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Review

Biotechnology and apple breeding in Japan

Megumi Igarashi[†1)], Yoshimichi Hatsuyama[†2)], Takeo Harada[3)] and Tomoko Fukasawa-Akada[*1)]

[1)] *Hirosaki Industrial Research Institute, Aomori Prefectural Industrial Technology Research Center*, Ogimachi 1-1-8, Hirosaki, Aomori 036-8104, Japan
[2)] *Apple Research Institute, Aomori Prefectural Industrial Technology Research Center*, Fukutami 24, Botandaira, Kuroishi, Aomori 036-0332, Japan
[3)] *Department of Agriculture and Life Science, Hirosaki University*, Bunkyouchou 3, Hirosaki, Aomori 036-8563, Japan

Apple is a fruit crop of significant economic importance, and breeders world wide continue to develop novel cultivars with improved characteristics. The lengthy juvenile period and the large field space required to grow apple populations have imposed major limitations on breeding. Various molecular biological techniques have been employed to make apple breeding easier. Transgenic technology has facilitated the development of apples with resistance to fungal or bacterial diseases, improved fruit quality, or root stocks with better rooting or dwarfing ability. DNA markers for disease resistance (scab, powdery mildew, fire-blight, Alternaria blotch) and fruit skin color have also been developed, and marker-assisted selection (MAS) has been employed in breeding programs. In the last decade, genomic sequences and chromosome maps of various cultivars have become available, allowing the development of large SNP arrays, enabling efficient QTL mapping and genomic selection (GS). In recent years, new technologies for genetic improvement, such as trans-grafting, virus vectors, and genome-editing, have emerged. Using these techniques, no foreign genes are present in the final product, and some of them show considerable promise for application to apple breeding.

Key Words: apple, breeding technology, trans-grafting, DNA marker, QTL-analysis, transgenic, NPBTs.

Introduction

Fruits are rich in fiber, antioxidants and phytochemicals that have beneficial effects for human health. Apple (*Malus* × *domestica* Borkh.) is one of the most popular fruits, and is considered to be a major functional food resource. Many new apple cultivars with improved fruit quality or growth habits have emerged as a result of the steady efforts of breeders worldwide.

Breeding of new apple cultivars is challenging. The main method of traditional apple breeding has been through the crossing and selection of superior individuals from thousands of seedlings. The lengthy juvenile period of the tree and its large size, requiring a long period of time for evaluation and a large field space, have imposed limitations on apple breeding programs.

Breeding apple cultivars that are resistant to diseases, especially scab caused by the fungus *Venturia inaequalis*, has also been a major aim of apple breeding programs worldwide. Genetic analyses and breeding programs for scab resistance were initiated in 1914, and Dayton *et al.* (1970) have reported that it took over 50 years to obtain apple cultivars with a scab-resistant trait derived from a wild apple species, although the resulting fruit still did not meet consumer expectations. In Japan, because of the climate and the favored cultivars, Alternaria leaf-blotch, a disease caused by the fungus *Alternaria alternarta* apple pathotype, has become a major concern among apple growers and breeders since the 1960s. The susceptibility of cultivars and its inheritance have been studied intensively (Saito and Takeda 1984). Five apple cultivars are recognized as the founders of cultivars developed in Japan, and two of them, ‘Indo’ and ‘Delicious’, are readily susceptible, with many progeny cultivars inheriting the trait in a dominant manner.

Commercial competition from other fruits has been steadily increasing, and changes in consumer demand have prompted breeders worldwide to develop new apple cultivars with more attractive characteristics and improved benefits.

Rapid developments in biotechnological breeding have shortened the period of time needed for fruit tree breeding, and such techniques are now being applied to apples. Decoding of the apple genome (Velasco *et al.* 2010) has provided insight into not only the evolution of this species,

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*Corresponding author (e-mail: tomoko_akada@aomori-itc.or.jp)
[†] These authors contributed equally to this work

but also information for clarifying the genetic basis of fruit quality, disease resistance, and growth habit. First, the number of solid markers of disease resistance and fruit character has been increasing, and the development of marker-assisted selection (MAS) strategies has accelerated. The development of molecular markers has also facilitated the construction of detailed linkage maps for QTL analysis, revealing chromosome regions associated with various apple traits (Bai *et al.* 2012a, Chagné *et al.* 2012a, Devoghalaere *et al.* 2012, Kunihisa *et al.* 2014). Apple breeding can thus be performed more efficiently using MAS strategies. Second, techniques for tissue culture and gene introduction in apple have been established (Puite and Schaart 1996, Schaart *et al.* 2011a, 2011b). A gene-modified (GM) line, which does not turn brown when cut, has finally been allowed to enter the marketplace in both Canada and the USA (Carter 2012). On the other hand, public concern about GM crops still persists, mainly with regard to the random insertion of a transgene in the genome and the remnant selectable marker gene. Third, in response to public concerns about GM crops, new plant breeding technologies (NPBTs; Lusser *et al.* 2012) have been introduced. NPBTs may allow breakthroughs in crop breeding, and have an enormous impact on apple breeding in the near future.

This review discusses the advances achieved so far through biotechnology in relation to apple breeding in Japan. As there have already been several distinguished reviews of apple breeding and biotechnology (Gardiner *et al.* 2007, Keller-Przybyłkowicz and Korbin 2013, Marić *et al.* 2010, Pereira-Lorenzo *et al.* 2009), the present review focuses mainly on recent developments.

Apple production and breeding in Japan

Over 140 years have passed since apple production was initiated in Japan. In the search for cultivars suitable for consumers and regional cultivation conditions, hundreds of introduced cultivars have been examined, and hundreds of unique cultivars have been newly developed. This section introduces the history of apple cultivars and breeding in Japan.

Apple production in Japan

Commercial apple production in Japan started in the 1870s using cultivars introduced mainly from the United States. By the 1900s, about 300 cultivars had been introduced from the USA, France, Canada, and other western countries, and seven cultivars, 'American Summer Pearmain', 'Ben Davis', 'Fameuse', 'Jonathan', 'Smith Cider', 'Ralls Janet', and 'Red Astrachan' had become dominant in the Japanese apple industry. During the period between 1940 and 1960, two cultivars, 'Jonathan' and 'Ralls Janet', accounted for over 85% of the total annual apple production in Japan. These old cultivars were then rapidly replaced by newly introduced cultivars such as 'Delicious' and 'Golden Delicious', and later by cultivars originally developed in Japan (**Table 1**, **Fig. 1**). In 2014, 'Fuji', 'Tsugaru', 'Orin', and 'Jonagold', accounted for 53.5%, 11.2%, 7.4%, and 6.9%, respectively, of the 816,300 tons of apples produced in Japan. Most of the remaining 21% consisted of new and old cultivars developed in Japan, each constituting less than 1% or a few percent of total apple production.

