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Induction and identification of tetraploids of pear plants (*Pyrus bretschneideri* and *Pyrus betulaefolia*)

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ABSTRACT

Polyploid plants usually exhibit broader leaves, thicker stems, bigger flowers and fruits, dwarfing stature, as well as improved biotic and abiotic resistance. Therefore, increasing the polyploidy is one of the most important strategies used in plant breeding. Here, we reported the successful induction of tetraploids of two pear varieties, 'duli' pear (*Pyrus betulaefolia*), a wildtype pear used for rootstocks in grafting, and 'Xinli No.7′' pear (*Pyrus bretschneideri*), a popular cultivated pear variety in China. This was achieved by treating their seeds, shoot tips of tissue cultured or field grown seedlings with colchicine and pendimethalin using three different methods: impregnation, mixed culture and smearing. The best tetraploids induction condition for 'duli' pear seeds was impregnating them with 0.4% colchicine for 24 h where the mutation rate of 2.0% was achieved with no chimera found. For shoot tips of tissue cultured seedlings the best condition was impregnating them with 0.2% colchicine where 6.67% of 'duli' (48 h) and 13.0% of 'Xinli No.7′' (24 h) were mutated. In addition, we found that in the mixed culture method the best induction condition for 'duli' was mixing with 1.0% colchicine while for 'Xinli No.7′' it was 0.5% colchicine. In the smear method, for the shoot tips of field grown seedlings of 'duli', the best induction condition was treating them with 0.4% colchicine for 24 h. Subsequently, the DNA content of these putative polyploid seedlings was estimated by the flow cytometry, confirming their ploidy nature. Also, we compared the morphological differences between the tetraploid seedlings and the diploid seedlings. Obviously, the tetraploid seedlings appeared to be dwarfed with shorter internode length and reduced internode number of their stems.

1. Introduction

Genome doubling could happen naturally, leading to polyploid plants with altered phenotype where 'mega-sized' flowers, fruits, stomata and guard cells were the common features (Niu et al., 2020; Dai, 1990; Li et al., 1999). In addition, increased chloroplast numbers (Wang et al., 1984; Li and Shang, 1993), glucose and sucrose contents (Wang et al., 2015), and aroma (Qian, 2004) and decreased cellulose (Fang, 2004) were also found in the cells of tetraploids. Therefore, polyploids in some crops are desirable.

Polyploid plants can be directly used as new cultivars (Wang et al., 2015), or as pollen donor in cross pollination. For example, pollens of tetraploid plants are used to pollinate diploid mother plants to produce triploid offsprings where less or no seeds were desired, such as seedless watermelon (Kihara, 1951; Thayyil et al., 2016). While polyploidization can happen naturally from spontaneous mutations, the mutation frequency is normally very low, about 0.3% (Einset, 1952; Ramsey and Schemske, 1998). Therefore, induced polyploidy is often applied for its high mutagenic efficiency and saving time. There are three methods generally used to induce polyploidy in plants.

The first one is physical mutagenesis where trauma, radiation, higher or lower temperatures and other mechanical stress were applied (Li et al., 2000; Han, 2004; Liu, 2018). Among them, gamma-radiation mutagenesis is the most common strategy where typically seeds were treated. For example, ^{60}Co-γ was employed to treat gutta percha tree seeds in order to obtain polyploid plants (Bi et al., 1999).

The second one is chemical mutagenesis, which is the most popular and frequently used method in polyploid mutation. It is achieved by

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treating plant meristematic tissues with colchicine, sodium azide, ethylene imine, etc., where colchicine is the most reliable choice due to its the high mutation rate for most of the plant species studied (Kadota and Niimi,2002; Kermani et al., 2003; Sun et al., 2009; Dhooghe et al., 2011; Urwin. 2014; Xie et al., 2015; Zhou et al., 2016). So far, successful polyploidy induction by colchicine has been achieved in many fruit species, such as apple (Shi et al., 1992), banana (Ganga and Chezhiyan, 2002), grapevine (Motosugi et al., 2002), citrus (Zeng et al., 2006) and pear (Kadota and Niimi, 2002). In terms of the plant tissue used in chemical mutagenesis, seeds, leaves and shoot tips are the most used and preferred tissues.

Biological mutagenesis is another method used in polyploidization where somatic hybridization, sexual hybridization and endosperm culture are used (Thomas et al., al.,2000; Kadota and Niimi, 2004; Liu, 2018). For example, tetraploids of citrus plant were obtained through the somatic hybridization method by fusing protoplasts isolated from cell suspension cultures of “Page” tangelo and mesophyll protoplasts of rough lemon (Guo et al., 1998). Triploids of Chinese wolfberry were isolated from cultured endosperms (Wang et al., 1985).

