Stable and efficient transformation of apple

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Received October 30, 2019; accepted June 2, 2020 (Edited by Y. Tabei)

Abstract  Apple is one of precious fruit crop grown in temperate zone. In the post genomic era, the analysis of gene functions in horticultural crops such as apple is required for agricultural utilization. For analysis of such crops, the protocol establishment of tissue culture and transformation is essential. Although transformation efficiency in family Rosaceae is generally very low, some cultivars of Malus species have high transformation ability. Apple cultivars are usually clonally propagated by grafting on rootstocks, which can affect fruit quality and maturity and scion productivity. Apple rootstock cultivar Japan Morioka 2 (JM2) was produced at the Division of Apple Research, Institute of Fruit and Tea Science, NARO, in Japan. JM2, which was developed for dwarfing scions and improving disease resistance, is easily propagated by hardwood cutting. Furthermore, JM2 can be stably transformed at a high efficiency, which is better than other JM series rootstocks derived from the same parent. Leaflets of cultured shoots of JM2 have been transformed using Agrobacterium (Rhizobium) with a transducing gene. In this article, the JM2 transformation protocol is introduced in detail. Various genes and promoters have been confirmed to function as expected, with the resultant transformants exhibiting specific staining and fluorescent signals, and modified floral organ shapes, precious blooming and other characteristics. JM2 is thus a useful rootstock material for the enhancement of genetic research on apple and its relatives.

Key words: apple, genome editing, JM2 (rootstock), tissue culture, transformation.

Introduction

Apple (Malus×domestica Borkh.) is a globally popular fruit. Apple is believed to have originated in central Asia, with worldwide expansion subsequently facilitated by migrating humans along ancient trade routes (e.g., the so-called Silk Road) over the course of several thousand years. As a result of both unconscious and conscious selection, thousands of apple cultivars have arisen in the Middle-East, Europe and America. Because their genomes harbor high diversity, cultivars are maintained by grafting on rootstock to ensure the availability of genetically uniform scions and rootstocks for commercial production (Cornille et al. 2014). Foreign apple cultivars from North America and Europe were introduced into Japan in Meiji era.

After almost 90 years, Fuji, a superior cultivar developed in 1939 by crossing Ralls Janet with Delicious in Japan, was released by the Horticulture Research Station, Ministry of Agriculture and Forestry of Japan, in 1962 (Sadamori et al. 1963). The popularity of Fuji is due to its good taste and flavor and long shelf life. Further additional features required of new apple cultivars are good pigmentation of fruit skin under global warming conditions and resistance to various diseases and pests. Such promising traits would reduce labor costs and agrichemical usage.

To produce new cultivars, genetic analysis for desirable traits is needed. Because the genetic background of apple cultivars is heterozygous and apple has a long juvenile phase, traditional genetic approaches are time consuming. A faster alternative is tissue culture or molecular biotechnology. The first application of apple tissue culture was for propagation of outstanding rootstocks and cultivars. In addition, tissue culture is useful for the production of virus-free planting materials, cryopreservation of genetic resources, development of synthetic seeds and improvement of traits via genetic engineering. The continuing development of biotechnological tools has enabled analysis of various...
apple genes, such as those related to fruit color, fruit shape, fruit size, fruit taste, tree shape, and disease and pest resistance, for agricultural advancement. Tissue culture is now a major component of apple molecular breeding and biotechnology (Bhatti and Jha 2010; Teixeira da Silva et al. 2019).