Apple cultivars and breeding programs in Japan

'Indo' is believed to be the first Japanese apple cultivar, having been found as a chance seedling of 'White Winter Pearmain' in 1884. Apple breeding by systematic crossing was initiated in the early 1900s in Japan, and subsequently many cultivars were developed through government-funded breeding programs at both national and local levels, and by universities and the private sector including growers and nurseries. According to the Japan plant registration website (http://www.hinsyu.maff.go.jp/en/en_top.html), 185 original apple cultivars have been registered since 1981. Including cultivars that were registered prior to 1980, those currently being assessed, and those commercialized without registration, our estimation for the number of Japanese cultivars is close to 300. Some of these are listed in **Table 1**.

The Aomori Apple Experiment Station, currently the Apple Research Institute, began apple breeding in 1928, and has registered 19 cultivars, and released 25 cultivars without registration. 'Mutsu' registered in 1949, known as 'Crispin' in the US, was the first apple cultivar to be registered in Japan. 'Tsugaru' has been the most popular early cultivar since the 1980s. 'Aori 27/Chiyuki™' is a unique non-browning apple developed by a crossing between 'Kinsei' and MaHe 7 (('Golden Delicious' × 'Indo') × 'Redgold'), and was patented in the USA in 2014. The flesh of the apple does not brown for more than several days after being sliced or grated, and has been shown to have low PPO activity and low polyphenol content (Kon *et al.* 2005, 2008, Noro *et al.* 2009). 'Aori 15'/Hoshi no Kinka™' is a sweet and juicy yellow apple harvested in late October in Aomori, and shows remarkable storage stability over 6 months when refrigerated (Kon *et al.* 2012). 'Aodai 3' is a dwarf root stock with easy propagation properties.

The national Institute of Fruit Tree Science, currently the NIAS Institute of Fruit Tree Science, began apple breeding in 1939, and in 1962 registered 'Fuji', the most widely cultivated apple cultivar in Japan. This cultivar was selected from 787 seedlings derived from the cross between 'Ralls Janet' and 'Delicious'. 'Fuji' has been considered the most widely produced apple cultivar in the world (O'Rourke 2003). The fruit is juicy, sweet, crispy, and shows remarkable storage ability with no loss of firmness for at least 1 month after harvest at room temperature. A new cultivar 'Mori no Kagayaki', harvested in mid-October, is a large yellow apple with a very sweet taste and attractive aroma. 'Ruby Sweet' is a large sweet apple with red flesh, and 'Rose Pearl' is an apple with red flesh and yellow skin. 'JM 1' and 'JM 7' are dwarf root stocks with easy propagation properties.

Many other research institutes funded by local governments, including Iwate, Yamagata, Miyagi, Fukushima,

Table 1. Cultivars originally developed in Japan

Breeding Programs or Organizations	Cultivar Names (year of registration or release[b])/Trade Marks
National	Fuji (1962), Himekami (1985), Iwakami (1985), Sansa (1988), Kizashi (1991), JM1[a] (1999), JM7[a] (1999), JM8[a] (1999), JM2[a] (2000), JM5[a] (2000), Kitaro (2000), Chinatsu (2001), Koutaro (2001), Santaro (2003), Hoiku Indo (2007), Mori no Kagayaki (2011), Ruby Sweet (2015), Rose Pearl (2015)
Hokkaido	Empire (1964[b]), HAC Nine (1986), North Queen (1989), Maoi (2004)
Aomori pref.	Mutsu (1949)/Crispin, Megumi (1950), Orei (1951), Toko (1963[b]), Sekaiichi (1974[b]), Tsugaru (1975), Kita no Sachi (1981), Hokuto (1983), Natsu Midori (1983), Mellow (1990), Aori 9 (2001), Aodai 3[a](2001), Aori 13 (2003)/Kita Kurenai™, Aori 11 (2004), Aori 12 (2004)/Shiori no Uta™, Aori 15 (2004)/Hoshi no Kinka™, Aori 16 (2004)/Koizora™, Aori 21 (2008)/Shunmei 21™, Aori 27 (2008)/Chiyuki™, Aori 24 (2013)/Hatsukoigurin™, Aori 25 (2013), etc.
Akita pref.	Senshu (1980), Akita Gold (1992), Akita Beni Akari (2005), Aki Shizuku (2007), Yume Akari (2007), Akita Beni Hoppe (2009)
Iwate pref.	Kiou (1994), Ouka (2006), Iwate 7 go (2009)/Beni Iwate™, Ooyume (2013)
Yamagata pref.	Syuyou (2008), First Lady (2009)
Miyagi pref.	Sour Rouge (2011)
Fukushima pref.	Hoozuri (1996), Hi no Azuma (2006)
Gunma pref.	Akagi (1973[b]), Youkou (1981), Shin Sekai (1998), Gunma Meigetsu (1991), Slim Red (1995), Honey Queen (1995), Oze no Kurenai (2009)[c]
Nagano pref.	Takane (1984), Shinano Sweet (1996), Shinano Red (1997), Shinano Gold (1999), Shinano Dolce (2005), Shinano Piccolo (2006), Shinano Petit (2010), Shinano Hoppe (2013)
Ishikawa pref.	Syusei (2005)
Regional[d]	Goshogawara (1996), Kuroishi 1 go (2006)
Universities	Koukou (1999), Haruka (2002), Kurenai no Yume (2010), Hirodai Misaki (2010)
Private Sectors[e]	Orin (1952[b]), Kinsei (1972), New Jonagold (1980[b]), Yataka (1987), Seirin (1990), Miki Life (1992), Akibae (1993), Beni Shogun (1993), Seimei (1995), Akiyo (1996), Ryoka no Kisetsu (1999), Gold Farm (2000), Aika no Kaori (2001), Toki (2004), Daikouei (2005), Takano 1 go (2010)/Beni Roman™, Takano 2 go (2010)/Gold Roman™, Takano 3 go (2010)/Fujiwara Roman™, etc.

[a] root stock cultivars.
[b] cultivars released without registration and listed with the year of public release.
[c] cultivars developed in corroboration with a national institute.
[d] regional government.
[e] growers and nurseries.
pref. = prefecture.

Gunma, Nagano, and Ishikawa have also developed original apple cultivars from individual breeding programs. 'Kiou', a yellow apple developed by the Iwate Agricultural Research Center, has become a very popular early cultivar that is widely cultivated in both Iwate and Aomori prefectures. 'Shinano Sweet' and 'Shinano Gold', developed by the Nagano Fruit Tree Experiment Station, are now becoming very popular, and their production is increasing, gradually replacing the established cultivars. 'Shinano Sweet' is a red sweet apple harvested between the seasons for 'Tsugaru' and 'Fuji', and has excellent eating quality. 'Shinano Gold' is a late yellow cultivar with a juicy texture and good taste, with a nice sugar and acid balance, and very good storage ability.

The private sectors, including growers and nurseries, have also developed many cultivars. Among Japanese apple cultivars, 'Orin', released in 1952, has grown to become the top-ranking yellow cultivar and the second-ranking late cultivar. 'Toki', released several years prior to its registration in 2004, is a juicy and very sweet yellow cultivar whose production is rapidly increasing.

