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Plant regeneration from apple (*Malus*×*domestica* Borkh.) anther culture-derived embryos

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To improve the adaptation process of the obtained androgenic plants and achieve high acquisition efficiency of plants in anther culture, six cultivars, 'ASP', 'Danxian', 'Fuji', 'Gala', 'SD', and 'Senshu', were used to investigate the effect of sucrose concentration on root formation from embryos in the cold treatment and the effect of plant regulators in the shoot regeneration medium for rooted and nonrooted embryos. The results indicated that cold treatment medium containing 1.5% sucrose showed the highest root formation in the three cultivars 'ASP', 'Senshu' and 'SD'. The rooted embryos readily developed into shoots compared to the nonrooted embryos. For rooted embryos, the preferred medium for shoot regeneration was 1/2 MS hormone-free medium containing 1.5% sucrose, whereas the preferred medium for nonrooted embryos was MS or 1/2 MS medium containing BAP and 3% sucrose. The genotype also played an important role in the process of plant regeneration from anther culture-derived embryos in apple. The cultivars 'ASP' (14.4%), 'SD' (6.2%) and 'Danxian' (6.0%) showed the highest rates of embryo induction. The cultivars 'Danxian', 'Gala', and 'Senshu' showed the highest rates of regeneration from embryos to shoots of 33.7%, 26.9% and 21.55%, respectively. Consequently, the total efficiency from anthers to shoots of 'Danxia' was 2.33%, followed by 'Gala', 'Fuji', 'Senshu', 'SD' and 'ASP', which had efficiencies of 0.35%, 0.30%, 0.30%, 0.27% and 0.25%, respectively.

Key words: cold treatment, rooted embryos, root induction, shoot induction

Introduction

Apple is one of the top three most important fruit crops grown in the wild worldwide. Limited by the high

degree of heterozygosity derived from self-incompatibility and the time-consuming nature of conventional breeding, increasing emphasis has been placed on haploid breeding and marker-assisted selection. The production of haploid plants from hybrids, followed by chromosome doubling, provides the apple breeder with a means of accelerating the process of true breeding line development.

It is well known that anther culture is the most effective technique for the production of doubled haploids in apples. Experiments using apple anther culture, which was developed in the early 1970s (Nakayama et al., 1972; Kubicki et al., 1975), have produced some plants or embryos (Fujii, 1989; Hidano et al., 1995; Höfer, 2004; Okada et al., 2009; Verdoodt et al., 1998; Xue and Niu, 1984; Zarsky et al., 1986). Nevertheless, the rates of induction and shoot formation from embryos have remained low. Moreover, most reports have only described the induction of embryos (Kadota et al., 2002). Improvement in shoot regeneration from embryos is quite difficult, and the low anther culture efficiency has limited its application.

In apples, there are two stages in the process of obtaining lines of doubled haploids using anther cultures. The first stage consists of embryo induction, which is fundamentally essential but not sufficient. Subsequently, it is necessary to develop a method for the efficient regeneration of plants from embryos. Usually, this stage creates a number of problems due to the large number of embryos, which are difficult to obtain and tend to die during the regeneration process (Górecka 2009).

For citrus anther culture, Hidaka (1990) reported that embryos derived from anther culture can produce roots and develop into plantlets when transferred to a medium without hormones. Moreover, in somatic embryo cultures of some plants (melon, spinach, cyclamen and asparagus), when somatic embryos had developed into a torpedo shape, the embryos were able to develop into plantlets on a medium without hormones (Kageyama and Yabe, 1991; Muto, 1991; Otani and Shimada, 1991; Saito, 1991). However, apple embryos failed to develop into plantlets when they were transferred to fresh medium. Some embryos only turned green, while other embryos developed deformities and thickening. Subsequently, these embryos developed into calli or died. When the embryos were transferred to dormant medium for another cold treatment, regeneration was achieved by culturing on regeneration medium (Fujii, 1989). In the literature, there is limited information regarding cold treatment and regeneration media for anther culture in apples.

To improve the adaptation process of the obtained androgenic plants, we investigated the effect of sucrose concentration on root formation from embryos in the cold treatment. We also sought to elucidate the effect of plant growth regulators in the shoot regeneration medium for rooted and nonrooted embryos to achieve high acquisition efficiency of plantlets in anther culture. Through these experiments, we produced more than 1000 androgenic plants and improved the final shoot acquisition efficiency for apple anther culture.