Given the lack of pear rootstocks in the current pear industry and so many positive attributes of polyploidy pear, such as more resistance to pear scab and cold than their diploid counterparts (Brown, 1960; Li et al., 2004), we attempted and successfully obtained tetraploids from the diploid ‘duli’ and ‘Xinli No.7′ pear using the chemical mutagenesis methods.

2. Materials and methods

2.1. Plant materials and reagents

Seeds of ‘duli’ pear were collected, dried and stored. The ‘duli’ and ‘Xinli No.7′ tissue cultured plants were maintained under 16-hour light (2000 lx light intensity) and 8-hour dark cycle at 23±2℃ in the tissue culture room. The ‘duli’ seedlings used in the smear method were grown in the field. 6-Benzylaminopurine (6-BA), Indole-3-butyric acid (IBA), thidiazuron (TDZ), dimethyl sulfoxide (DMSO), MS medium, of Solarbio, and colchicine of Aladdin, were used in related experiment as well as Sucrose, agar, $AgNO_3$, and N-(1-ethylpropyl)−2,6-dinitro-3,4-xylidine (Pendimethalin) of analytical grade.

2.2. Induction of tetraploids by different chemical mutagens

2.2.1. Mutagenesis by the impregnation method

One thousand and two hundred plump ‘duli’ seeds were selected and stratified. Briefly, the seeds were washed with tap water firstly and then they were mixed with clean wet sand (humidity about 60%) in 1:3 ratio. Then the mixture was covered with a layer of 5 cm thick wet sand and was left in the dark at 2–7℃ for 2 months. After that, the seeds were collected and divided into 4 groups with 300 seeds in each group. Each group was again divided into 3 subgroups with 100 seeds in each. They were treated by soaking in 0%, 0.2%, 0.4%, and 0.6% colchicine or pendimethalin solution for 24 h, 48 h and 72 h, respectively. Then the seeds were washed with distilled water for three times to remove traces of the mutagens before being laid on the two layers of wet filter paper. The seeds were kept at 25±2℃ with humidity of 75–80% until they were germinated. The germination rate was counted and percentage of seeds with radicles were calculated. When the length of the radicles reached 0.5 cm they were transferred to soil-based compost. When the height of the seedlings reached 6 cm they were transferred to large pots where the survival rate (number of survival seedlings/total seedlings×100%) was recorded. Subsequently, they were transferred to glass house where the temperature and the humidity were kept at 25±2℃ and 60–90%, respectively.

The shoot tips of the tissue cultured seedlings were isolated from the 30-day old ‘Xinli No.7′ and ‘duli’ pear plants. They were treated under sterile conditions with 0%, 0.2%, 0.4%, and 0.6% colchicine solution for 24 h, 48 h and 72 h, respectively. Regular shaking was applied to make sure the shoot tips in constant contact with colchicine. After treatments, the shoot tips were washed with distilled water for three times and dried with sterile filter paper. Then they were transferred to medium (pH 5.8–6.0) containing 2.22 $g{\cdot}L^{-1}$ MS, 1.5 $mg{\cdot}L^{-1}$6-BA, 0.1 $mg{\cdot}L^{-1}$IBA, 30 $g{\cdot}L^{-1}$ sucrose and 6 $g{\cdot}L^{-1}$ agar and cultured in the tissue culture room under long day condition as described before. The rate of mortality (number of dead seedlings/total seedlings×100%) and the rate of mutation (number of mutated seedlings or shoot tips/total seedlings or shoot tips×100%) of each treatment were recorded and analyzed.

2.2.2. Mutagenesis by the mixed culture method

Under sterile conditions 1.5 cm long shoot tips containing axillary buds of ‘Xinli No.7′ and ‘duli’ tissue cultured plants, which were grown in the tissue culture room mentioned above, were transferred to the “mixed medium” for 30 days. The “mixed medium” was the medium for tissue culture materials described formerly mixed with 1.5% (v/v) DMSO after sterilization, as well as 0%, 0.1%, 0.5% and 1.0% (w/v) colchicine, respectively. Then they were washed, transferred and cultured on media without colchicine and DMSO. The mortality and the mutation rate were recorded and analyzed as described above.