Japan has been mostly self-sufficient in the development and utilization of apple cultivars as well as in the production and consumption of apples.

DNA markers and genomic information

The development of molecular markers and chromosome maps facilitates the location of genes responsible for important agronomic traits, and helps the breeding process through MAS. Any individual trait may be related to a major gene or several genes distributed in multiple chromosomal regions as quantitative trait loci (QTLs). Markers linked to specific traits, such as disease resistance, fruit quality, and growth habit, have been developed (reviewed by Gardiner *et al.* 2007, Keller-Przybyłkowicz and Korbin 2013, Marić *et al.* 2010, Pereira-Lorenzo *et al.* 2009). These markers are expected to provide solutions to several problems of fruit tree breeding such as the lengthy tree juvenile period and the large field space required for growing populations.

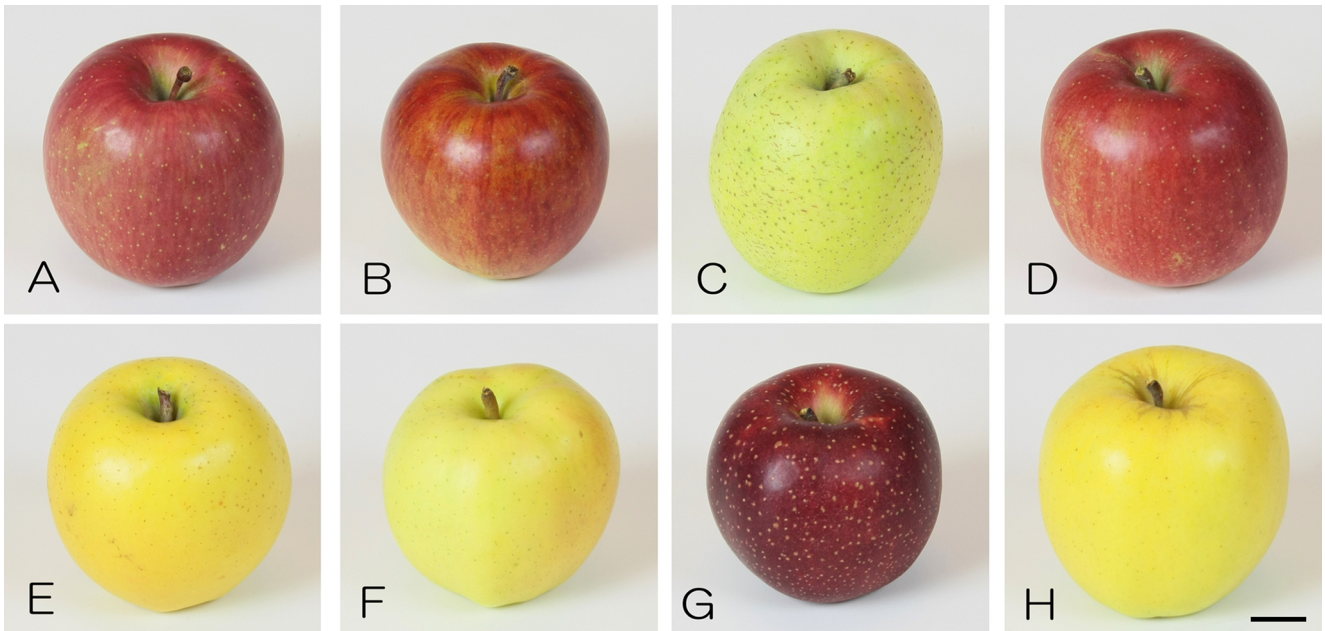

Fig. 1. Some of the apple cultivars developed in Japan. The cultivars dominating the current apple market are (A) 'Fuji' registered in 1962, (B) 'Tsugaru' registered in 1975, (C) 'Orin' released in 1952, and the promising new cultivars are (D) 'Shinano Sweet' registered in 1996, (E) 'Shinano Gold' registered in 1999, (F) 'Toki' registered in 2004, (G) 'Aori 27/Chiyuki™' registered in 2008, and (H) 'Mori no Kagayaki' registered in 2011. Scale bar represents 2 cm.

Disease resistance

In apple, scab, powdery mildew (caused by the fungal pathogen *Podosphaera leucotricha*), and fire blight (caused by *Erwinia amylovora*) are the major diseases affecting commercial apple production in many countries. For breeding of resistant apple cultivars, genes and QTLs related to disease resistance, and the linked DNA markers, have been successively identified.

Development of DNA markers for scab resistance has preceded that of markers for other diseases. Seventeen genes for apple scab resistance have been identified, and their global positions have been located on the apple genetic map (Bus *et al.* 2011). Among them, the most intensively studied has been the *Rvi6* (*Vf*) gene from *M. floribunda* 821. This was the first fine-mapped scab resistance gene, and defined as a receptor-like gene showing homology to candidate tomato genes for *Cladosporium fulvum* resistance (Vinatzer *et al.* 2001). The *Rvi15* (*Vr2*) locus was found to contain three candidate genes (of the Toll and mammalian interleukin-1 receptor protein nucleotide-binding site leucine-rich repeat structure resistance gene family) (Galli *et al.* 2010a, 2010b, Schouten *et al.* 2014), and the *Rvi1* (*Vg*) locus was shown to contain 6 ORFs of four putative TIR-NBS-LRR (TNL) genes, a TNL pseudogene, and a serine/threonine protein phosphatase 2A gene (Cova *et al.* 2015). Furthermore, Soriano *et al.* (2014) have developed SSR markers linked to the broad-spectrum resistance of the selection 1980-015-025 (*V25*), and fine-mapped them on LG11 as *Rvi18*. This region contains a lectin-like receptor kinase (LRK) as a candidate gene for resistance. Clark *et al.* (2014) have also identified two novel scab resistance loci in 'Honeycrisp', and mapped the loci as *Rvi19* and *Rvi20* on LG1 and LG15, respectively. They suggest that genes containing a leucine rich repeat region (LRR), a motif common in R genes, would be the prime candidate at each locus. Bastiaanse *et al.* (2015) have reported that resistance in 'Geneva' is conditioned by at least five NBS-LRR candidate genes clustered on LG4. Padmarasu *et al.* (2014) have mapped *Rvi12* (*Vb*) on LG12 of *Malus baccata* Hansen's baccata #2, and developed 16 SNP markers for resistance selection. Among the identified scab-resistance genes, *Rvi15* (*Vr2*) and *Rvi6* (*Vf*) have been proven to be practical for transformation of common susceptible cultivars (*Vr2*: Shouten *et al.* 2014, *Vf*: Belfanti *et al.* 2004, Joshi *et al.* 2011, Würdig *et al.* 2015).