Materials and Methods

1. *Plant materials*

Flower bud clusters from apple (*Malus* × *domestica* Borkh.) cultivars 'American Summer Pearmain' ('ASP'), 'Danxia', 'Fuji', 'Gala', 'Senshu' and 'Starking Delicious' ('SD') were used in this experiment. Flower bud clusters of 'ASP', 'Senshu' and 'SD' were collected for three years during the time period of 2009-2011, and those of 'Danxia', 'Fuji' and 'Gala' were collected for four years during the time period of 2013-2016 (Table 1).

All flower buds in clusters of six apple cultivars were collected during the "First pink" stage of flower bud development (Zhang et al., 2013), in which most microspores were at the stage of early uninucleate to mid-uninucleate.

2. Cold pretreatment and flower bud sterilization

Flower buds were placed in a cold (4 °C), dark chamber for 25 days for pretreatment (Zhang et al., 2013). Immediately before anther isolation, buds were surface sterilized for 7-8 min with 0.1% sodium hypochlorite, with subsequent washing with sterilized water a total of three times.

3. Embryo induction

The embryo induction medium consisted of N6 medium (Chu et al., 1975) supplemented with 10 μM BAP, 0.5 μM naphthaleneacetic acid (NAA) and 5% sucrose. The cultures were incubated in the dark at 25 °C. The compositions of the embryo induction medium and the anther culture were identical to those reported previously (Zhang et al., 2013, 2017). The embryos were counted after being transferred to the other medium for cold treatment.

4. Shoot induction from embryos

(1) Cold treatment for embryos

After 7–18 weeks from the beginning of anther culture, embryos grown to 3–8 mm were isolated from the anthers (Zhang et al., 2013). The embryos were transferred to cold treatment medium consisting of MS basal medium, for which all components were half-strength (1/2 MS) supplemented with 1.5% sucrose and 0.8% agar. No hormones were added to the cold treatment medium. The plates with embryos were maintained for 12–20 weeks in a dark chamber at 4 °C.

(2) Shoot induction from embryos

After cold treatment, the embryos were transferred onto shoot regeneration medium and incubated at 25 °C under cool white fluorescent light with a 16/8-h (light/dark) photoperiod (ca. 45 μmol·m^{-2}·s^{-1} PPFD). All embryos were subcultured every 4 weeks. Regeneration rates were counted once all shoots were transferred to the shoot multiplication medium.

5. Shoot multiplication

The shoot multiplication medium for propagation of the regenerated shoots consisted of MS medium (Murashige and Skoog, 1962) supplemented with 4.44 μM (1 mg·L^{-1}) BAP, 0.49 μM (0.1 mg·L^{-1}) 4-(3-indolyl) butyric acid (IBA), 3% sucrose and 0.65% Bacto agar (Li et al., 2011).

6. Root induction

For root induction, adventitious shoots more than 10 mm in size and grown on shoot multiplication medium were transferred to root induction medium consisting of 1/2 MS medium supplemented with 2.45 μM (0.5 mg·L^{-1}) IBA, 3% sucrose and 0.65% Bacto agar (Shi et al.,2018). Then, they were incubated for 7 days in the dark at 25 °C. The resulting cultured shoots were maintained under cool white fluorescent light with a 16/8-h (light/dark) photoperiod (ca. 45 μmol·m^{-2}·s^{-1} PPFD).

7. Acclimatization of the rooted shoots

After 6 weeks of root induction, cultured shoots were washed in sterilized water and planted in 60 × 60 mm plastic pots with sterilized vermiculite. Then, they were covered with a polyethylene bag to maintain humidity. These plants were grown in the culture room, and the polyethylene bag was gradually removed.

8. Improvement of anther culture

1) Root induction from embryos during the cold treatment period to increase the plantlet acquisition rate

To investigate the effect of sucrose concentration on root formation of embryos during the cold treatment period, the embryos were cultured on media used for the cold treatment for root induction, consisting of 1/2 MS, 0.8% agar and sucrose of different concentrations. The control treatment medium consisted of 1.5% (0.04 M) sucrose and 2.5% (0.07 M) maltose (total 0.11 M). The sucrose concentrations were established at 4.125% (0.11 M), 3% (0.08 M), 2.25% (0.06 M) and 1.5% (0.04 M).

The rooted embryos for which cold treatment had been completed were transferred to 1/2 MS hormone-free medium containing 1.5% sucrose for shoot regeneration in the experiment shown in Table 2.

