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Efficient mesophyll protoplast isolation and development of a transient expression system for castor-oil plant (*Ricinus communis* L.)

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Introduction: We investigated the main factors affecting the efficacy of protoplast isolation, including leaf-obtaining period, cutting shapes of leaf material, enzyme concentration, enzymolysis time, and centrifugal speed. *Methods:* Protoplast isolation was optimal on the condition of 20 days of leaf materials, 2-mm filament of leaves, 1.6% RS and 0.8% R-10, 80 min of enzymolysis, and 700 rpm of centrifugation, resulting in the best yield (1.19×106 protoplasts/g FW) and vitality (80.34%) of mesophyll protoplasts. The transient expression vector pGFP1 with green fluorescent protein was transfected into the obtained protoplasts from castor by polyethylene glycol-mediated method with a transformation efficiency of 12.37%. *Results:* Moreover, the applicability of the system for studying the subcellular localization of RcFATA (an acyl-ACP thioesterase) was validated via the protoplast isolation and transient expression protocol in this study. *Discussion:* Collectively, the efficient mesophyll protoplast isolation and protoplast transient expression system facilitate to analyze the function of specific gene in castor (*Ricinus communis* L).

Original Article

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INTRODUCTION

In recent years, transient expression of gene in protoplasts of plant is a useful method for studying subcellular localization of proteins, gene silencing *in vivo*, gene and promoter activities, protein–protein interactions, signal transduction, and protein complexes (De Caroli et al., 2015; Faraco et al., 2011; Miao & Jiang, 2007; Sheen, 2001; Siritunga et al., 2007; Wang et al., 2015; Wu et al., 2017; Yoo et al., 2007). Compared to the transgenic plants with steady gene expression, a rapid, easy, and effective protocol with high level of expression is implemented via transient expression of gene (Chen et al., 2010; Kim et al., 2009; Nanjareddy et al., 2016). Furthermore, the large-scale analysis of gene functions can be accomplished by the transient expression of gene, but the methods of steady gene expression are comparatively expensive and take longer period to carry out, so that the applications of those protocols for massive analyses of plant genes are naturally limited (Chen et al., 2010; Kim et al., 2009; Nanjareddy et al., 2016). Although there are different kinds of transfection techniques for transient expression of gene, such as electroporation, microinjection, and polyethylene glycol (PEG)-mediated, which have been exploited to transport DNA recombinant plasmids into plant protoplasts, the method of PEG-mediated has been utilized commonly with high transformation efficiency and extensively used in the studies of molecular and cellular in plants (Nanjareddy et al., 2016; Wu et al., 2017; Yoo et al., 2007).

Castor-oil plant (*Ricinus communis* L.) is an annual or perennial herb of the *Euphorbiaceae* cultivated in tropical and subtropical regions for its oil storage seeds

(Shao et al., 2012). Oil extracted from the seed of castor-oil plant is an important industrial oil with extensive applications (Li et al., 2012). Since castor-oil plant is one of the major oil crops, a great deal of studies on castor-oil plant molecular breeding have been centrally developed, which involves analyzing functions of agronomic traits gene (Arroyo-Caro et al., 2013; Eastmond, 2004; He et al., 2004; Kim et al., 2011; Sujatha et al., 2009). For the past few years, the whole-genome sequencing of castor-oil plant has been well studied and published communally (Chen et al., 2010). Because more and more important genes in castor-oil plant are needed to assay the functions, a method for function analysis.

Steady gene transfection can be employed for studying gene function in the whole plant. However, there are few research report about genetic transformation in castor-oil plant whether *Agrobacterium tumefaciens*-mediated or biolistic bombardment-mediated (Gressel, 2008; Sujatha et al., 2009; Sujatha & Sailaja, 2005). Nevertheless, the results of transformation in castor-oil plant are comparatively inefficient and genotype difference (Auld et al., 2001; Malathi et al., 2006); meanwhile, excellent tissue culture systems are needed in the procedures, which are time-consuming and poor repeatability (Auld et al., 2001; Sailaja et al., 2008). Therefore, a valid protocol for transient expression of gene is required eagerly, which can be developed and applied to discover the functions and molecular pathways of gene in castor-oil plant.