Many apple cultivars with the *Rvi6* (*Vf*) gene have been developed by MAS, and are now in commercial use. In Japan, a scab-resistant cultivar 'Aori 25' has been developed, and the presence of the *Rvi6* (*Vf*) gene has been identified on the basis of DNA markers (Kudo *et al.* 2013). However, as breakdown of resistance conferred by a single gene has been observed at several experimental farms (Bénaouf and Parisi 2000, Parisi *et al.* 2006), accumulation of multiple resistance genes has become an essential strategy.

Although each of the developed molecular markers is a powerful tool for the pyramiding of resistance genes, it is necessary to include reference cultivars or strains derived from original studies for the appropriate use of such markers. Patocchi *et al.* (2009) standardized SSR markers linked to nine scab resistance genes (*Rvi2*, *Rvi4*, *Rvi5*, *Rvi6*, *Rvi11*,

Rvi12, *Rvi13*, *Rvi14*, and *Rvi15*) by sizing the alleles of four cultivars ('Fiesta', 'Prima', 'Gala', 'Golden Delicious'). These markers make it possible to breed durable scab-resistant cultivars through the pyramiding of resistant genes.

With regard to powdery mildew resistance, DNA markers have been reported for five major genes, *Pl1* on LG12, *Pl2* on LG11, *Plw* on LG8, *Pld* on LG12, and *Plmis* on LG11 (Dunemann *et al.* 2007, Fernández-Fernández *et al.* 2008, Gardiner *et al.* 2003, 2004, James *et al.* 2004, James and Evans 2004, Rikkerink *et al.* 2010). Calenge and Durel (2006) analyzed QTLs in F1 progenies (Discovery and TN10-8) over a four-year period (five seasons), and detected seven QTLs. They reported that the QTL regions on LG2 included most members of a RGA cluster, and that the QTL on LG8 was located 2~4.3 cM from *Plw* derived from 'White Angel'. The QTL on LG8 exhibited a major effect whereas QTLs on LG2 and LG13 exhibited intermediate but stable effects over the five seasons. These results suggest that a combination of these QTLs, the major effect QTL and the stable QTLs, would be required in order to obtain sufficient resistance to powdery mildew.

Khan *et al.* (2006) have identified a major QTL for fire-blight resistance on LG7 in the apple cultivar 'Fiesta'. From this QTL, a SCAR marker suitable for MAS was also developed (Khan *et al.* 2007). An ornamental cv.'Evereste' exhibited a strong QTL effect of the resistance on LG12 (Durel *et al.* 2009). Within the QTL interval, a 189 kb sequence was identified and cloned, on which new microsatellite markers were developed (Parravicini *et al.* 2011). The annotation of genetic elements contained in this fragment revealed the presence a cluster of eight genes, the *Pto/Prf* complex, showing homology to known genes conferring resistance to bacterial disease in tomato. Peil *et al.* (2007) mapped a major QTL on LG3 in *Malus* × *robusta 5*. By analyzing three populations derived from 'Robusta 5' accessions (Gardiner *et al.* 2012), three fire-blight resistance QTLs were also detected. The QTL identified on LG7, using a population grown in the USA, was co-located with a heat shock 90 family protein gene (HSP90) and a WRKY transcription factor gene. Analysis carried out on a population grown in Germany instead allowed the identification of a QTL on LG3, co-located with another HSP90. Finally, a QTL identified on LG3 was also discovered in New Zealand, which was co-located with a leucine-rich repeat family receptor-like protein gene (MxdRLP1).

Khan *et al.* (2012) reported information (linkage groups, closest marker, marker type etc.) for 27 QTLs obtained in individual studies using different genetic backgrounds and strains, and presented a scheme for breeding fire-blight resistant cultivars by utilizing three strong stable QTLs (LG3/*Malus* × *robusta 5*, LG12/*M. floribunda* clone 821, 'Evereste', LG7/'Fiesta'). Khan *et al.* (2013) then identified additional QTLs on LG2, LG6 and LG15 and a total of 34 significant associations for resistance to fire blight through a genome wide association study (GWAS). Within the QTL region on LG3 of *Malus* × *robusta 5*, a candidate resistance gene Fb_MR5, which belongs to the CC-NBS-LRR resistance gene family, was detected (Fahrentrapp *et al.* 2013). It was suggested that this gene was a determinant of resistance because transgenic 'Gala' showed significantly less severe fire blight symptoms (Broggini *et al.* 2014).

The development of new varieties with multiple disease resistance and high fruit quality has become a major goal in many apple breeding programs. For this purpose, MAS would be a highly effective approach. Kellerhals *et al.* (2008) reported the selection of multi-disease resistant seedlings by pyramiding two scab resistance genes (*Rvi6* (*Vf*) and *Rvi2* (*Vh2*) or *Rvi4* (*Vh4*)) and a mildew resistance gene (*Pl1* or *Pl2*) using DNA markers specific to each of the genes. They also attempted to construct a MAS system combining the QTLs for fire blight resistance with scab resistance.

Jänsch *et al.* (2015) developed SNPs linked to eight disease resistance genes (scab: *Rvi2*, *Rvi 4*, *Rvi 6*, *Rvi 11*, *Rvi 15*, powdery mildew: *Pl2*, fire blight: *FB_E*, *FB_MR5*), and refined the locus of *Rvi2*, *Rvi4*, and *Rvi11*. They then validated specificity of their alleles in coupling with resistance by determining the allele composition in eight apple genotypes ('Golden Delicious', 'Delicious', 'Cox's Orange Pippin', 'Jonathan, McIntosh', 'Granny Smith', 'Braeburn', and selection F2-26829-2-2 derived from *M. floribunda 821*) by systematic high throughput analysis in marker-assisted breeding (MAB). Baumgartner *et al.* (2015) bred valuable homozygous lines as breeding parents for pyramiding of resistance genes using markers of the resistance genes or QTLs for scab (*Rvi2*, *Rvi4*, *Rvi6*), powdery mildew (*Pl1*, *Pl2*) and fire blight (*FBF7*).

Although the occurrence of fire-blight has not yet been reported in Japan, Alternaria leaf-blotch has been the most problematic disease for apple growers and researchers since the 1960s. Based on detailed genetic analyses of F1 populations derived from crosses between cultivars, Saito and Takeda (1984) reported that susceptibility to Alternaria blotch was determined by a major gene (*Alt*), and was a dominantly inherited trait. Fukasawa-Akada *et al.* (1999, 2000a, 2000b, 2003) identified RAPD markers linked to the susceptibility of 'Kaori', an offspring of 'Delicious'. By analyzing 108 cultivars and strains, they demonstrated a strong correlation between the susceptible phenotype and the presence of the markers, tracing their origin back to the cultivars 'Delicious' and 'Indo'. Moriya *et al.* (2011) mapped *Alt* between two SSRs at the upper end of LG11 of 'Starking Delicious', and defined DR033892 as the nearest marker (Moriya *et al.* 2013). They also developed a MAS system for apple seedlings with these SSRs linked to Alternaria blotch resistance and fruit skin color (Moriya *et al.* 2012b). Later, Moriya *et al.* (2013) narrowed the region to 102 kb (containing ten candidate genes) using 32 newly developed SSR markers. Tabira and Otani (2004) validated the use of SNP in the alpha subunit of the chloroplast chaperonin (cpn-alpha) gene in a system for selection of seedlings resistant to Alternaria blotch (Japan Patent Kokai

2004-283002). However, Okada *et al.* (2011) reported that cpn-alpha is linked to *Alt*, but not *Alt* itself. Abe *et al.* (2012a, 2012b) studied the inheritance of moderate susceptibility in 'Sekai-ichi', 'Golden Delicious' and 'Orin', and suggested the presence of a dominant gene (*Alt-2*), different from *Alt*, that was common among the cultivars. However, Moriya *et al.* (2012a) mapped the QTL for moderate susceptibility on Orin LG11, in the same region of *Alt*. It is not yet clear whether *Alt* and *Alt-2* are the same loci. On the other hand, there is a report that SSR on other LGs was linked to the susceptibility of 'Golden Delicious' (Li *et al.* 2011).