2) Effects of hormone concentrations and strength of MS medium on shoot regeneration

The embryos used for this experiment received cold treatment on the cold treatment medium. After cold treatment for 12–20 weeks, the embryos were divided into two groups: rooted and nonrooted embryos. Embryos from each group were cultured separately to determine a suitable hormone concentration and strength of MS medium for shoot regeneration (Table 3-6). The media contained 0.65% Bacto agar.

9. SSR and ploidy analysis

SSR analysis was conducted to confirm whether the plants were derived from pollen grains after acclimatization. Ten SSR markers showing a heterozygous genotype on the donor genotypes of 'Senshu' and 'SD' were used (Okada et al., 2009), and seventeen SSR markers on the donor genotype 'Gala' were used (Wen et al., 2017) for identification.

The ploidy level of the plants was determined by flow cytometry (Wen et al., 2017).

Results

1. Embryos and callus formation from anther cultures

The embryos emerged from anthers after 7–18 weeks of culturing. Some anthers formed several embryos (Fig. 1. A). These embryos included various developmental stages, from globular embryos to cotyledonary.

The data for ‘ASP’, ‘Senshu’ and ‘SD’ from 2009-2011 are shown in a previous report (Zhang et al., 2017). ‘ASP’ showed the highest embryo formation rate, with an average rate over three years of 14.4%. In 2009, the embryo formation rate of ‘ASP’ reached 26.7%. The average rates of ‘Senshu’ and ‘SD’ were 2.4% and 6.2%, respectively. For the callus, ‘ASP’ also showed the highest callus formation, with a rate as high as 10.2%. The average rates of ‘Senshu’ and ‘SD’ were 2.5% and 2.1%, respectively.

The data for ‘Danxia’, ‘Fuji’ and ‘Gala’ from 2013-2014 are shown in Table 1. ‘Danxia’ had the highest average rate (6%) of embryo formation over the four-year period. The average rates of ‘Fuji’ and ‘Gala’ were 1.5% and 1.3%, respectively. ‘Gala’ showed the highest callus formation of 5.4%, followed by Danxia (4.2%) and Fuji (3.3%).

2. Root formation from the cold treatment for root induction medium

For root formation from embryos, the effects of sucrose concentration in the cold treatment for root induction media are shown in Table 2. In 2009, the highest rates of root formation for ‘ASP’ and ‘Senshu’ were observed in 3% and 1.5% sucrose, respectively. However, the root formation rate was the highest (23.1%) with 1.5% sucrose for ‘SD’. In 2010, the root formation rate was the highest (32.6%) with 1.5% sucrose. Therefore, the cold treatment medium containing 1.5% sucrose showed the highest root formation rates in all three cultivars.

3. Effects of hormone concentrations on shoot regeneration

After cold treatment, the rooted and nonrooted embryos (Fig. 1. B) were cultured separately to elucidate the optimum culture conditions for plant regeneration. Table 3-6 presents the results of the shoot regeneration media for rooted and nonrooted embryos.

(1) Rooted embryos

The rooted embryos more readily developed into shoots (Fig. 1. C) compared to the nonrooted embryos (Fig. 1. D). As shown in Table 3, the highest shoot regeneration rates of ‘Senshu’ and ‘SD’ in 2009 were obtained from the rooted embryos cultured in 1/2 MS hormone-free medium with 1.5% sucrose, with values almost identical to that of 1/2 MS with 1.5% sucrose and 1 mg/L BAP of ‘Senshu’. In 2010, the highest shoot regeneration of ‘ASP’ was shown on 1/2 MS medium containing 1 Mm BAP, 5 μM GA and 1.5%

sucrose. In 2011, the highest shoot regeneration of ‘ASP’ was shown on the 1/2 MS hormone-free medium with 1.5% sucrose, and the highest shoot regeneration of ‘Senshu’ was shown on the 1/2 MS medium containing 1 mg/L BAP and 1.5% sucrose.

For the cultivars ‘Danxia’, ‘Fuji’ and ‘Gala’, the rates of shoot regeneration were almost identical among each culture medium. In 2013 and 2014, all three cultivars showed the highest shoot regeneration rates on the 1/2 MS hormone-free medium with 1.5% sucrose (Table 4). In 2015, ‘Danxia’ showed a higher shoot regeneration of all three years on all four media. The highest rate was 58.6% on MS medium containing 0.5 mg·L-1 BAP, 2 mg·L^{-1} GA and 3% sucrose; the lowest rate was 54.3% on MS medium containing 0.5 mg·L^{-1} BAP and 3% sucrose. In ‘Fuji’, only two or three embryos were tested in each medium, and they reached shoot regeneration rates of 50~100%. In 2016, ‘Danxia’ and ‘Fuji’ showed higher shoot regeneration on both MS+ BAP 0.5 mg·L-1 and MS+ BAP 0.5 mg·L^{-1}+2 mg·L^{-1} GA media. Regeneration of ‘Gala’ was shown on both MS+ BAP 0.5 mg·L-1 and MS+ BAP 0.5 mg·L^{-1}+0.1 mg·L^{-1} IBA media.