2.2.3. Mutagenesis by the smear method

The main growing tips of the 30-day old field grown ‘duli’ pear seedlings were exposed after removing the young leaves. They were then treated with 0%, 0.2%, 0.4% and 0.6% colchicine for 24 h, 48 h and 72 h respectively, by smearing twice daily, once at 7:00am and another at 6:00pm. The treated tips were wrapped with wet cotton to maintain humidity. After treatment the tips were rinsed with water and kept in dark and moist by covering with sun-shading net or black plastic for 5 days. The growth inhibition rate (number of seedlings that were ceased growth /total treated seedlings×100%) and mutation rate were recorded and analyzed.

2.3. Ploidy verification and phenotypic analysis

The putative polyploidy of ‘Xinli No.7′ and ‘duli’ that showed significant difference compared to the diploid plants were analyzed to confirm their polyploidy nature by the flow cytometry (Javadi et al., 2013; Postman et al., 2015; Puskás et al., 2016; Niu et al., 2020). Briefly, 1 g of young leaves were collected from the putative tetraploid seedlings and cut into small pieces. Lysis buffer (2 ml) was added, and the mixture was incubated at 4 °C for 5 min before being filtered through a membrane (pore diameter=80 μm). Then single cells were collected by centrifugation at 1000 rpm for 5 min at 4℃ and stained with propidiumiodide (PI) in the dark for 20 min. Finally, the detection was carried out by the flow cytometry (FACSCalibur, BD company, USA). Diploid plants were served as controls. Further, morphological differences of the tetraploid and the diploid pear seedlings were observed and compared in terms of the height, the number of internodes, the internode length, the thickness of the hypocotyl, the leaf length, the leaf width and the leaf thickness. All the experiments were repeated three times.

2.4. Data analysis

The data obtained were subjected to one-way ANOVA followed by Fisher’s least significant difference (LSD) or Student’s *t*-test analysis using the SPSS 20.0 software (IBM, Armonk, NY, USA). Statistically significant differences were indicated at levels of $p < 0.05$ and $p < 0.01$.

3. Results

3.1. The effects of colchicine and pendimethalin on tetraploids induction of ‘duli’ seeds

Different concentrations of colchicine and pendimethalin were used to treat seeds of 'duli' pear, aiming to obtain tetraploid rootstock for grafting. The results showed that none of the concentrations of pendimethalin used could induce tetraploids, and all concentrations of colchicine except 0.2% could yield tetraploids (Table 1). The mutation rate of 'duli' seeds treated with colchicine ranged from 2% (impregnated with 0.4% colchicine for 24 h) to 1% (impregnated with 0.6% colchicine for 24 h, or impregnated with 0.4% colchicine for 72 h). Therefore, our data clearly demonstrated that colchicine can induce polyploids in 'duli' pear seeds, and hence it is the mutagen of choice for polyploidization of 'duli' pear under the current experimental conditions.

The letters in capital and lower case indicate significant differences at the $p<0.01$ level and the $p<0.05$ level analyzed by one-way ANOVA followed by Fisher's least significant difference (LSD).

3.2. The effects of impregnation method on the induction of tetraploids of 'duli' and 'Xinli no.7′ shoot tips

Different concentrations of colchicine and different duration of time were used for impregnating the shoot tips of 'duli' and 'Xinli No.7′. Overall, the mutation rate of tetraploids of 'Xinli No.7′ was much higher than that of 'duli' (Table 2). For example, mutation rate of 6.67% was achieved when 'duli' shoot tips were treated with 0.2% colchicine for 48 h. However, the mutation rate could reach 13% when 'Xinli No.7′ shoot tips were treated with 0.2% colchicine for 24 h or 0.6% colchicine for 24 h or 72 h.

Colchicine can cause mortality of shoot tips where 'duli' shoot tips were more sensitive with the mortality rate ranging from 40% to 90% while that of 'Xinli No.7′ ranging from 10% to 80% (Table 2).

The letters in capital and lower case indicate significant differences at the $p<0.01$ level and the $p<0.05$ level analyzed by one-way ANOVA followed by Fisher's least significant difference (LSD).

3.3. The effects of mixed culture method on induction of tetraploids of 'duli' and 'Xinli no.7′

Different amount of colchicine was added to the growth media to induce mutation. It showed that the higher the colchicine concentration the higher the mutation rate was, and the highest mutation rate of 20% and 13% was achieved for 'duli' and 'Xinli No.7′, respectively, when treated with 1.0% colchicine (Table 3). Compared with the impregnation method, the mortality caused by this method was much lower. In

Table 2
The effects of impregnation method on mortality and mutation rate of 'duli' and 'Xinli No.7′.