Because there is difficultly for castor-oil plant to regenerate whole plant from protoplasts natively, and to the best of our knowledge no result about tissue culture and isolation of protoplasts in castor-oil plant has been reported. To date, transient expression of gene in castor-oil plant protoplasts has not been accomplished. An effect and credible system for separation of mesophyll protoplast and PEG-mediated transient expression of gene in castor-oil plant were demonstrated elaborately in this paper. This method has been applied successfully to implement the subcellular localization of an acyl-ACP thioesterase (RcFATA protein) in castor-oil plant protoplasts.

MATERIALS AND METHODS

Plant material

Castor-oil plant seeds named Fen-Bi 10 were supplied by the Economic Crop Research Institute, Shanxi Academy of Agricultural Sciences, Fenyang city, Shanxi Province.

Seeds of castor-oil plant were surface-sterilized for 60 s with 75% (v/v) ethanol after stripping the outer seed coat, immersed in 2% (v/v) sodium hypochlorite (NaClO) for 20 min, and conclusively rinsed five times in sterile-distilled water. The embryos were gained from the sterile seeds and placed on hormone-free Murashige and Skoog salt medium (Murashige & Skoog, 1962) at 25 °C, under lighting with a period of 12/8 h (light/dark) for different days (10, 20, and 40 days) to obtain completely expanded *in vitro* leaves.

Preparation of the filterable device

A nylon membrane with 8.47 μm was cut into 10 × 10 cm small pieces, and two pieces of the nylon films were aligned to place them on a tea leak and then covered with another tea leak of the same specification. Finally, the protoplast filterable device was prepared.

Protoplast isolation

Protoplast isolation was implemented on the basis of the reported methods with little modification (Anthony et al., 1995; Yoo et al., 2007; Zhang et al., 2011). The completely expanded leaves were cut into different shapes (2-mm filament, 0.5 × 0.5 cm small piece, and 1 × 1 cm small piece) using sharp blades. The cut leaf materials of 0.4 ± 0.02 g (for each treatment) were rapidly placed into W5 solution (154 mM NaCl, 125 mM CaCl2, 5 mM KCl, and 2 mM MES at pH 5.8) for 10 min in the dark. After getting rid of the W5 solution, the cut leaf materials were immediately transferred into 20 ml of the enzyme solution (0.5 M mannitol, 10 mM MES at pH 5.7, 10 mM $CaCl_2$, and 0.1% bovine serum albumin) supplemented with different combinations of cellulase *Rhizopus* sp. (RS; cellulase derived from RS) and Macerozyme R-10 (Macerozyme derived from RS no. 10; R-10) (0.8% RS and 0.4% R-10, 1.6% RS and 0.8% R-10, 3.2% RS and 1.6% R-10; Yakult, Japan) for various periods (20, 40, 60, 80, 100, and 120 min) in the dark (25 °C) with gentle shaking (50 rpm).

After treatment with enzymolysis, the digested leaf materials were filtered using the aforementioned filterable device, gathered by centrifugation (300, 700, and 1,400 rpm for 5 min) and suspended in 20 ml W5 solution. The protoplasts were depurated in W5 solution twice via resuspension and centrifugation (700 rpm for 5 min), and then the protoplasts were gently suspended with 2 ml of W5 solution and transferred into 2 ml round-bottom centrifuge tubes. After centrifugation (700 rpm for 10 min), the supernatant was removed and finally the protoplasts were resuspended with 1 ml of MMg solution (0.5 M mannitol, 2 mM MES, and 15 mM $MgCl_2$, pH 5.8).

The protoplasts obtained were measured under a microscope equipped with a hemocytometer. The activity of protoplasts was observed with 1% Evans blue staining (Tang et al., 2015) and calculated as follows: yield of protoplasts (g/g FW) = (number of protoplasts in five large squares × 1,000 × dilution factor)/fresh weight of material; activity of protoplasts (%) = (number of unstained protoplasts/total number of protoplasts) × 100%.