(2) Nonrooted embryos

In 2009, the highest regeneration rate of ‘Senshu’ was shown on MS medium with 1 mg·L^{-1} BAP and 3% sucrose (Table 5), while the highest regeneration rate of ‘SD’ was shown on 1/2 MS medium with 1 mg·L^{-1} BAP and 1.5% sucrose. In 2010, the highest regeneration rate of ‘ASP’ was shown on 1/2 MS medium with 1 mg·L^{-1} BAP and 1.5% sucrose. In 2011, the highest regeneration rate of ‘ASP’ was shown on MS medium with 1 mg·L^{-1} BAP and 3% sucrose, while that of Senshu was shown on MS medium with 1 μM BAP, 5 μM GA and 3% sucrose.

In 2013, the highest regeneration rates of ‘Danxia’ and ‘Gala’ were shown on MS medium with 0.5 mg·L^{-1} BAP, 2.0 mg·L^{-1} GA and 3% sucrose (Table 6). In 2014, the highest regeneration rates of ‘Danxia’ and ‘Gala’ were shown for MS medium with 0.5 mg·L^{-1} BAP, 0.1 mg·L^{-1} IBA and 3% sucrose. The values were almost identical to that of ‘Gala’ for MS medium with 0.5 mg·L^{-1} BAP, 2.0 mg/L GA and 3% sucrose. The highest ‘Fuji’ regeneration was shown on MS medium with 0.5 mg·L^{-1} BAP and 3% sucrose. In 2015, the highest regeneration rate of ‘Danxia’ was shown on MS medium with 0.5 mg·L^{-1} BAP, 2.0 mg·L^{-1} GA and 3% sucrose, while that of ‘Gala’ was shown on MS medium with 0.5 mg·L^{-1} BAP, 0.1 mg·L^{-1} IBA and

3% sucrose. In 2016, the highest regeneration rate of 'Danxia' was shown on MS medium with 0.5 mg·L^{-1} BAP, 2.0 mg·L^{-1} GA and 3% sucrose. The highest rate of 'Fuji' regeneration was shown on MS medium with 0.5 mg·L^{-1} BAP and 3% sucrose, while that of Gala was shown on MS medium with 0.5 mg·L^{-1} BAP, 0.1 mg·L^{-1} IBA and 3% sucrose.

4 SSR and ploidy analysis

In the SSR analysis, 10 SSR markers showed heterozygous genotypes on the donor genotypes of 'Senshu' and 'Starking Delicious'. Seventeen SSR markers were used for the donor cultivar 'Gala'. Only one allele from each donor cultivar was amplified in each sample. SSR marker analysis confirmed that all individuals had a haploid origin.

Discussion

1. Effects of cold treatment on shoot regeneration

In Brassica species (Zhou et al., 2002; Da Silva Dias, 2001; Ferrie, 2003), treatments at a temperature below 10 °C for 1-3 weeks at the start of the regeneration phase are recommended to increase regeneration from embryos. In apples, Wang and Ogata (1988) reported that when they placed three embryos from M9 in a cold (4 °C) chamber for 45 days, one of the three embryos developed plantlets and an embryonic axis with root formation. Moreover, Fujii (1989) reported that there were 8 embryos that formed roots when they treated 18 embryos for 3 months, and 9 of 21 embryos formed roots after 5 months of cold treatment.

In our experiment, all of the tested cultivars showed root formation after cold treatment (Table 2 and Table 7). In 2009 and 2010, we investigated the effect of sucrose concentration on the cold treatment of root induction media to increase the rates of root formation from embryos. The cold treatment for root induction medium containing 1.5% sucrose showed the highest root formation rates among all three cultivars. Therefore, 1.5% sucrose was advantageous for root formation using the cold treatment method.