Crown gall is also a serious disease affecting Japanese apple production, caused by *Agrobacterium tumefaciens*. The Japanese wild apple *Malus sieboldii* Sanashi 63 is reported to carry the crown gall resistance gene Cg, against the strain Peach CG8331, and has been identified as a crown gall-resistant rootstock (Moriya *et al.* 2008). Moriya *et al.* (2010) mapped Cg to LG 2 of the wild apple, and developed selectable markers for MAS.

Fruit quality

Improvement of fruit quality and growth habit is also a major goal of apple breeding programs worldwide. Many important fruit quality traits are regulated by multiple genes, and thus more information about the QTLs for these traits is required for MAS. Since the previous reviews (Gadiner *et al.* 2007, Keller-Przybyłkowicz and Korbin 2013, Marić *et al.* 2010, Pereira-Lorenzo *et al.* 2009), many new molecular markers related to fruit characteristics have been reported. The relevant reports are listed in **Table 2**.

Most of the QTL analyses reported so far have been performed using populations derived from cultivars ('Prima', 'Fiesta', 'Discovery', 'Telamon' etc.) developed in Europe, the USA, or New Zealand. Apple breeding programs in Japan have been performed using cultivars and strains with a rather unique genetic background, and thus evaluating the effects of genetic background has become important. Kunihisa *et al.* (2014) analyzed QTLs for 16 traits using a Japanese cultivar F1 population ('Orin' originated from 'Indo' × 'Golden Delicious', yellow fruit skin cultivar, × 'Akane' from 'Jonathan' × 'Worcester Pearmain', scab resistant). They identified QTLs that overlapped those reported previously for foreign cultivars, and also detected novel QTLs for harvest time, Brix, flowering date and juice browning. Among four QTLs for harvest time, a novel one was detected near the locus of the *MdACS1* gene on LG15 of 'Orin'. Examining relationships among the directions of the allelic effects of linked QTLs, they also demonstrated that the allele for early ripening and the *ACS1-1* allele for fruit drop were in a coupling phase on LG15, and that the effects of two QTLs (harvest time and firmness) on 'Akane' LG3 for earlier ripening and softer flesh were also similar. They investigated the actual validity of MAS using QTL markers by evaluating the broad-sense heritability of each trait, and concluded that these four QTLs (detected in LG3, 10, 15 and 16) can be used on a practical level for MAS of harvest time as well as for acidity based on the QTLs in LG8 and LG16. Their reports indicated two QTLs for depth of fruit skin color, one located close to the locus of the *MdMYB1* gene responsible for apple skin color at the lower end of LG9, and the other located on the hot spot for QTLs related to polyphenolic compounds of LG16. These results are important for breeding of Japanese apple cultivars, because fruit appearance is an important factor for consumers in Japan.

With regard to other traits related to the economically important aspects of fruit appearance, such as bitter pit, skin russeting, and fruit size, many reports have identified related QTL regions and candidate genes (Buti *et al.* 2015, Chang *et al.* 2014, Devoghalaere *et al.* 2012, Falginella *et al.* 2015, Kunihisa *et al.* 2014, Potts *et al.* 2014, Sun *et al.* 2015), as well as those for other fruit quality traits such as fruit texture (Chagné *et al.* 2014, Kunihisa *et al.* 2014, Longhi *et al.* 2012, 2013a, 2013b, Sun *et al.* 2015), ingredients (Bai *et al.* 2012b, Guan *et al.* 2015, Kunihisa *et al.* 2014, Morimoto *et al.* 2014, Potts *et al.* 2014, Sun *et al.* 2015) and tree habit (Bai *et al.* 2012a, Celton *et al.* 2014, Guitton *et al.* 2012, Morimoto and Banno 2015, Moriya *et al.* 2012c, 2015). Among them, firmness is a trait important for the texture and storage of fruit, and therefore a number of related QTLs have been identified (Chagné *et al.* 2014, Kunihisa *et al.* 2014, Marondedze and Thomas 2013, Sun *et al.* 2015). Costa *et al.* (2010) have already mapped *Md-PG1* to a locus of LG 10, within the QTL region associated with fruit firmness. Three haplotypes of the *Md-PG1* marker have been validated as selectable for fruit firmness (Longhi *et al.* 2013b), and a SNP marker (PG_FEM_LC_19) of *Md-PG1* has been used for MAS in the FruitBreedomics program.

Advanced technology

Since any given locus captures only a small proportion of the total genetic variance for complex traits, a large number of genome-wide markers are required for making accurate selection decisions. A project involving eleven European research groups, HiDRAS (High-quality Disease Resistant Apples for Sustainable Agriculture), has been supplying molecular markers linked to fruit quality and pathogen resistance QTLs through a dedicated website (URL: http://www.hidras.unimi.it/) to facilitate identification of the genetic factors that control fruit quality. The international RosBREED SNP consortium (IRSC, URL:www.rosbreed ORG), an American team centered at Michigan State University, has developed a total of 7867 apple SNPs (single nucleotide polymorphisms) through next-generation sequencing of 27 cultivars used as founders in global apple breeding programs. This consortium has initially developed the 8K SNP array (Chagné *et al.* 2012b), which is anticipated to be effective for a wide range of germplasms and applications such as high-resolution genetic mapping, QTL detection and characterization, and marker-assisted introgression.