Variety	Cochicine concentration (%)	Treatment time (h)	Mortality (%)	Mutation rate(%)
'duli'	0	24	0^{Ef}	0^{Bb}
	0	48	0^{Ef}	0^{Bb}
	0	72	0^{Ef}	0^{Bb}
	0.2	24	43.0^{Dde}	0^{Bb}
	0.2	48	40.0^{De}	6.67^{Aa}
	0.2	72	53.0^{BCDcde}	0^{Bb}
	0.4	24	50.0^{CDbc}	0^{Bb}
	0.4	48	67.0^{BCDbcd}	0^{Bb}
	0.4	72	77.0^{ABab}	0^{Bb}
	0.6	24	60.0^{BCDbcd}	0^{Bb}
	0.6	48	67.0^{ABCbc}	0^{Bb}
	0.6	72	90.0^{Aa}	0^{Bb}
'Xinli No.7′	0	24	0^{Ce}	0^{Ab}
	0	48	0^{Ce}	0^{Ab}
	0	72	0^{Ce}	0^{Ab}
	0.2	24	10.0^{BCde}	13.0^{Aa}
	0.2	48	20.0^{BCcd}	6.67^{Aab}
	0.2	72	27.0^{Bc}	0^{Ab}
	0.4	24	13.0^{BCcde}	0^{Ab}
	0.4	48	17.0^{BCcd}	10.0^{Aab}
	0.4	72	13.0^{BCcde}	6.67^{Aab}
	0.6	24	63.0^{Ab}	6.67^{Aab}
	0.6	48	67.0^{Aab}	0^{Ab}
	0.6	72	80.0^{Aa}	13.0^{Aa}

Table 3
Effects of the mixed culture method on the mortality and mutation rate of 'duli' and 'Xinli No.7′.

Variety	Colchicine concentration (%)	Mortality (%)	Mutation rate (%)	Ratio of chimera (%)
'duli'	0	0	0^{Bb}	0
	0.1	0	0^{Bb}	
	0.5	0	0^{Bb}	
	1.0	0	20.0^{Aa}	
'Xinli No.7′	0	0	0^{Bb}	50.00
	0.1	0	0^{Bb}	
	0.5	0	13.0^{Aa}	
	1.0	0	13.0^{Aa}	

Table 1
Effects of colchicine and pendimethalin on tetraploid induction rate of 'duli' seeds.

Mutagen	Concentration(%)	Time(h)	Germination rate(%)	Seedling rate(%)	Mutation rate(%)
Colchicine	0	24	98.0^{Aa}	98.0^{Aa}	0^{Ab}
	0	48	98.0^{Aa}	98.0^{Aa}	0^{Ab}
	0	72	98.0^{Aa}	98.0^{Aa}	0^{Ab}
	0.2	24	27.0^{BCbc}	67.0^{BCb}	0^{Ab}
	0.2	48	32.0^{Bbc}	66.0^{Bb}	0^{Ab}
	0.2	72	17.0^{CDd}	24.0^{Dd}	0^{Ab}
	0.4	24	36.0^{Bb}	42.0^{BCbc}	2.0^{Aa}
	0.4	48	36.0^{Bb}	25.0^{CDcd}	0^{Ab}
	0.4	72	5.0^{De}	20.0^{Dd}	1.0^{Aa}
	0.6	24	25.0^{BCcd}	36.0^{CDcd}	1.0^{Aa}
	0.6	48	4.0^{De}	25.0^{Dd}	0^{Ab}
	0.6	72	6.0^{De}	33.0^{Dd}	0^{Ab}
Pendimethalin	0	24	98	98	0
	0	48	98	98	0
	0	72	98	98	0
	0.2	24	1.0	0	0
	0.2	48	0	0	0
	0.2	72	0	0	0
	0.4	24	3.0	0	0
	0.4	48	1.0	0	0
	0.4	72	0	0	0
	0.6	24	0	0	0
	0.6	48	0	0	0
	0.6	72	4.0	0	0

addition, we found that half of the mutants of 'Xinli No.7′ induced by this method (with 1.0% and 0.5% colchicine) were chimera of diploid and tetraploid while all the mutants of 'duli' were tetraploid (Table 3).

The letters in capital and lower case indicate significant differences at the $p<0.01$ level and the $p<0.05$ level analyzed by one-way ANOVA followed by Fisher's least significant difference (LSD).

3.4. The effects of smear method on induction of tetraploids of 'duli'

Smearing colchicine solution directly onto the tissues resulted in very low mutation rate where only 6.67% mutants were obtained when the main meristems of 'duli' seedlings were treated with 0.4% colchicine for 24 h (Table 4). In addition, the growth inhibition rate was increased with the increased treatment time under the same concentration of colchicine and with increased concentrations of colchicine within the same duration of treatment time.