2. *Relationship between rooted and nonrooted embryos*

In our experiment, all of the tested cultivars showed that the shoot regeneration rates of rooted embryos were higher than those of nonrooted embryos (Table 7). The cultivar ‘Danxia’ reached 54.1%, which was much higher than the 23.4% of the nonrooted embryos. In 2011, there was no shoot regeneration from 178 nonrooted embryos of ‘SD’, and the average shoot regeneration rate of nonrooted embryos was only 2.6%. Moreover, the green shoot apex appeared from the rooted embryos one week after transplantation into the shoot regeneration medium. The nonrooted embryos started to regenerate adventitious shoots approximately 3 weeks after transplantation in the medium. It can be inferred that the embryos that formed roots had bipolarity. Fujii (1989) observed that embryos induced from anther culture in apples were unable to develop into shoots if the embryos were not cultured at a low temperature (4 °C). Approximately half of the embryos developed into shoots, and most of them were accompanied by roots after cold treatment. These results were not contradictory to our results for rooted embryos after cold treatment. Maximova et al. (1998) used leaves from rooted shoots in *Agrobacterium*-mediated apple transformation. These results suggest that shoot formation is closely related to root formation. The rooted embryos must be ideal for obtaining haploid or homozygous plants.

3. Effects of genotypes on shoot regeneration

In our previous experiments (Zhang et al., 2013, 2017), the ability to induce embryos from anthers depended mostly on the applied cultivar. This study revealed that the genotypes also played an important role in the process of plant regeneration from apple anther culture-derived embryos. The cultivars ‘ASP’, ‘SD’ and ‘Danxia’ showed the highest rates of embryo induction, followed by ‘Senshu’, with approximately 2.43%, and ‘Fuji’ and ‘Gala’, each with less than 2% (Table 1). The regeneration rates from embryo to shoot of ‘Danxia’, ‘Gala’ and ‘Senshu’ were all more than 20%; however, the rates of ‘ASP’ and ‘SD’ were 1.55% and 5.22%, respectively (Zhang et al., 2017). Consequently, the total efficiency from anthers to shoots of ‘Danxia’ was 2.33%, followed by ‘Gala’ with approximately 0.35% (Table 1). ‘Fuji’, ‘Senshu’, ‘SD’ and

‘ASP’ had efficiencies of 0.30%, 0.30%, 0.27% and 0.25%, respectively (Zhang et al., 2017).

Previous reports have been very limited in apple anther culture regarding the description of the total efficiency, meaning the rate of shoot regeneration per anther number, and stepwise efficiencies, such as the shoot regeneration rate from embryos. Höfer (2004) stated that the shoot regeneration rate of anther culture varied from 0 to 33.5% depending on the genotype, and the average rate of shoot regeneration of Alkmene, Remo, Rene and Reka was 26.6%. Kadota et al. (2002) reported that the regeneration rates of shoots from embryos of six cultivars of apple (Nagafu No. 6 (sport of Fuji), Himekami, Homei (sport of Tsugaru), Kotoku, ‘SD’ and Yataka (sport of Fuji)) were between 14.3% and 100%; however, only shoots from ‘SD’ developed roots and grew into plantlets. Vanwynsberghe et al. (2005) reported that the efficiency of anther culture in apple was both genotype and stepwise dependent. The total production efficiency was generally low at only 0.01% in Braeburn, and an average of only 14 shoots could be obtained from more than 120,000 anthers per experiment. Höfer et al. (1999) obtained one homozygous line of apple cultivar Remo from 537 anthers, with a total efficiency of 0.19%. Zhang et al. (1987) stated that the embryo induction rate was dependent on the culture medium, which varied from 0 to 1.3% for ‘SD’. The efficiency of apple anther culture was highly genotype dependent; therefore, the selection of cultivars possessing both embryo and shoot induction abilities is important.

4. Effects of hormone and MS medium concentrations on shoot regeneration

For rooted embryos, all six cultivars demonstrated regeneration on 1/2 MS medium without hormones each year (Tables 3 and 4). When comparing the same component media except for MS strength (the MS + BAP pair of ‘Senshu’ and ‘SD’ in rooted embryos, the MS + BAP + GA pair and the MS + BAP pair of ‘Senshu’ and ‘SD’ in nonrooted embryos), the shoot regeneration rates of 1/2 MS were higher than those of MS, except for the MS + BAP pair of ‘Senshu’ in nonrooted embryos (Table 3). For ‘Danxia’, ‘Fuji’ and ‘Gala’, the values were almost identical to those of 1/2 MS hormone-free medium. Therefore, 1/2 MS was useful for shoot regeneration. In particular, 1/2 MS without hormones was the most advantageous for shoot regeneration of rooted embryos.