Genomic selection (GS) can be used to obtain genomic

Table 2. QTLs and genes reported recently in apple fruit character and growth habit

Target traits		Reference
Fruit quality		
Coloration	Depth of skin color	Kunihisa *et al.* 2014
	Skin color	Zhang *et al.* 2014
	Skin/leaf/flesh	Morimoto *et al.* 2013
	Weighted cortical intensity	Kumar *et al.* 2012, 2013(GS)
Fruit size components	Weight, length, diameter	Chang *et al.* 2014
	Circumference, diameter, length, weight	Potts *et al.* 2014
	Weight	Devoghalaere *et al.* 2012, Mellidou *et al.* 2012, Sun *et al.* 2015
Russet	Average russet coverage	Kumar *et al.* 2012, 2013(GS)
	Calyx, pedicel	Kunihisa *et al.* 2014
		Falginella *et al.* 2015
Bitter pit		Kumar *et al.* 2013(GS), Buti *et al.* 2015
Fruit splitting (cracking)		Kumar *et al.* 2013(GS)
Fruit firmness and/or related qualities		Mellidou *et al.* 2012, Kumar *et al.* 2012, 2013(GS), Kunihisa *et al.* 2014, Marondedze and Thomas 2013, Chagné *et al.* 2014 (at harvest, after storage, Loss), Sun *et al.* 2015
	Texture sub-traits (14 parameters)	Longhi *et al.* 2012, 2013a, 2013b
Browning	Fruit flesh	Mellidou *et al.* 2012 (color, time), Kumar *et al.* 2012, 2013(GS), Di Guardo *et al.* 2013, Sun *et al.* 2014
	Fruit juice	Kunihisa *et al.* 2014, Morimoto *et al.* 2014
Flesh astringency		Kumar *et al.* 2012
Dry matter		Chagné *et al.* 2014
Soluble solids content (Brix)		Kumar *et al.* 2012, 2013(GS), Mellidou *et al.* 2012, Kunihisa *et al.* 2014, Guan *et al.* 2015
Individual sugars	Sucrose, glucose, fructose, sorbitol	Kunihisa *et al.* 2014
	Sucrose, glucose, fructose, sorbitol	Guan *et al.* 2015
	Sucrose, fructose	Sun *et al.* 2015
Acidity		Kumar *et al.* 2012, 2013(GS), Kunihisa *et al.* 2014, Morimoto *et al.* 2014
	Titratable acidity	Bai *et al.* 2012b
	Malic acid, citric acid, acetic acid, total acid	Sun *et al.* 2015
Vitamin C contents	AsA concentrations, dehydroascorbate conc., ascorbate-glutathione conc., total antioxidant activity	Mellidou *et al.* 2012
Phenolic compounds		Chagné *et al.* 2012b, Khan *et al.* 2012a, 2012b, Verdu *et al.* 2014
Ethylene production		Costa *et al.* 2014
Volatile compounds	57 individual esters and alcohols	Souleyre *et al.* 2014 (QTL, AAT1)
		Costa *et al.* 2013
Growth habits		
Biennial bearing		Guitton *et al.* 2012
Flowering date		Kunihisa *et al.* 2014
Fruit self-thinning		Celton *et al.* 2014
Preharvest fruit drop		Kunihisa *et al.* 2014
Earliness of fruit maturity		Morimoto *et al.* 2013
Harvest date		Chagné *et al.* 2014, Kunihisa *et al.* 2014
Columnar (fine mapping, specific marker)		Bai *et al.* 2012a, Moriya *et al.* 2012 c, Morimoto and Banno 2015
Rooting capability		Moriya *et al.* 2015

breeding values for selection of next-generation parents or potential cultivars for further testing at a very early stage (Desta and Ortiz 2014). By using an 8K SNP array and a population of 1,200 seedlings, Kumar *et al.* (2012) evaluated the accuracy of GS, and demonstrated its suitability as an alternative approach for fruit trait selection. Furthermore, Bianco *et al.* (2014) have developed a more high-throughput whole-genome genotyping array (20K) for apple.

Most of the QTL analyses reported so far have involved one or two crossing populations. For practical use of QTL markers among different genetic backgrounds, Costa (2015) performed MetaQTL analysis using four populations from six parental lines, 1,289 SNP genotypes and phenotypes for fruit quality traits, and incorporated the most relevant QTLs associated with important fruit quality traits into a consensus map. He suggested that this method would become more powerful for identification of candidate genes controlling relevant fruit quality traits through improvement

with additional pedigreed families and genotyping tools facilitating a higher marker density.

In 2011, the FruitBreedomics Project, an international consortium composed of 18 research institutes from Europe, Israel, South Africa, New Zealand and China and 6 small to medium enterprises, was launched to improve the efficiency of fruit breeding by bridging the gap between genomics and breeding (http://www.fruitbreedomics.com/, Laurens *et al.* 2010). The project ended on August 31st, 2015 with the generation of new tools for phenotyping, genotyping and transcriptomics such as ASSIsT, an automatic SNP scoring tool for the selection of high-quality reliable markers (Di Guardo *et al.* 2015).

Transgenic technology

Twenty years have passed since the first gene modified (GM) crop (Flavor Savor tomato) was commercialized in 1994 (Kramer and Redenbaugh 1994). Cultivation of GM crops has continued to increase steadily and globally over the past few years, and at present, over 15 GM crops are being cultivated in about 25 countries. There is now general scientific agreement that food derived from GM crops poses no greater risk to human health than crops bred using conventional techniques. Recently, the world's first GM apple, which does not turn brown when cut or bruised, was approved for consumption by the United States Department of Agriculture (Xu 2013). However, GM cultivation itself has not yet been introduced in several countries including Japan, mainly because of general public skepticism about the safety of GM crops. On the other hand, transgenic apple trees have been created experimentally to investigate the applicability of GM technology. This section introduces the development of the GM apple for breeding systems using transgenic techniques.

Root stock improvement

In early transgenic studies of apple, the *rol* genes isolated from *Agrobacterium rhizogenes* were used to improve the rooting ability of the M.26 and M.9, dwarf root stocks. These root stocks are propagated vegetatively, but are often difficult to root. To improve their rooting ability, transgenic technology has been applied using the *rol* genes, which are plant oncogenes carried in the Ri plasmids of *A. rhizogenes* (Lambert and Tepfer 1992). *Rol* genes induce not only enhancement of rooting initiation, but also a dwarfing character. Therefore, they have also been used to induce dwarfing in wild-type rootstock (Holefors *et al.* 1998, Welander *et al.* 1998, Zhu *et al.* 2001a, 2001b). In Japan, Igarashi *et al.* (2002) incorporated the *rolC* gene into the most popular rootstock in Japan, 'Marubakaidou' (*M. prunifolia*), which has high rooting ability but no dwarf character. The transgenic Marubakaidou exhibited a dwarfing trait with a reduction of internode length. Furthermore, these transgenic lines showed enhanced rooting ability. However, development of the transgenic apple was suspended because it proved difficult to obtain public understanding of GM apple production in Japan, and thus accurate greenhouse evaluation of the trait could not be performed.

Fruit improvement

Research aimed at improvement of apple fruit has focused mainly on storage quality and shelf-life. In Japan, Wakasa *et al.* (2006) reported that cultivars retaining firm flesh during storage showed very weak or transient expression of an endo-polygalacturonase (*MdPG1*), irrespective of the ethylene production rate and transcription levels of other ripening-related genes. These results strongly suggested that softening during ripening may depend on the expression pattern of *MdPG1*. Indeed, *MdPG1*-suppressed 'Royal Gala' apples harvested at various seasons were firmer than controls after ripening (Atkinson *et al.* 2012).