Castor-oil plant mesophyll protoplast transformation

The protoplasts were obtained by centrifugation (700 rpm for 10 min) and resuspended in MMg solution to a density of 1.0 × 106 protoplasts/ml. About 10 μg of pUC18-35S-eGFP (pGFP1) plasmid (10 μl), which had a green fluorescent protein (GFP) tag, were mingled softly with 100 μl of protoplasts (Wang et al., 2013; Zhang et al., 2011). Then, 110 μl of freshly prepared 40% PEG4000 solutions (0.2 mM mannitol and 100 mM $CaCl_2$) were subsequently

supplemented and mixed lightly with protoplasts and pGFP1 plasmid. The mixture was placed in the dark at 25 °C for 15 min, separately. Later, the transfected mixture was lightly washed with 1 ml of W5 solution after incubation. The transfected protoplasts were centrifuged (700 rpm for 10 min) twice, then resuspended with 1 ml WI solution (4 mM MES, 0.5 mM mannitol, 20 mM KCl, pH 5.8), and placed overnight at 25 °C in dark. The efficiency of transformation and the expression of GFP fusion protein were measured by means of fluorescence microscopy (Olympus MF30, Olympus company, Japan). Transformation efficiency was calculated as follows: transformation efficiency (%) = (number of bright green fluorescent protoplast in view/total number of protoplast in view) × 100%. Then, fluorescent and optical pictures were processed by LSM 5 Image Browser (Carl Zeiss company, Germany) and Adobe Photoshop 5.0 software (Adobe Photoshop 5.0, USA).

Plasmid construction and subcellular localization of RcFATA protein

Total RNA was extracted from castor-oil plant leaves with plant RNA Kit (Solar, USA) and cDNA was produced by making use of the Prime Script RT reagent Kit with gDNA Eraser (Takara, Japan) with following the operating instructions. The specific primers with cleavage sites (PstI and EcoRI) of restriction enzyme introduced, RcFATA-F (5′-AAAACTGCAGATGTTAAAAGTACCTTGTTG-3′) and RcFATA-R (5′-AAAAGAATTCTCTCGCAGATTT CTTTCTCC-3′), were designed, respectively, on the basis of the CDS sequence of RcFATA (NCBI GenBank no. EF495065). The gene was amplified by utilizing high-fidelity thermostable DNA polymerase KOD-plus (Toyobo, Japan) under the following conditions: 94 °C for 4 min followed by 35 cycles of amplification (94 °C for 30 s, 54 °C for 30 s, and 68 °C for 30 s) and finally 68 °C for 10 min for ultimate extension.

The pGFP1-eGFP plasmid and the RcFATA fragment were digested with PstI and EcoR I, respectively, and the double-digested plasmid and the target fragment were recovered and ligated with T4 DNA ligase. The connection product was transformed into DH5α competent *Escherichia coli* (Solarbio, China), and then identified through the engineering bacterium culture in shake-flask and double digestion. The positive clone was named pGFP1-RcFATA-GFP, and finally the recombinant plasmid was sequenced, and the correctly sequenced recombinant plasmid was transferred into DH5α. Subcellular localization of RcFATA protein in castor-oil plant mesophyll protoplasts was implemented in accordance with the transformation protocol mentioned above.

Data analysis

Statistical analysis of the data was carried out using SPSS 17.0 software (IBM corporation, USA), and data in the same column followed by different letters were significantly different at $p \leq 5\%$ level as determined by Duncan's multiple range test. The results were expressed as mean ± standard deviation of three independent experiments.

RESULTS

Effects of leaf-obtaining periods on the yield and vigor of protoplasts

The obtaining time of completely expanded *in vitro* leaves was optimized, and the protoplasts were filtered by a self-prepared filtration unit (Fig. 1). As shown in Table 1 and Fig. 2, the yield and vigor of protoplasts were influenced significantly by obtaining periods of leaves. When the acquisition time of leaf material was 20 days, the highest yield (1.19 × 106 protoplasts/g FW) and the best vitality (85.34%) of protoplasts were gained.

Results of the cutting shapes of leaf material on yield and vigor of protoplasts

It was found that the yield of castor-oil plant protoplasts was affected by the shapes of leaf material, but the vigor of protoplasts was not effected visibly. It is clear that the maximum yield of protoplasts (1.19 × 106 protoplasts/g FW) could be obtained from cutting the leaves into 2-mm filaments (Table 2).