The letters in capital and lower case indicate significant differences at the $p<0.01$ level and the $p<0.05$ level analyzed by one-way ANOVA followed by Fisher's least significant difference (LSD).

3.5. Confirmation of ploidy of pear seedlings by flow cytometry

Within the pear seedlings treated with colchicine we screened out some putative mutants from both 'duli' and 'Xinli No.7′. They appeared to have shorter internodes, dwarf and compact stature (Fig. 1). In order to confirm the ploidy nature of the putative mutated seedlings of both 'duli' and 'Xinli No.7′ pear, DNA was isolated and quantified by flow cytometry at the cytological level (Fig. 2). The results showed that the fluorescence of diploids of 'duli' and 'Xinli No.7′ appeared at 50 (FL2-A) (Fig. 2A, D) while that of the tetraploids appeared at 100 (FL2-A) (Fig. 2B, E). Interestingly, the fluorescence of the chimera appeared at both 50 and 100 (Fig. 2C, F). Further, we found that the effective cell number of diploids (about 800) was much higher than that of the tetraploids of 'duli' pear (about 275) (Fig. 2A, B). The chimera of 'duli' pear had the effective cell number of 60 when the FL2-$A = 50$, and 150 when the FL2-$A = 100$ (Fig. 2C). However, the opposite results were observed in diploids, tetraploids and chimera of 'Xinli No.7′ pear where the effective cell number was about 350 for diploids and 800 for tetraploids, respectively, while that of the chimera at FL2-$A = 50$ and at FL2-$A = 100$ was nearly the same (about 350) (Fig. 2D, E, F).

3.6. Morphological identification of tetraploids of 'duli' and 'Xinli no.7′ pear seedlings

After identifying the ploidy of putative mutated seedlings of 'duli' and 'Xinli No.7′, we further compared the morphological differences of the tetraploid seedlings and diploid seedlings. The thickness of the hypocotyl of 40-day old seedlings of 'duli' tetraploids was 4.0 ± 0.16 mm, which was twice as thick as those of the diploids (1.8 ± 0.11 mm, Table 5 and Fig. 3A). These seedlings grew much slower and their leaves were darker green compared to the untreated diploids (Fig. 3A). In older seedlings (120-day old), the height, length and number of the internodes of the tetraploids were 52.0 ± 0.12 mm, 2.0 ± 0.08 mm and 6.0 ± 1.5), respectively, which were much reduced compared to the diploids (175.0 ± 0.20 mm, 15.0 ± 0.12 mm and 11.0 ± 1.0, respectively (Fig. 3A, B). However, the average leaf length, width and thickness were 47.5 ± 0.50 mm, 30.0 ± 0.55 mm and 0.50±0.18 mm for the tetraploids, while they were 50.0 ± 0.38 mm, 20.0 ± 0.52 mm and 0.42±0.14 mm in diploids (Table 5). Therefore, the leaves of the tetraploids were much bigger and thicker, and had more teeth around the edge than those of the diploids (Fig. 3A).

The letters in capital and lower case indicate significant differences at the $p<0.01$ level and the $p<0.05$ level analyzed by one-way ANOVA followed by Fisher's least significant difference (LSD). The short horizontal line means data missing.

Table 4
Effects of the smear method on the growth inhibition rate and mutation rate of 'duli'.

Colchicine concentration (%)	Treatmenttime (h)	Germination rate (%)	Growth inhibitionrate (%)	Mutation rate (%)
0	0	93.0^{Aa}	7.00^{Ee}	0^{Bb}
0.2	24	90.0^{ABa}	10.00^{DEe}	0^{Bb}
0.2	48	83.0^{ABa}	17.00^{DEe}	0^{Bb}
0.2	72	40.0^{CDEd}	60.00^{ABCb}	0^{Bb}
0.4	24	80.0^{ABab}	20.00^{DEde}	6.67^{Aa}
0.4	48	63.0^{BCbc}	37.00^{CDcd}	0^{Bb}
0.4	72	33.0^{DEd}	67.00^{ABb}	0^{Bb}
0.6	24	50.0^{CDcd}	50.00^{BCbc}	0^{Bb}
0.6	48	33.0^{DEd}	67.00^{ABb}	0^{Bb}
0.6	72	13.0^{Ee}	87.00^{Aa}	0^{Bb}

4. Discussion

In this study we first tested the effectiveness of the two most used chemical mutagens and found that only colchicine can successfully induce tetraploids in 'duli' pear (Table 1). Subsequently, colchicine was employed to induce 'duli' and 'Xinli No.7′ tetraploids using three different methods (Tables 2, 3, 4). We screened out some putative polyploid plants by observing their phenotypes (Fig. 1), and conformed their ploidy nature by the flow cytometry (Fig. 2). Finally, we compared the morphological differences of the tetraploid seedlings and the diploid seedlings of 'duli' pear, such as the height, the internode length, the internode number, the leaf color, leaf size, leaf thickness, number of teeth on the edge of leaves, etc. (Fig. 3 and Table 5; Zhang, 2018).