Apple flesh turns brown after being sliced or grated, due to a phenolic polymer produced by polyphenol oxidases (PPOs) (Murata *et al.* 2000). Although browning is a natural phenomenon, it is usually an undesirable feature for consumers. A company in Canada (Okanagan Specialty Fruits Inc.) has genetically modified 'Granny Smith' and 'Golden Delicious' by inhibiting four *MdPPO* genes using the RNAi approach. The resulting transgenic line showed a strong reduction of the browning process compared with the control. This GM apple showed a 90% reduction of the enzyme activity in mature fruit. The decision to deregulate these GM apple varieties was announced by the USDA Animal and Plant Health Inspection Service in February 2015. However, this GM apple genome retains a selectable marker gene for kanamycin resistance (Carter 2012). Therefore, it may not easy to convince consumers about the acceptability of this GM apple.

Disease resistance

The most serious diseases that hamper apple cultivation are scab and fire blight. Over the last few decades, apple varieties have been bred for production of better resistance. For more than half a century (1914 to 1970), the scab resistance gene *Rvi6/Vf*, originating from the wild species *Malus floribunda* 821, was incorporated into a wide variety of apple cultivars through crossing. However, the creation of a variety possessing the *Vf* resistance gene, but with commercially sufficient fruit quality, was not easy (Dayton *et al.* 1970). In order to improve the scab resistance of apples, genes encoding chitinolytic enzymes from a bio-control organism *Trichoderma harzianum* were introduced into apple (Bolar *et al.* 2000, 2001). The resulting transgenic lines expressing the genes were more resistant than non-transformed controls. Analysis of the *Vf* region led to the identification of a cluster of genes homologos to the tomato *Cladosporium fulvum* resistance gene family (Vinatzer *et al.* 2001). One of these genes, *HcrVf2*, was used to transform the susceptible apple cultivar 'Gala'(Belfanti *et al.* 2004).

As a candidate gene conferring resistance to fire blight disease, the attacin E gene derived from *Hyalophora*

cecropia (the North America silkworm moth) has been used. Attacin E exhibits substantial lytic activity against many important plant pathogenic bacteria, and apple trees transformed with this gene exhibited resistance to fire blight (Borejsza-Wysocka *et al.* 2010, Ko *et al.* 2002). Apples incorporating the *Lc* gene, a bHLH transcription factor of maize, exhibited resistance to both scab and fire blight (Li *et al.* 2007). It is considered that the effect is likely related to enhancement of biosynthesis of a specific flavonoid, which plays important roles in the plant response to pathogens. Moreover, Krens *et al.* (2011) have reported that transgenic apple lines carrying the barley hordothionin gene (*hth*), which inhibits *in vitro* growth of a number of fungi and bacteria (Terras *et al.* 1993), were significantly less susceptible to scab disease.

Cisgenesis and marker free

Public concern about the suggested or perceived risks of GM crops centers mainly around the presence of foreign gene(s) in the genome. To circumvent this issue, cisgenesis technology has been implemented. Cisgenesis uses all parts of the transgenes including the promoter and terminator, derived from sexually compatible plants (Holme *et al.* 2013). Furthermore, it has become possible to delete transformed selection markers, such as antibiotic resistance genes, using recombinase systems. These techniques have been utilized in some transgenic apples (Joshi *et al.* 2011, Krens *et al.* 2015, Schaart *et al.* 2011b, Vanblaere *et al.* 2014, Würdig *et al.* 2015). On the other hand, genetic transformation in apple has been achieved without the use of a selectable marker gene (Krens *et al.* 2015, Malnoy *et al.* 2010).

Early flowering

Apple trees have a long juvenile phase of 5-12 years or more, during which young seedlings cannot be induced to flower. This places a constraint on genetic analysis and the creation of new apple cultivars through cross-breeding. When seedlings are obtained from crosses with wild apple species, an even longer period is needed. Studies of a model plant, *Arabidopsis thaliana*, have identified several flowering-related genes during the last fifteen years. Apple homologues of the *Arabidopsis TFL1* and *FT* genes have been identified in Japan, and up-regulation of the *MdFT* gene in apple has resulted in precocious flowering (Kotoda *et al.* 2010). On the other hand, Flachowsky *et al.* (2007) have succeeded in breaking the juvenile phase through overexpression of the *BpMADS4* gene of silver birch (*Betula pendula*). The resulting transgenic apples came into flower only 3 to 4 months after planting in a glass house. The transgenic line was crossed with a fire blight-resistant wild species, and the resulting F1 seedlings were selected for *BpMADS4* and fire blight resistance, then backcrossed with another cultivar to integrate the scab resistance gene (Flachowsky *et al.* 2011, Le Roux *et al.* 2012). Recently, *BpMADS4* has been integrated into various linkage groups of four different apple cultivars (Weigl *et al.* 2015) with the intention of selecting non-transgenic null segregates at the end of the breeding program. Another outstanding technique for shortening the generation period of apple seedlings using a plant virus vector (Yamagishi *et al.* 2014) will be described in the following section.

New plant breeding technology

Although, transgene technology has provided opportunities to go beyond natural crossing barriers, it has also raised questions about possible effects on human health and the environment. In 2000, the Cartagena Protocol on Bio-safety was adopted at the Convention on Biological Diversity, and almost all countries have signed up to gene modified organism (GMO) legislation. Consequently, approval for introducing a GM crop to the market has become a very expensive issue, as documents pertaining to experimental safety verification must be submitted (Hartung *et al.* 2014). Thus, development has been preferentially focused on major crops such as soybean and maize (Heap 2013), whereas that of minor crops, such as fruit trees and apple, has been greatly delayed. Another reason for the delay is that development and evaluation of GM fruit trees requires a long period in comparison with annual crops. In the early 2010s, new plant breeding techniques (NPBT; Lusser *et al.* 2012) have emerged. Here, some of them that are closely applicable to apple breeding are introduced.

Trans-grafting

Grafting is a cultivation method that exploits the cooperative relationship between partner plants possessing different genomes (Mudge *et al.* 2009). In apple cultivation, it has been used mainly for maintenance and propagation of clone strains, and for altering plant vigor, architecture, and precocity. Since the rootstock interacts with soil, it greatly affects the growth and production ability of the scion through water and mineral uptake. Trans-grafting refers to grafting a GM part with a non-GM part. The GM-root provides the potential of using transgenic rootstocks to improve the performance of commercially approved scion varieties, and produce non-GM products. Therefore, trans-grafted plants have the potential to address the public's concerns about transgene flow and exogenous transgene products in most transgenic organisms. As a matter of course, GM parts can be used for cisgenic (genetic modification by disusing a non-crossable species or a synthetic gene) strategies (Lusser *et al.* 2012). In the scion on *rolB*-induced rootstock, no *rolB* gene was detected by PCR analysis, suggesting that the transgene was not translocated from rootstock to scion (Smolka *et al.* 2010).

