shandong and Shaanxi, are very cold in winter with temperatures well below 0°C, whereas other regions have warm winters, e.g., south Jiangxi (Gannan), Guangxi, Yunnan, and even Guangdong with limited kiwifruit plantings. Variation in the winter-chilling requirements of different selections could determine their productivity and hence profitability in the different parts of China. Because in China kiwifruit are grown widely in areas that differ considerably in climate, it is important to recognise how these different climatic conditions may affect commercial kiwifruit cultivation, especially quality, e.g., flesh colour (Man et al., 2014). Experiments should be carried out to establish which cultivars will grow well and be productive under particular conditions, or, conversely, which climatic conditions are ideal for a certain cultivar. Too often, planting has gone ahead without a proper understanding of the suitability of the cultivars grown or of the site. It pays to be cautious.

MANAGEMENT CHANGES TO IMPROVE FRUIT NUMBER, SIZE AND QUALITY

Productivity has significantly increased over the last thirty years because of new, inherently more productive cultivars and changes in orchard management following improved understanding of kiwifruit canopy and growth characteristics. For example, in New Zealand, 'Hayward' productivity has increased from 30 t ha^{-1} in 1980 to 50 t ha^{-1} in 1990 through adoption of the pergola training system and other management improvements such as use of bio-stimulants. With some of the newer cultivars, production can reach 70 t ha^{-1} in 2000. The aim is for all to average100 t ha^{-1} in the near future.

K.J. Patterson (PFR) compared a kiwifruit canopy to a solar panel. Maximum productivity requires maximum interception of light (i.e., maximum photosynthesis). The canopy should not be too dense, because all the leaves should be photosynthesizing to provide for fruit growth and sugar accumulation during the season. If the canopy is too

dense, lower leaves may be net importers not exporters of carbohydrate. In dense canopies, lower leaves may also die, replacement canes may be weak, lacking strong buds for the following year's flowers and fruit, and there may be outbreaks of diseases and pests. The canopy should consist of a maximum of three or four layers of leaves without overlays together, and some spots of lights should reach the orchard floor.

Management of vine canopies is achieved by techniques such as crushing the tips of young canes during the growing season, spacing canes to ensure complete canopy development and hence maximise interception of light, removal of overlying layers of canes, zero-leaf pruning of the end of fruit canes, and girdling at different times during the season to improve fruit size or dry matter content e.g., increases of 5% in fruit size and of 0.5% units dry matter can be achieved in 'Hort16A' by girdling at 3-4 weeks and 10-14 weeks respectively after mid-bloom. It is important to prevent the spread of Psa by sterilizing equipment used in girdling and pruning and protecting any wounds. Valagro®, popularly called "Benefit KIWI" or "Benefit-PZ", was widely applied three times during early fruit development of 'Hort16A' to increase average fruit size from 95 to 115 g. It is described as a liquid organic nitrogenous fertilizer containing nucleotides, amino acids and other nutrients such as vitamins, co-factors essential to cell metabolism. Although the active ingredient is not specified as it is a proprietary product, the effect is similar to application of auxins or cytokinins; overuse will affect fruit dry matter content. K.J. Paterson commented on its use. In China, CPPU (1-(2-chloro-4-pyridyl)-3-phenylurea has been used widely, but illegally, to increase fruit size in kiwifruit. Recent studies have found that the application of CPPU to increase fruit size can significantly reduce fruit quality (dry matter content), resulting in bland, insipid fruit that cannot be stored for long, because CPPU increases fruit size mainly through increased water uptake (Nardozza et al., 2017). M. Currie (PFR) presented the results of trials with Shaanxi Rural Science and Technology Development Centre and confirmed that good production of high quality green-fleshed kiwifruit could be achieved without application of CPPU.

Fruit yield and quality determine the profitability of kiwifruit production. K.J. Patterson demonstrated the relationship, in New Zealand, between grower returns and the three components: taste (as defined by dry matter), fruit size and fruit number.

Orchard management practices have been modified to optimise profitability by balancing between these three components:

- the ratio of males: females (and the strip-male layout currently) (Figure 1C);
- establishment of the vine structure as quickly as possible, so that the canopy forms completely in the second year after planting or top-working, e.g., use of the string-and-pole system to grow shoots;
- canopy management and pruning of males to give most canopy space to females;
- design of pollination systems to ensure fruit size by adequate pollination either by bees or artificially;
- polleniser selection (pollenisers can affect the expression and stability of colour in red-fleshed kiwifruit) (Seal et al., 2013; Wu, 2014, 2015);
- fruit thinning as soon as possible after flowering to remove crowded or blemished fruitlets;
- summer control of canopy growth;
- girdling to increase fruit size and dry matter content or flow differentiation;
- separation of fruiting and replacement canes;
- string and pole system: tying down of strings along the leader wire where the side shoots will grow;
- winter pruning to spread and tie down only a few low-to-medium vigour canes for next-season flowering along the leader wire, with 35-cm distances between side canes;
- establishment of windbreak system before planting of new orchards.