Previous researches showed that the successful polyploidy induction is determined by the type of plant species, tissues and importantly the methods used. The most common used methods are impregnation, mixed culture and smearing. For example, when shoot tips of garlic plants were treated with 0.1% colchicine the mutation rate was much higher than that of materials treated with lower at 0.05% or higher at 0.2% colchicine (Zhou and Cheng, 2008). Similarly, the mutation rate could reach 56.1% by treating tissue cultured apple leaves with 0.5% colchicine by the impregnating method (Wang et al., 1999). In grapevine, it was found that the susceptibility of embryoid to colchicine was different from that of young seedlings. This could be due to the fact that the cells of young seedlings were more closely packed than those of embryoids (Wang et al., 2000). In line with these findings, we also found that colchicine had different effects on mutation rate of pear plants where the mixed culture method appeared to be the best for mutating shoot tips of both 'duli' and 'Xinli No.7′ where the mutation rate of 20% and 13% respectively were achieved (Table 3).

It appears that the concentration of colchicine and the duration of treatment time had the biggest effect on the mutation rate of both 'duli' and 'Xinli No.7′ pear plants. In general, the higher the concentration and the longer the treatment time were, the higher the mutation rate was (Tables 2 and 3). However, when a threshold was reached the mortality rate also became high (Table 2). Therefore, determining the appropriate concentration and duration of treatment time for each plant species and choosing a specific plant tissue are vital for the success to obtain viable polyploids (Chang et al., 2007). This was also supported by the induction of banana and money tree polyploids (Kanchanapoom and Koarapatchaikul, 2012; Xie, 2010).

Interestingly, we also found that half of the induced 'Xinli No.7′ mutants by the mixed culture method were chimera while this did not happen to 'duli' mutants. This indicates that genotype was one of the most important factors affecting the mutation rate as well as the method of mutation when colchicine was used as the mutagen. Similar observations were also made in papaya, apple, Chinese kiwifruit and cowberry (Diao et al., 2019; Su et al., 2021; Wei et al., 2020; Li et al., 2010; Shi et al., 2012). This could be caused by cell division in these tissues being out of sync as the chromosome doubling event only

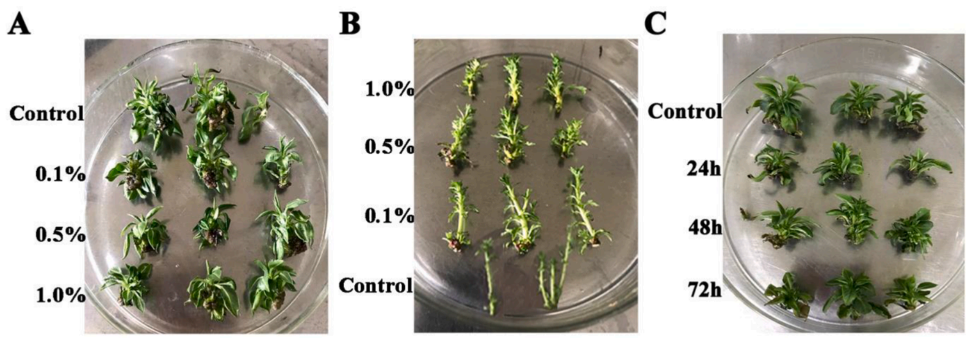

Fig. 1. Effects of different concentrations of colchicine and treatment methods on the growth of ‘duli’ and ‘Xinli No.7′ pear seedlings. A. ‘Xinli No.7′ in mixed culture method. 0.1%, 0.5% and 1% colchicine were added to the culture media. B. ‘duli’ in mixed culture method. 0.1%, 0.5% and 1% colchicine were added to the culture media. C. ‘Xinli No.7′ in impregnation method. Seedlings were treated with 0.2% colchicine for 24, 48 and 72 h, respectively. All seedlings were cultured in normal medium for 30 days after each treatment.