Transformation conditions for apple cultivars/rootstocks are based on methods originally developed for model plants such as tobacco (Horsch et al. 1985) and carrot (Fujii and Uchimiya 1991). Shoot tips or nodal shoot segments or leaflets are usually selected as explants for the culture of apple shoots. The phytohormones cytokinin and auxin are included in the Murashige–Skoog (MS) culture medium (Murashige and Skoog 1962) for shoot multiplication (Figure 1). Benzylaminopurine (BAP) is the preferred cytokinin for apple, and indole butyric acid (IBA) is used as the auxin. IBA also induces rooting from apple shoots, while adventitious shoot regeneration from leaf explants of apple is induced by BAP. Although various cultivars have similar reactions to these phytohormones (Teixeira da Silva et al. 2019), the regeneration rate and transformation efficiency depends on the individual scion cultivar or rootstock cultivar (Dobránszki and Teixeira da Silva 2010). In most cases, apple leaf explants were cut across veins are cocultured with an appropriate strain (e.g. EHA101, EHA105, or LBA4404) of Agrobacterium harboring a Ti plasmid carrying genes to be introduced, and regenerated shoots from cut ends are selected with suitable antibiotics, typically kanamycin.

The generation of transgenic plants by this method has been attempted with many apple cultivars. High transformation efficiencies have been obtained using the cultivars Jonagold (De Bondt et al. 1996), Royal Gala (Maximova et al. 1998; Yao et al. 1995), Greensleeves (James et al. 1989; Maximova et al. 1998), Orin (Murata et al. 2000), Elstar (Szankowski et al. 2001) and Pinova (Flachowsky et al. 2007). Exogenous genes that have been expressed in transgenic apple plants include β-glucuronidase (GUS) (Malnoy et al. 2010), green fluorescent protein (GFP) (Maximova et al. 1998), nptII (James et al. 1989), antibiotic protein (attacin E) (Malnoy et al. 2010), flowering inducing regulators MdTFL1 (Kotoda et al. 2006) and BpMADS4 (Flachowsky et al. 2007), phytohormone metabolizer (iaaM, iaaH or IPT) (Li et al. 2011) and sorbitol metabolizer S6PDH (Kanamaru et al. 2004). During early transformation events, the GFP reporter gene was introduced into several apple cultivars including Golden Delicious. After coculture with Agrobacterium solution, leaflets of Golden Delicious formed calli and GFP fluorescence was observed (Maximova et al. 1998), but Golden Delicious calli never regenerated. This result demonstrates that transformation bottleneck is shoot regeneration from callus or explants, not the insertion and expression of T-DNA in the apple genome.

Apple cultivars are usually grown as scions grafted on to a rootstock, an approach that can influence productivity as well as factors such as precocity, yield, environmental and edaphic adaptability, light interception, and disease and pest resistance (Rom et al. 1990). Rootstock cultivars M.26 (Malus × domestica Borkh.) and Mo 84-A (Malus prunifolia var. ringo Asami Mo 84-A) are reported to give high transformation efficiencies (Igarashi et al. 2002; Maheswaran et al. 1992; Malnoy et al. 2010; Norelli et al. 1994; Smolka et al. 2009). In this article, we introduce a rootstock JM2, which exhibits stable transformation efficiency for the introduction of various genes or control under various promoters. The JM2 rootstock can serve as a powerful tool for research on connections between causative genes and desirable traits in apple.

Application of the JM2 transformation system

Our research institute (Division of Apple Research, Institute of Fruit Tree ad Tea Science, National Agriculture and Food Research Organization [NARO]) produced the JM rootstock series, with traits such as dwarfing and disease resistance, all from the same parents (Malus prunifolia Borkh. ‘Seishi’ × Malus pumila Mill. var. paradisiaca Schneid.). The rootstock cultivars were analyzed for their transformation efficiency. Sarcotoxin (Oshshima et al. 1999; Okada and Natori 1983) or MybA (Kobayashi 2002) genes were introduced by the Agrobacterium method (Takahashi et al. 2007) into rootstock cultivars M. prunifolia maruba-kaido, M.26,
JM1, JM2 and JM7 (Soejima et al. 2010, 2013). Analysis of the resultant transformants, each harboring an introduced gene, revealed that the most stable and efficient transformation was achieved with JM2. Compared with other rootstock cultivars, JM2 yielded more regenerated shoots and surviving transformed shoots. The stable transformation efficiency was confirmed by additional transformation experiments in our laboratory (Table 1, Figure 2). In this transformation experiments, ultrasonication was tried to enhance the transformation efficiency. Ultrasound in the presence of Agrobacterium causes micro wounds on plant tissue surfaces and allowed the Agrobacterium easy access to plant tissues (Szankowski et al. 2001; Trick and Finer 1997).