Influence of enzyme concentration on the yield and viability of protoplasts

The experimental results are shown in Table 3. When the enzyme concentration was 1.6% RS and 0.8% R-10, the highest yield (1.19 × 106 protoplasts/g FW) and the strongest viability (85.34%) of protoplasts isolated from leaves of castor-oil plant were acquired subsequently.

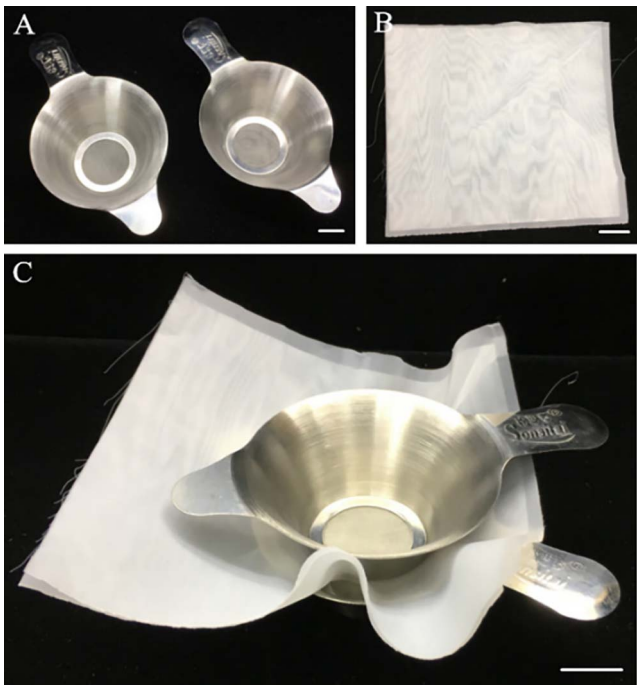

Fig. 1. Simple filter for protoplast isolation. (A) Two tea leaks, (B) two layers of 8.47-μm nylon membrane, (C) assembled protoplast filtration device (bar = 1 cm)

Table 1. Results of the acquisition time of leaf materials on the yield and vitality of protoplasts in castor-oil plant*

Time of leaves (days)	Protoplast yield ($\times 10^6$/g FW)	Total unbroken protoplasts ($\times 10^6$/g FW)	Integrated rate (%)***	Protoplast viability (%)
10	0.74 ± 0.06^{b}**	0.53 ± 0.05^{b}	71.57 ± 4.56^{b}	70.32 ± 3.42^{b}
20	1.19 ± 0.08^{a}	0.97 ± 0.07^{a}	81.54 ± 3.41^{a}	80.34 ± 2.78^{a}
40	0.58 ± 0.11^{c}	0.35 ± 0.09^{d}	60.29 ± 2.18^{c}	59.45 ± 3.05^{c}

Note. *Data were obtained on the condition of 2-mm filaments of leaves, 1.6% RS and 0.8% R-10, 80 min of enzymolysis, and 700 rpm of centrifugation. **Data in the same column followed by different letters were significantly different at $p \leq 5\%$ level as determined by Duncan's multiple range test. ***Integrated rate (%) = (number of intact protoplasts/total number of protoplasts) × 100%.

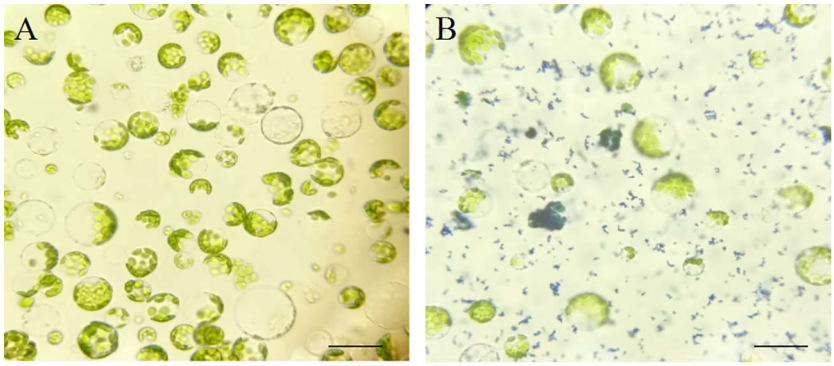

Fig. 2. Detection of yield and vigor of protoplasts from castor-oil plant leaves. (A) Castor-oil plant protoplasts were obtained from the optimized experimental system, in the condition of 20 days of leaf materials, 2-mm filament of leaves, 1.6% RS and 0.8% R-10, 80 min of enzymolysis, and 700 rpm of centrifugation; (B) vigor detection of castor-oil plant protoplasts (only dead cells stain with Evans blue) (bar = 50 μm)