For nonrooted embryos, the regeneration rate of ‘Senshu’ was the highest in MS medium containing 1 mg·L^{-1} BAP and 3% sucrose, ‘SD’ regeneration was highest using 1/2 MS medium containing 1 mg·L^{-1} BAP and 3% sucrose, ‘Danxia’ and ‘Gala’ were highest using MS medium containing 1 mg·L^{-1} BAP and 2 mg/L GA, and ‘Fuji’ was highest using MS medium containing 1 mg·L^{-1} BAP.

Moreover, plantlet development was difficult in nonrooted embryos on 1/2 MS medium without hormones. We believe that rooted embryos have completed the postripening process after completing the cold treatment, similar to zygotic embryos, and have the conditions for germination. Therefore, the quality of embryos may be more important than the composition of the medium for regeneration in apple anther culture. It is therefore necessary to further explore the effects of cold treatment and the composition of the medium on root formation from embryos.

In conclusion, our experiments clarified that several factors are responsible for the regeneration efficiency of apple anther culture, including donor plant genotype, cold treatment of embryos and shoot regeneration medium. It is important to select genotypes possessing excellent abilities for both embryo formation and shoot regeneration among many cultivars. We also elucidated that the abilities of embryo formation and shoot regeneration from embryos were controlled by different genes. The induction of rooted embryos to the greatest degree possible is important for the acquisition of many plantlets. The rooted and nonrooted embryos produced by the cold treatment should be cultured separately. The preferred medium for shoot induction of rooted embryos is 1/2 MS hormone-free medium containing 1.5% sucrose, whereas the preferred medium for shoot induction of nonrooted embryos is MS or 1/2 MS medium containing BAP and 3% sucrose. This improved procedure of cold treatment and separate shoot induction in rooted and nonrooted embryos is expected to improve the acquisition rate of plantlets using apple anther culture.

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Legends

Table 1. Rates of embryo formation and shoot regeneration in each cultivar of anther culture.

Surviving anthers (%) = (Number of surviving anthers/Number of cultured anthers)×100

Calli formation (%) = (Number of Calli formed/Number of surviving anthers)×100

Embryo formation (%) = (Number of embryos formed/Number of surviving anthers) * 100

Shoot formation from embryos (%) = (Number of shoots formed/Number of embryos formed) ×100

Shoot formation from anthers (%) = (Number of shoots formed/Number of surviving anthers) ×100

Table 2. Effects of sucrose concentration on the cold treatment of root induction media on embryoid root formation.

Rooted embryos (%) = (Number of rooted embryos/Number of embryos tested)×100

Table 3. Effects of shoot regeneration media for rooted embryos (2009-2011).

Shoot regeneration (%) = Number of shoots regenerated/Number of embryos tested)×100

BAP: 1 mg･L-1 = 4.4 μM

GA: 1 mg･L-1=2.9 μM

Table 4. Effects of shoot regeneration media for rooted embryos (2013-2016).

Shoot regeneration (%) = Number of shoots regenerated/Number of embryos tested)×100

BAP: 1 mg･L-1 = 4.4 μM

GA: 1 mg･L-1=2.9 μM

Table 5. Effects of shoot regeneration media for nonrooted embryos (2009-2011).

Shoot regeneration (%) = Number of shoots regenerated/Number of embryos tested)×100

BAP: 1 mg·L-1 = 4.4 μM

GA: 1 mg·L-1=2.9 μM

Table 6. Effects of shoot regeneration media for nonrooted embryos.

Shoot regeneration (%) = Number of shoots regenerated/Number of embryos tested)×100

BAP: 1 mg·L^{-1}= 4.4 μM

GA: 1 mg·L-1= 2.9 μM

Table 7. Expected final shoot formation rate in apple anther culture.

Root formation (%) = (Rooted embryos/Number of embryos tested)×100

Shoot regeneration of rooted embryos (%) = (Number of shoot/rooted embryos)×100

Shoot regeneration of nonrooted embryos (%) = (Number of shoot/nonrooted embryos)×100

Fig. 1. Anther culture in apple.

(A) Embryo formation from anthers; (C) rooted embryos and nonrooted embryos during cold treatment; (C) regenerated shoots from rooted embryo; (D) regenerated shoots from nonrooted embryo.

Figures

Figure 1

Anther culture in apple. (A) Embryo formaiton from anthers; (C) Rooted embryos and Nonrooted embryos during cold treatment; (C) Regenerated shoot from rooted embryo; (D) Regenerated shoots from nonrooted embryo.

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