GFP is a visible, nondestructive reporter widely used in transformation experiments. The cauliflower mosaic virus 35S promoter or the Agrobacterium rhizogenes (Rhzobium rhizogenes) rolC promoter conjugated with GFP (p35S: GFP or prolC: GFP) was transferred to JM2, and green light emission was observed in a whole plant and in phloem (transgenic shoots per explants: 0.6%; Table 1, Figure 2a–d) (Tanaka et al. 2014). Another reporter gene, GUS driven by promoters from various flowering-control genes such as MdTFL1, AFL1 and AFL2, was transformed into JM2, and GUS-specific staining was observed at shoot apices and in floral organs (0.5–3.1%; Table 1, Figure 2e, f).

The effects of plant growth regulators in response to specific promoters (MdTFL1 or AFL) were further analyzed (Mimida et al. 2011; Wada et al. 2009). The anthocyanin synthesis regulator gene Myb was overexpressed with 35S promotor in JM2 (Umemura et al. 2013). Red pigments in the resulting transgenic JM2 were accumulated in the callus and shoots (1.75%; Table 1, Figure 2g, h). Homeotic changes in floral organs due to antisense suppression of MdPI, an apple floral organ identity gene, were observed in flowers with malformed petals and no stamens in transgenic JM2 (3.8%; Table 1, Figure 2i, j) (Tanaka et al. 2016). The control of flowering genes by transformation of JM2 resulted in precocious, perpetual blooming in the transgenic plants (0.4–0.6%; Table 1, Figure 2k, l) (Kotoda et al. 2006, 2010; Tanaka et al. 2014; Yamagishi et al. 2011).

In addition to the above experiments, the PDS gene, required for carotenoid biosynthesis, was repressed in JM2 by genome editing using the CRISPR/Cas9 system. The resultant plants with repressed PDS exhibited albinism (Nishitani et al. 2016). Expression of dormancy-related gene (PmDAM6 from Prunus mume, Japanese apricot) transformed in JM2 was chemically induced by external addition of dexamethasone, and dormancy release was subsequently decreased in the transgenic JM2 (0.25–1.7% in Table 1) (Yamane et al. 2019).

These results demonstrate that JM2 is a useful cultivar for apple transformation. The average transformation rate from transgene experiments, approximately 1 to 3%, is constant thus far, but the number of transgenic plants obtained is dependent on the function of the transgene (e.g., MdPI, MdTFL1, MdFT, AFL1, AFL2 or PmDAM6) related to growth, differentiation or dormancy. The number of transgenic plants carrying these genes was decreased in some cases by 90% compared with transgenic apple harboring innocuous genes such as reporters (Table 1). This result suggests that the type of introduced gene also influences the generation of transformants and sometimes even their survival. In particular, 35S:: PmDAM6-GM did not affect transformation efficiency in the absence of dexamethasone. A better approach is the use of induction promoters such as heat shock (Herzog et al. 2012) or chemical reagents, as listed in Table 1 (Yamane et al. 2019). Furthermore, rootstocks are advantageous, causing reduction in tree height, when studying signal transduction in non-transgenic scions (Xu et al. 2013). It shows a possibility to improvement of scion by modified JM2.