Table 2. Effects of the cutting shapes of leaf material on yield and vigor of protoplasts*

Shapes of leaf material	Protoplast yield ($\times 10^6$/g FW)	Total unbroken protoplasts ($\times 10^6$/g FW)	Integrated rate (%)***	Protoplast viability (%)
2-mm filament	1.19 ± 0.08^{a}**	0.97 ± 0.07^{a}	81.54 ± 3.41^{a}	80.34 ± 2.78^{a}
0.5 × 0.5 cm piece	0.64 ± 0.09^{b}	0.51 ± 0.09^{b}	79.58 ± 3.73^{a}	78.15 ± 3.75^{a}
1 × 1 cm piece	0.32 ± 0.12^{c}	0.25 ± 0.13^{c}	78.28 ± 4.25^{a}	76.69 ± 2.31^{a}

Note. *Data were obtained on the condition of 20 days of leaf materials, 1.6% RS and 0.8% R-10, 80 min of enzymolysis, and 700 rpm of centrifugation. **Data in the same column followed by different letters were significantly different at $p \leq 5\%$ level as determined by Duncan's multiple range test. ***Integrated rate (%) = (number of intact protoplasts/total number of protoplasts) × 100%.

Table 3. Results of enzyme concentration on the yield and vigor of protoplasts*

Enzyme (%)		Protoplast yield ($\times 10^6$/g FW)	Total unbroken protoplasts ($\times 10^6$/g FW)	Integrated rate (%)***	Protoplast viability (%)
RS	R-10				
0.8	0.4	0.78 ± 0.09^{b}**	0.56 ± 0.10^{b}	71.69 ± 4.35^{b}	70.12 ± 3.72^{b}
1.6	0.8	1.19 ± 0.08^{a}	0.97 ± 0.07^{a}	81.54 ± 3.41^{a}	80.34 ± 2.78^{a}
3.2	1.6	0.47 ± 0.13^{c}	0.23 ± 0.15^{c}	48.91 ± 3.01^{c}	40.17 ± 3.21^{c}

Note. *Data were obtained on the condition of 20 days of leaf materials, 2-mm filament of leaves, 80 min of enzymolysis, and 700 rpm of centrifugation. **Data in the same column followed by different letters were significantly different at $p \leq 5\%$ level as determined by Duncan's multiple range test. ***Integrated rate (%) = (number of intact protoplasts/total number of protoplasts) × 100%.

Effects of enzymolysis time on the yield and viability of protoplasts

It is clear that the influences of enzymolysis time on the yield and viability of protoplasts isolated from leaves of castor-oil plant were all significant (Table 4). Enzymolysis for 80 min was the most suitable period in which the highest yield of protoplasts (1.19 × 106 protoplasts/g FW) and the largest protoplast viability (85.34%) were achieved successfully.

Influence of centrifugal speed on the yield and viability of protoplasts

The yield and viability of protoplasts were influenced by the centrifugal speed obviously. When the centrifugal speed was at 700 rpm, the most viability of protoplasts (85.34%) was obtained subsequently (Table 5). However, when the centrifugal speed was higher than 700 rpm, the viability of protoplasts was dramatically decreased (Table 4), although the yield of protoplasts was continued to increase clearly.

Efficiency of transient transformation of mesophyll protoplast expression

Castor-oil plant protoplasts were obtained from the optimized experimental system, in the condition of 20 days of leaf materials, 2-mm filament of leaves, 1.6% RS and 0.8% R-10, 80 min of enzymolysis, and 700 rpm of centrifugation. The obtained protoplasts were applied into transiently transformed. Castor-oil plant protoplast transformation efficiency was investigated with the transient expression vector pGFP1. After transformation, transient expression of GFP had been verified as GFP signal in the protoplasts, and the average efficiency of transient transformation was 12.37% (Table 6; Fig. 3A1–A4 and B1–B4).