All these horticultural practices have to be integrated with soil and water management practices (Wu and Dan, 1993), as possible magnesium deficiency and other

nutrient deficiencies have been found to weaken vines in some areas of China. Trials of horticultural techniques should be carried out before they are widely used, as the environment and the patterns of vine growth can vary greatly in different growing areas in China, even the same cultivar. Furthermore, different cultivars can vary in their responses: in New Zealand commercial orchards of 'Hort16A' and 'Zesy002' can respond differently to management techniques. The factors that mostly limit fruit yields or fruit quality have to be identified.

FRUIT QUALITY AT HARVEST IS VITAL

J.-P. Rao from Northwest A&F University reviewed the characteristics of chilling injury in cool-stored kiwifruit. This is a serious problem in China. J. Burdon from PFR stressed that fruit quality could not be improved during storage, that quality could only deteriorate during storage, and that the quality of harvested fruit entering storage was therefore critical. High dry matter content and physiological maturity were important. He described the changing approaches to harvest indices during the history of the industry (Burdon et al., 2016). It was now realised that the appropriate time to harvest fruit depended to a large extent on the length of time for which those fruit were to be stored.

Growers need to make a profit. No matter how high the quality of the fruit when they are harvested and then removed from long-term storage, they still need to be promoted and marketed. A.R. Ferguson commented that with the selections developed by PFR for Zespri, a major part of the development costs was the promotion of new cultivars in the marketplace.

PEST AND DISEASE MANAGEMENT

X.-N. Gao from Northwest A&M University and X.-H. Yi from Guizhou University presented studies on Psa in the north-west and southwest of China. Some pests such as scale and some diseases, e.g., *Sclerotinia sclerotiorum*, and other stem pests and diseases, have been serious problems (pers. comm. Y.-H. Long, Guizhou University; L.-H. Cai, Huazhong Agricultural University), causing premature leaf drop and affecting fruit storage, further reducing the life of kiwifruit orchards, especially in the south of China. All these problems could be related to planting density and the selection of orchard sites. The high-density planted orchards in China have already encountered problems such as scale and early leaf drop: we have observed such problems in some orchards in the provinces we have recently visited (Figure 1G-I). Water flooding and nutrient disorders have also been found to cause problems in some areas of China (Figure 1E-F). All of these caused premature leaf drop (pers. comm. D.-M. Tang, Guizhou Academy of Agricultural Sciences; kiwifruit team of Forestry Bureau Office in Yichang Government, Hubei; Cangxi Kiwifruit Institute, Sichuan), but leaves are important not only for production but also for red-flesh pigmentation (Nardozza et al., 2015). During the growing season, premature leaf drop caused by pests and diseases or by unsuitable growing conditions such as nutrient deficiencies or flooding stimulates budbreak and production of many short and unhealthy shoots in autumn after fruit harvest. This significantly weakens the vines, ultimately reducing the life of the vines and the orchard itself. Integrated management including pest and disease protection is necessary for high quality and sustainable production (Wu and Dan, 1993).

CONCLUSION

At this conference all colleagues expressed a keenness to co-operate, to learn from each other and to make progress that contributes to the kiwifruit industry in the world. All wish to make the industry stronger, the fruit growers more profitable, fruit-growing villages more beautiful (a slogan of the Shaanxi government), and the environment better – these are the aims of many horticultural scientists around the world and are also reflected in the posters everywhere in the countryside of Meixian, Shaanxi, and other major fruit-growing counties in China.

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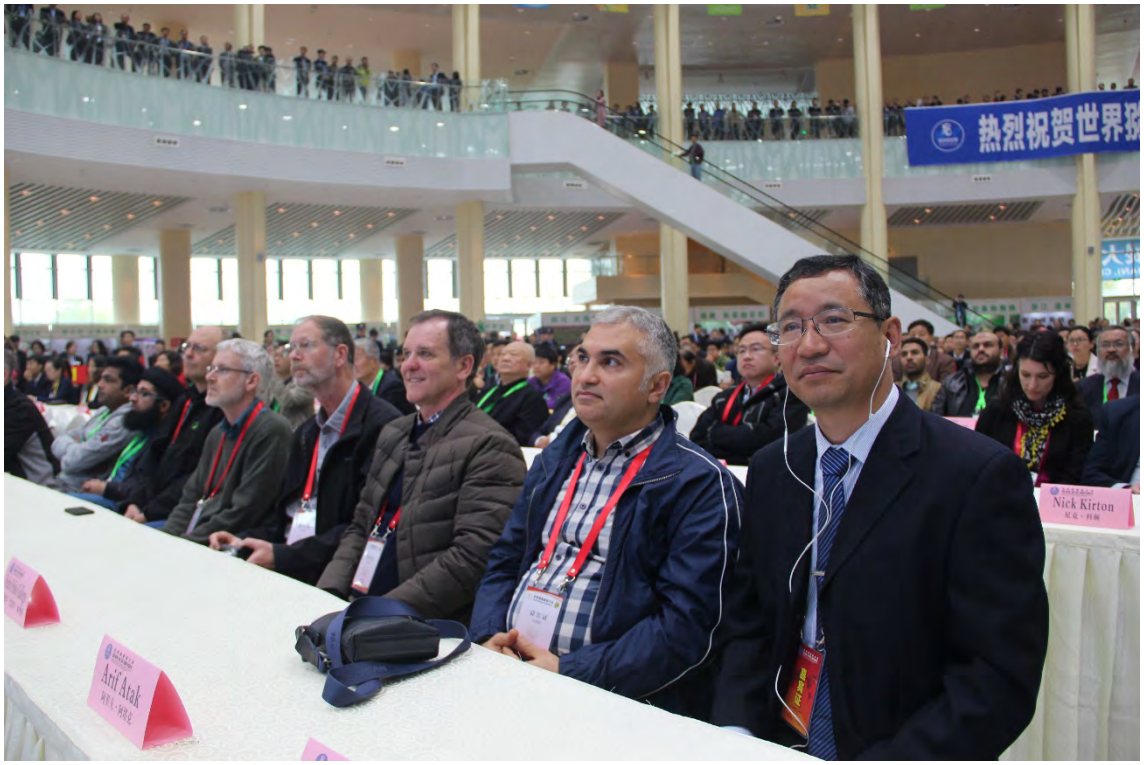
Photos (Credits: NWAFU Kiwifruit experimental station)