Table 1. Number of regenerated shoots and transgenic JM2.

| Introduced genes | Promoters | Explants | Km resistant shoots (%) | Transforms (%) | Number of experiments | References |
|------------------|-----------|----------|-------------------------|---------------|----------------------|-----------|
| VvMybA           | 3SS       | 1598     | 57 ( 3.6)               | 28 (1.75)     | 1                    | Takahashi et al. 2007 |
| GUS              | AFL1p2    | 300      | 11 ( 3.6)               | 7 (2.3)       | 1                    | Wada et al. 2009 |
| GUS              | AFL2p2    | 576      | 34 ( 5.9)               | 18 (3.1)      | 2                    | Wada et al. 2009 |
| GUS              | MdTFL1-1  | 973      | 78 ( 8.0)               | 5 (0.5)       | 1                    | Mimida et al. 2011 |
| GUS              | MdTFL1-2  | 1040     | 89 ( 8.5)               | 8 (0.7)       | 1                    | Mimida et al. 2011 |
| MdMYB110a_JP     | 3SS       | 1372     | 107 (7.7)               | 26 (1.8)      | 2                    | Umemura et al. 2013 |
| AhFT             | 3SS       | 550      | 22 ( 4.0)               | 3 (0.5)       | 1                    | Tanaka et al. 2014 |
| AhFT:GFP         | 3SS       | 3400     | 19 ( 0.5)               | 0 (0)         | 3                    | Tanaka et al. 2014 |
| AhFT:rolC        | 3SS       | 2675     | 20 ( 0.7)               | 11 (0.4)      | 3                    | Tanaka et al. 2014 |
| GFP              | rolC      | 2575     | 23 ( 0.8)               | 18 (0.6)      | 2                    | Tanaka et al. 2014 |
| antisense-MdPI   | 1P-2      | 1120     | 116 (10.3)              | 43 (3.8)      | 2                    | Tanaka et al. 2016 |
| 91071(C11-1)     | 3SS       | 997      | 6 ( 0.6)                | 6 (0.6)       | 3                    | Okada et al. 2016 |
| PmDAM6           | 3SS       | 2000     | 16 ( 0.8)               | 5 (0.25)      | 2                    | Yamane et al. 2019 |
| PmDAM6-GR        | 3SS       | 1096     | 21 ( 1.9)               | 19 (1.7)      | 1                    | Yamane et al. 2019 |
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JM2 transformation protocol

Sterilization
The protocol presented in this article was developed using shoot tips and axillary buds of apple-cultivar JM2 harvested from an orchard at Division of Apple Research, Institute of Fruit Tree and Tea Science, NARO. After harvest, tips including petioles are trimmed to a length of 2 to 3 cm, dipped in 70% ethanol for a few seconds, immediately immersed in 1% sodium hypochlorite solution (approximately 0.14% w/v available Cl) with stirring for 3 min and washed three times with sterile distilled water. The tips are dissected to remove cut ends. All manipulations are carried out under sterile condition. The aseptic tips and buds are then cultured on 0.5×MS medium with 0.1 mg l⁻¹ BAP, 0.65% Bacto agar and 30 g l⁻¹ sucrose instead of sorbitol at 23°C under fluorescent light (16 h light/8 h dark) in a growth room. After 3 to 4 months, vigorous growth shoots are planted in JM2 propagation medium.

Preparation of apple leaves
To prepare apple leaves for transformation, micropropagated JM2 shoots are cultured in JM2 propagation medium at 23°C under fluorescent light (16 h light/8 h dark) in a growth room. The shoots are maintained by subculturing every 4 weeks. In preparation for transformation, only leaflets unfolding at the apex should be selected. Nipped-out leaflets (approximately 500 pieces) are transferred to 0.5×MS medium (Figure 3a).
Preparation of Agrobacterium solution

*Agrobacterium* (*Rhizobium radiobacter*: LBA4404) harboring the objective plasmid is grown in 20 ml of ψB medium containing antibiotic (e.g., 50 mg ml⁻¹ kanamycin) in a 50 ml tube with shaking at 28 °C for 24 h (OD 600 ≦ 0.8–1.2). Agrobacterium cells in the tube are collected by centrifugation at 2,000 × g for 10 min at room temperature and then washed twice with 10–20 ml of 0.5 × MS medium. After re-centrifugation of the Agrobacterium solution, the pellet is resuspended with 20 ml of the same medium in a 50 ml tube, and the mixture is shaken at 28 °C for 2 to 3 h.