Hetero-grafting experiments using herbaceous plants, such as tomato and pumpkin, have revealed that specific mRNAs, such as *GAI* (*GIBBERELLIC ACID INSENSITIVE*), are capable of long-distance transport in phloem (Haywood *et al.* 2005), as well as viral RNA and viroids.

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Furthermore, recent studies using deep sequencing techniques have disclosed that over 2000 transcripts are translocated between shoots and roots in *Arabidopsis* (Thieme *et al.* 2015) and grapevines (Yang *et al.* 2015). It has been considered that in apple, as a representative woody plant, the same RNA transportability would be observed. In fact, Kanehira *et al.* (2010) in Japan have shown that phloem cells obtained by laser-capture micro-dissection from sub-cultured apple shoots harbor mRNAs that have already been reported as phloem-transported RNAs in other plants. Furthermore, they have confirmed that transport of *GAI* mRNA between 'Fuji' and a root stock occurs in 3-year-old shoots in both upward and downward directions via the graft union (Xu *et al.* 2010). It has also been demonstrated that *gai* transgenic Marubakaidou rootstock conferred a dwarf phenotype to the 'Orin' scion and lower sensitivity to GA enhancement of stature (Xu *et al.* 2013). Thus, the trans-grafting system has the potential to improve the scion through transport of a specific gene mRNA from the GM-stock (Harada 2010).

Small RNAs have also been shown to be transportable in phloem. Flachowsky *et al.* (2012) reported that small RNAs can be transported through the graft union to apple sub-cultured shoots. In sweet cherry, Zhao and Song (2014) have confirmed long-distance (1.2 m) transfer of siRNA targeting the plum necrotic ringspot virus (PNRSV-hpRNA) from a transgenic rootstock to a non-transgenic scion. They also demonstrated that the transferred siRNAs enhanced the virus resistance of scions grafted on the GM stock. Therefore, trans-grafting technology to achieve transport of siRNA would provide an opportunity for apple improvement Kasai *et al.* (2011) and Bai *et al.* (2011) in Japan using tobacco plants have demonstrated that small RNA transported through the graft-union induce not only post-transcriptional gene silencing (PTGS) but also transcriptional gene silencing (TGS) of the target gene in the grafted partner. Thus, the combination of small RNA and grafting may allow the creation of innovative approaches for improvement of fruit crops (Kasai and Harada 2015).

ALSV vector

Viral vectors are a tool commonly used by molecular biologists to deliver genetic material into cells (Vainstein *et al.* 2011). This is the most effective means of gene transfer because of the ease of infection and the high degree of gene expression through spontaneous propagation of virus molecules in the cell. Furthermore, there is no need for tissue culture. Apple latent spherical virus (ALSV) is an ideal virus for this purpose because it produces no disease symptoms, and vertical transmission occurring via seed is extremely low (Nakamura *et al.* 2011). In addition, as horizontal transmission of ALSV via pollen does not occur among apple trees in an orchard (Nakamura *et al.* 2011), this vector is easy to manage safely in terms of bio-containment.

Yamagishi *et al.* (2011) in Japan have successfully induced rapid flowering in apple seedlings using the ALSV vector expressing the *AtFT* gene. Moreover, Yamagishi *et al.* (2014) have reported a novel technique that simultaneously promotes expression of the *AtFT* gene and silencing of the apple *MdTFL1-1* using the ALSV vector to greatly accelerate the flowering time and life cycle of apple seedlings. This system can reduce the time required for completion of the apple life cycle to 1 year or less. Furthermore, it was proved that all of the 47 next-generation seedlings were virus-free. Therefore, the authors claim that the successive progeny obtained using this technique would satisfy the regulations for GM organisms (Yamagishi *et al.* 2014).

Genome and epi-genome editing

Breeding strategies using mutations induced by irradiation or chemicals have played an important role in the development of new cultivars in fruit species. However, their effectiveness is very low, and the loci of mutations are random and not controllable. Recent biological technologies (ZFN, TALENS, CRISPR/Cas9) allow direct manipulation of target genetic sequences, leading to the desired phenotype. Since this technique, genome editing is a type of genetic engineering in which DNA is inserted, replaced, or removed from a genome using artificially engineered nucleases, legal regulations are expected to be much less stringent than for transgenic plants. Already, several countries, such as the USA and Israel, have approved plants derived by targeted mutagenesis as non-GM products. Peer *et al.* (2015) have reported the efficacy of ZFN technology in apple plants. Once genome editing has induced a mutation, it is desirable that the starter fragment (ZFNs gene) is not retained in the genome in order to ensure non-transgenic breeding. The authors have proposed using *Agrobacterium*-mediated transient expression or virus inoculation techniques combined with a high-efficacy regeneration system. In particular, direct delivery of endo-nuclease into target cells by virus vectors provides a unique non-transgenic approach for the production of gene-edited apple plants (Vainstein *et al.* 2011). The CRISPR/Cas9 genome-editing system will soon be applied to breeding of both apple and sweet orange (Jia and Wang 2014).

Epigenetic mechanisms including histone modifications and DNA methylation are critical for accurate gene expression. In view of the long-term memory afforded by epigenome editing, it would be a novel and attractive technology for the improvement of vegetatively propagated species, because it does not involve a germplasm in which epigenetic reprogramming occurs (Crevillén *et al.* 2014). Although detailed epigenetic research, such as that for *Arabidopsis* and rice, has not been carried out for apple, many phenotypic variations in apple may be correlated with epigenetics (Telias *et al.* 2011). Furthermore, editing using CRISPR-Cas9-base acetyl transferase may be adaptable to apple breeding systems (Hilton *et al.* 2015). Apple varieties improved by these modern techniques are considered to be equivalent to naturally occurring sports, and development of many improved apple cultivars is expected in the near future.

Conclusions and future prospects

Following developments in molecular biology, genomics, and bioinformatics, new breeding technologies are being developed rapidly. Traditional apple breeding involves the deliberate crossing of closely or distantly related individuals to produce new varieties with desirable properties. For such breeding, MAS is being steadily applied. Simultaneously, for improvement of existing trusted cultivars, new transgenic technologies can be applied in order to quickly eliminate any foreign genes. Furthermore, genome editing, by which only the target gene can be accurately modified, is emerging as a novel breeding technology. These new technologies will undoubtedly facilitate apple breeding, and yield novel and attractive apple cultivars.

In Japan, techniques, such as MAS, trans-grafting, and reduction of generation time by virus vectors, are being studied for practical use. On the other hand, technologies utilizing a large volume of genomic information and molecular markers, such as GS and Meta QTL, are yet to be acquired. It will also be necessary to adopt information on markers to Japanese apple cultivars, which have emerged via unique evolution. The importance of individual diseases and fruit characteristics may differ among countries, as growth conditions and consumer preference vary internationally. Development of apple cultivars that satisfy consumers and related industries will be accelerated by the integration of new genomic information, new technologies and existing breeding programs.

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