Experts attending The World Kiwifruit Conference

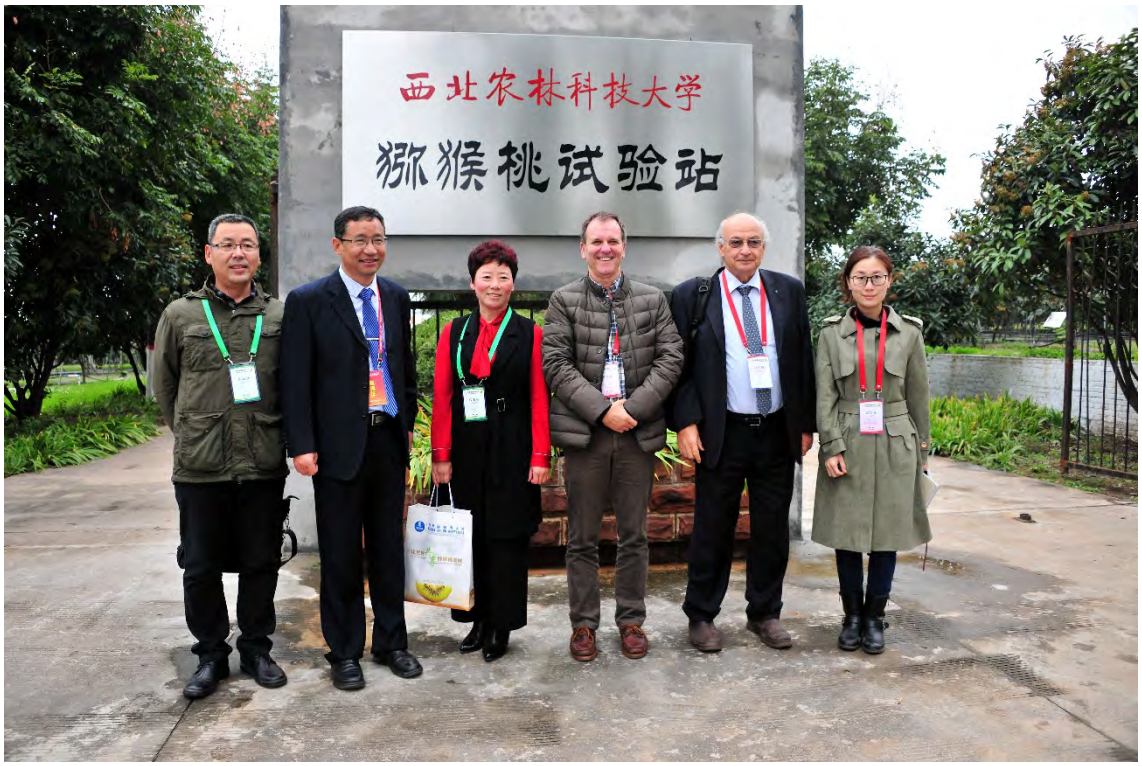
Dr. Ross Ferguson

Mei County – The main producing area of kiwifruit in Shaanxi


Kiwifruit in Mei County

A good harvest of Qihong is in sight

The orchard of the kiwifruit experimental station of NWAFU



Delegates of The World Kiwifruit Conference visited the kiwifruit experimental station of NWAFU


Prof. Zhande Liu

Technology exchange of experts from NWAFU in the field

The research team of the kiwifruit experimental station of NWAFU

Cultivar ‘Qihong'

Cultivar ‘Nongda Jinmi’

Group photo of The World Kiwifruit Conference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