Transformation

Collected unfolding leaflets are moved to a 50 ml tube containing the prepared *Agrobacterium* solution. The tube is ultrasonicated in a water-filled ultrasonicator (UT-105S; Sharp Corp.) at 100 W and 37 kHz for exactly 10 s (Figure 3b). The *Agrobacterium* leaflet solution is then transferred to a plastic Petri dish on a clean bench and incubated for 15 min. The leaflets are placed on sterilized paper in remove excess *Agrobacterium* solution and then arranged on sterilized filter paper on a Petri dish containing co-incubation medium (25 leaflets per plate) (Figure 3c). After covering and sealing with parafilm, the plates are kept at 20–23 °C in the dark for 1 week.

Regeneration and selection of transgenic apple

Leaflets are cut on an angle vertical to leaf veins and divided into two to three fragments. The fragments are arranged, 20 per plate, on regeneration medium including kanamycin (25 mg l⁻¹) and meropenem (50 mg l⁻¹) (Figure 3c) (Komori et al. 2009). The plates are sealed with surgical tape (3M Healthcare, 2.5 cm width) and kept in darkness at 20–23 °C. After 1 month, the leaflets are transferred to fresh medium under light, with planting repeated monthly. After 3 months culture, green shoots will have regenerated from the leaflets on regeneration medium (Figure 3d). The regenerated shoots are then moved to propagation medium for transformed JM2 containing kanamycin (25 mg l⁻¹) and meropenem (50 mg l⁻¹).

Agrobacterium elimination and confirmation of transformants

To check for residual *Agrobacterium* in the regenerants, three leaflets from regenerants are collected and, chopped in distilled water, and the mixture is shaken for 1 h. The supernatant is applied to LB agar medium with kanamycin (50 mg l⁻¹), and the plates are incubated at 28 °C for 2 to 3 weeks. The absence of colonies indicates *Agrobacterium* has been eliminated. If colonies appear, PCR should be carried out with the suitable primer set for the objective plasmid. After amplification, the PCR solutions are checked by agarose gel electrophoresis. If an expected amplification band is observed, culture of the transformant on propagation medium for transformed JM2 containing meropenem (50 mg l⁻¹) is continued until colonies are no longer detected.

Rooting and acclimatization

Transformant shoots are planted under sterile conditions in pots containing a mixture of perlite and vermiculite (1:1) and rooting solution. The transformants are covered with a clear plastic bag or cup to prevent drying and then cultured for 1 month under light/dark conditions until roots develop. To acclimatize the developed plantlets, small holes are made in the bag or cup and gradually enlarged.

Protocol tips

1. *Agrobacterium* harboring a plasmid proliferates in ψB medium containing antibiotic (e.g., 50 mg ml⁻¹ kanamycin) in one night (14–18 h). Inoculation of *Agrobacterium* should thus begin a day before the transformation. Adequate quantities of bacterium should be prepared.

2. JM2 growth and leaf conditions are critical determinants of the transformation rate. Vigorous cultured shoots and unfolding leaflets are desirable. When nipping out leaflets, expanded leaflets are often

Figure 3. Procedure for apple JM2 transformation. a: Cultured JM2 shoots exhibiting vigorous growth are selected. Unfolding leaflets are nipped out from the apices with ceramic forceps. b: Collected leaflets soaking in *Agrobacterium* solution in a 50 ml plastic tube are sonicated with water in an ultrasonicator. c: After 1 week of co-incubation with *Agrobacterium* on paper, leaflets are cut and transferred to regeneration medium plates containing antibiotics. d: A green transgenic shoot (white arrow) is regenerated from a leaflet after approximately 3 months.
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3. Agrobacterium solution (with an OD_{600}<0.4) is unavailable.