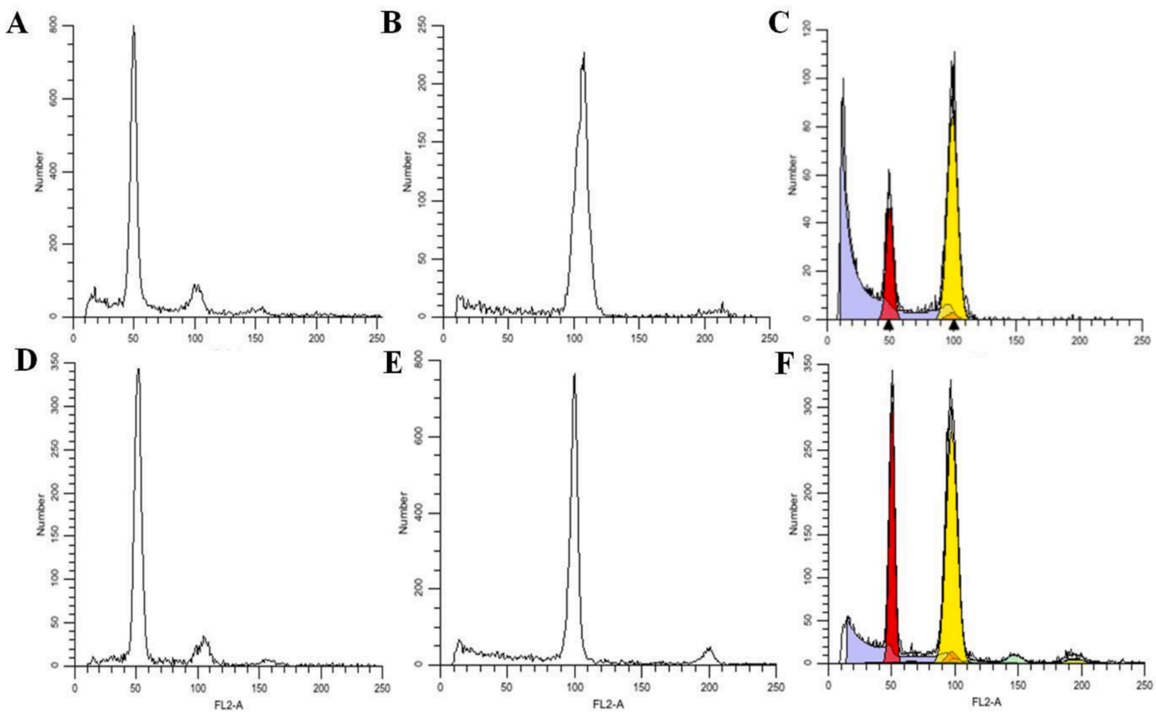

Fig. 2. Flow cytometry histogram of DNA isolated from diploid, tetraploid and chimera of ‘duli’ and ‘Xinli No.7′ seedlings. A. The diploid control of ‘duli’ DNA
B. The induced tetraploid of ‘duli’ DNA
C. The induced chimera of ‘duli’ DNA
D. The diploid control of ‘Xinli No.7′ DNA
E. The induced tetraploid of ‘Xinli No.7′ DNA
F. The induced chimera of ‘Xinli No.7′ DNA
The X axis indicates the DNA content, and the Y axis indicates the effective cell number.

Table 5
Morphological comparation of the tetraploids and the diploids of ‘duli’.

Variety	Age (d)	Thickness of the hypocotyl (mm)	Leaf length (mm)	Leaf width (mm)	Leaf thickness (mm)
Tetraploid seedling	40	4.0 ± 0.16^{Aa}	—	—	—
	120	—	47.5 ± 0.50^{Bb}	30.0 ± 0.55^{Aa}	0.50 ± 0.18^{Aa}
Diploid seedling	40	1.8 ± 0.11^{Bb}	—	—	—
	120	—	50.0 ± 0.38^{Aa}	20.0 ± 0.52^{Bb}	0.42 ± 0.14^{Ab}

happens during cell division.

In conclusion, we have successfully induced tetraploid seedlings for both ‘duli’ and ‘Xinli No.7′ pear plants. Preliminary observation indicates that these plants have the desirable traits, such as dwarfism, enlarged leaves and thick stems which are suitable for rootstock (‘duli’) and cultivar (‘Xinli No.7′) in pear cultivation.