4. Ultrasonication of the Agrobacterium leaflets solution should be performed for exactly 10 s. If a shorter time is used, no transformants are obtained, while a longer time kills leaflets.

5. Arrangement of the leaflets on filter paper on co-incubation medium must be carried out quickly to ensure a high transformation rate.

**Culture media and reagents**

**ψB medium for Agrobacterium**

A solution of 2% (w/v) Bacto tryptone, 0.5% (w/v) Bacto yeast extract and 1% (w/v) MgSO_{4}·7H_{2}O is prepared in distilled water, adjusted to pH 7.2 with 1M KOH and then autoclaved at 121°C for 20 min.

1× MS medium

MS-1, MS-2, MS-3, MS-4, MS-5 and MS-6 stock solutions are mixed in distilled water to obtain 1× MS medium consists of 1% (v/v) MS-1 (100×) and 0.5% (v/v) each of 200×MS-2, 200×MS-3, 200×MS-4, 200×MS-5 and 200×MS-6 and 0.5% (v/v) of 1,000× MS vitamin solution in distilled water.

MS-1 (100×): 165 g l^{-1} NH_{4}NO_{3} and 190 g l^{-1} KNO_{3} in distilled water.

MS-2 (200×): 34 g l^{-1} KH_{2}PO_{4} in distilled water.

MS-3 (200×): 88 g l^{-1} CaCl_{2}·2H_{2}O in distilled water.

MS-4 (200×): 74 g l^{-1} MgSO_{4}·7H_{2}O in distilled water.

MS-5 (200×): 3.38 g l^{-1} MnSO_{4}·H_{2}O, 1.24 g l^{-1} H_{3}BO_{3}, 1.72 g l^{-1} ZnSO_{4}·7H_{2}O, 0.166 g l^{-1} KI, 0.05 g l^{-1} NaMoO_{4}·2H_{2}O, 0.005 g l^{-1} CuSO_{4}·6H_{2}O and 0.005 g l^{-1} CoCl_{2}·6H_{2}O in distilled water.

MS-6 (200×): 5.56 g l^{-1} FeSO_{4}·7H_{2}O and 7.46 g l^{-1} Na_{2}-EDTA in distilled water.

1,000× MS vitamin solution

This solution contains 0.1 g l^{-1} nicotinic acid, 0.02 g l^{-1} thiamin hydrochloride, 0.1 g l^{-1} pyridoxine hydrochloride, 20.0 g l^{-1} myo-inositol, and 0.4 g l^{-1} glycine in distilled water.

0.5× MS medium

A solution of 0.5% (v/v) of 100×MS-1, 0.25% (v/v) each of 200×MS-2, 200×MS-3, 200×MS-4, 200×MS-5, 200×MS-6, 0.5% (v/v) of 1,000×MS vitamin solution, and 20 g l^{-1} sorbitol is prepared in distilled water and autoclaved at 121°C for 20 min.

1× N6-macro element solution (Chu et al. 1975)

N6-1, N6-2, N6-3 and N6-4 stock solutions are mixed in distilled water to obtain 1× N6-macro element solution containing 1% (v/v) of 100×N6-1, 0.5% (v/v) of 200×N6-2, 0.5% (v/v) of 200×N6-3, and 0.5% (v/v) of 200×N6-4.

N6-1 (100×): 283.0 g l^{-1} KNO_{3} and 46.3 g l^{-1} (NH_{4})_{2}SO_{4} in distilled water.

N6-2 (200×): 80.0 g l^{-1} KH_{2}PO_{4} in distilled water.

N6-3 (200×): 32.2 g l^{-1} CaCl_{2}·2H_{2}O in distilled water.