Author contributions

Rui Liu treated seedlings to mutate tetraploids, confirmed the ploidy levels, determined nuclear DNA contents, characterized plant morphological changes and growth characteristics, collected and analyzed data, prepared figures, and drafted the manuscript. Chengyu Gao, Jiangzhou Jin and Yiheng Wang germinated ‘Duli’ seeds, prepared the tissue cultured seedlings and seedlings in the field. Xiaoqing Jia helped collecting and analyzing data, preparing figures, and drafting the manuscript. Jianfeng Xu designed and supervised the experiments. Hui Ma and Yuxing Zhang helped supervising the project. Haixia Zhang, Baoxiu Qi, and Jianfeng Xu reviewed the experiments and data, revised and finalized the manuscript.

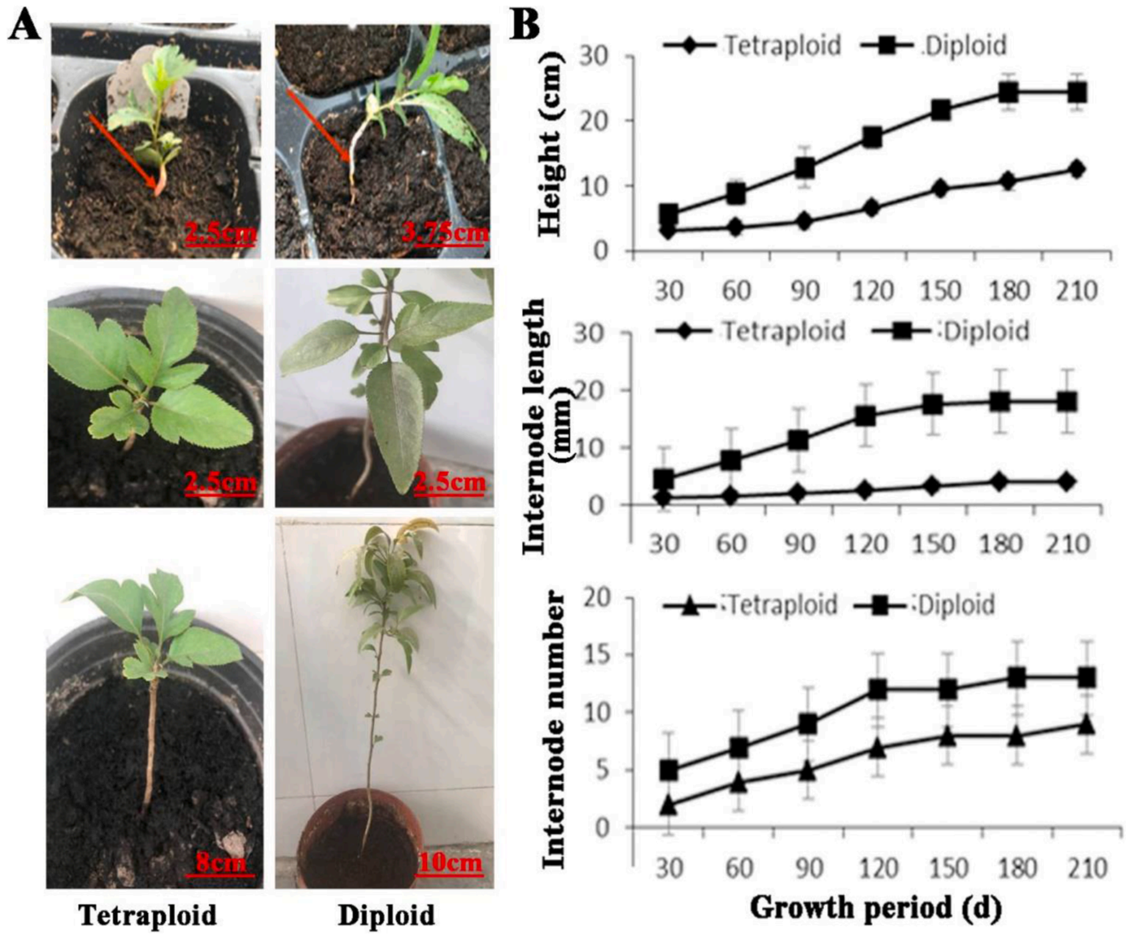

Fig. 3. Morphological differences between the induced tetraploids and the wild type diploids of 'duli'. A. Seedlings of the induced tetraploids (left) and wild type diploids (right) of 'duli' seedlings at 40 days (top), 90 days (middle) and 360 days (bottom). Arrows indicate the hypocotyls of the seedlings. B. The height, internode length and internode number of tetraploids and diploids of 'duli' pear seedlings.

Declaration of Competing Interest

The authors declare that they have no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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