N6-4 (200×): 37.0 g l^{-1} MgSO_{4}·7H_{2}O in distilled water.

**Co-incubation medium**

To prepare co-incubation medium, a mixture of 1×N6-macro element solution, 1×MS-micro element solution, and 0.5% (v/v) MS vitamin solution containing 5 mg l^{-1} BAP, 0.2 mg l^{-1} NAA, 20 µM acetosyringone, 3% (w/v) sorbitol and 0.2% (w/v) phytagel is adjusted to a pH of 5.6 with 1N KOH and then autoclaved at 121°C for 20 min.

**Regeneration medium**

To prepare regeneration medium, a mixture of 1×N6-macro element solution, 1×MS-micro element solution, and 0.5% (v/v) MS vitamin solution containing 5 mg l^{-1} BAP, 0.2 mg l^{-1} NAA, 3% (w/v) sorbitol, 50 mg l^{-1} meropenem, 25 mg l^{-1} kanamycin, and 0.25% (w/v) phytagel. The medium pH was adjusted at 5.6 with 1N KOH and then autoclaved at 121°C for 20 min.

**Propagation medium for JM2**

JM2 propagation medium is prepared by combining 1×MS medium with 0.2 mg l^{-1} BAP, 0.1 mg l^{-1} IBA, 3% (w/v) sucrose, and 0.7% (w/v) Bacto agar. The medium pH was adjusted at 5.8 with 1N KOH and then autoclaved at 121°C for 20 min.

**Propagation medium for transformed JM2**

This medium is prepared by mixing 1×MS medium with 1 mg l^{-1} BAP, 0.1 mg l^{-1} IBA, 3% (w/v) sucrose, 50 mg l^{-1} meropenem, 25 mg l^{-1} kanamycin, and 0.7% (w/v) Bacto agar, followed by pH adjustment to 5.8 with 1N KOH, and then autoclaved at 121°C for 20 min.

**LB medium**

To prepare LB medium, a solution of 1% (w/v) Bacto tryptone, 0.5% (w/v) Bacto yeast extract, 1% (w/v) NaCl, 50 µg l^{-1} kanamycin, and 0.7% (w/v) Bacto agar. The medium pH was adjusted at 7.0–7.2 with 1N KOH, and then autoclaved at 121°C for 20 min.

**Future perspectives**

As reported in this article, the availability of apple JM2 rootstock cultivar allows molecular techniques and tools to be applied for stable and efficient Agrobacterium...
mediated transformation of apple, similar to various other model plants. The development of the JM2 transformation system has thus paved the way for the next stage of apple genetic analysis and breeding. Because the population genetic structure of apple is heterogeneous, some commercial cultivars are not as easily transformed as JM2. Improved methods are thus needed to facilitate the transformation of cultivars. Apple cultivars with high transformation efficiency are limited. Intriguingly, one common parent of these cultivars is the cultivar Golden Delicious. The transformation efficiency of Golden Delicious itself, however, is much lower than that of its offspring (Maximova et al. 1998), which indicates that the genetic factor in the Golden Delicious genome may be recessive. Research on the genetic background of easily transformable cultivars may open a gateway for understanding transformation events and can lead to the production of new cultivars. Another strategy is the development of a technique based on nucleotide genome editing (Corte et al. 2019). In a trial reported by Charrier et al. (2019), targeted mutagenesis using genome editing tools, followed by transient expression of guide nucleotides, resulted in production of low frequencies of cultured shoots with targeted edits. Methodological research on genome editing of apple cultivars is becoming increasingly important. Because rootstocks affect the physiological traits of fruit tree scions, a transgenic rootstock may be able to confer new traits on non-transgenic scions. Such an achievement will accelerate the acceptance of genetically modified organisms in society.

Acknowledgements

We thank Edanz/Group (www.edanzediting.com/ac) for editing the English text of a draft of this manuscript.

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