Subcellular localization of GFP-fused RcFATA protein in castor-oil plant mesophyll protoplasts

To verify the feasibility of the optimized protocol of transient expression for the subcellular localization of RcFATA protein in castor mesophyll protoplasts, the transient expression of RcFATA:GFP fusion protein in castor-oil plant mesophyll protoplasts was performed. After transfection, green fluorescence distributed inside cells was viewed explicitly. RcFATA:GFP was definitely localized in chloroplasts (Fig. 3C1–C4), but empty vector (pGFP1-GFP) acted as controls was found in all over the cytomembrane and cytoplasm (Fig. 3B1–B4).

Table 6. Efficiency of transient transformation of mesophyll protoplast expression*

Number of views	Number of protoplasts	Number of protoplasts with GFP signal	Transformation efficiency (%)
1	47	6	12.77
2	45	5	11.11
3	28	3	10.71
4	37	5	13.51
5	29	4	13.79
Total	186	23	12.37

Note. GFP: green fluorescent protein.
*The exposure time was 600 ms.

DISCUSSION

A credible and efficient system for protoplasts isolation is the premise for the transient expression of gene successfully. The dissociation of protoplasts is dramatically influenced by source of plant materials (Davey et al., 2005; Wu et al., 2017; Yoo et al., 2007). Moreover, leaf materials derived from the plants, which are grown in field or greenhouse, universally contain thick epidermis and hard cytoderms (Guo et al., 2012). As a result, the isolation of protoplasts may be limited subsequently (Guo et al., 2012). Hence, in

Table 4. Influence of enzymolysis time on the yield and viability of protoplasts*

Enzymolysis time (min)	Protoplast yield ($\times 10^6$/g FW)	Total unbroken protoplasts ($\times 10^6$/g FW)	Integrated rate (%)***	Protoplast viability (%)
20	0.25 ± 0.14^{d}**	0.18 ± 0.06^{d}	72.12 ± 3.12^{b}	70.12 ± 2.46^{bc}
40	0.69 ± 0.09^{c}	0.52 ± 0.12^{bc}	75.25 ± 2.69^{ab}	73.25 ± 3.45^{b}
60	1.08 ± 0.07^{a}	0.84 ± 0.13^{a}	77.44 ± 2.67^{ab}	76.27 ± 1.89^{ab}
80	1.19 ± 0.08^{a}	0.97 ± 0.07^{a}	81.54 ± 3.41^{a}	80.34 ± 2.78^{a}
100	0.94 ± 0.04^{b}	0.65 ± 0.04^{b}	69.32 ± 4.56^{b}	65.34 ± 3.73^{c}
120	0.75 ± 0.03^{c}	0.42 ± 0.05^{c}	56.31 ± 4.20^{c}	45.34 ± 3.19^{d}

Note. *Data were obtained on the condition of 20 days of leaf materials, 2-mm filament of leaves, 1.6% RS and 0.8% R-10, and 700 rpm of centrifugation. **Data in the same column followed by different letters were significantly different at $p \leq 5\%$ level as determined by Duncan's multiple range test. ***Integrated rate (%) = (number of intact protoplasts/total number of protoplasts) × 100%.

Table 5. Results of enzyme concentration on the yield and vigor of protoplasts*

Centrifugal speed (rpm)	Protoplast yield ($\times 10^6$/g FW)	Total unbroken protoplasts ($\times 10^6$/g FW)	Integrated rate (%)***	Protoplast viability (%)
300	0.51 ± 0.11^{c}**	0.41 ± 0.06^{b}	80.27 ± 3.12^{a}	79.62 ± 3.29^{a}
700	1.19 ± 0.08^{b}	0.97 ± 0.07^{a}	81.54 ± 3.41^{a}	80.34 ± 2.78^{a}
1,400	1.98 ± 0.15^{a}	0.68 ± 0.12^{c}	34.45 ± 4.78^{c}	32.15 ± 1.68^{b}

Note. *Data were obtained on the condition of 20 days of leaf materials, 2-mm filament of leaves, 1.6% RS and 0.8% R-10, and 80 min of enzymolysis. **Data in the same column followed by different letters were significantly different at $p \leq 5\%$ level as determined by Duncan's multiple range test. ***Integrated rate (%) = (number of intact protoplasts/total number of protoplasts) × 100%.

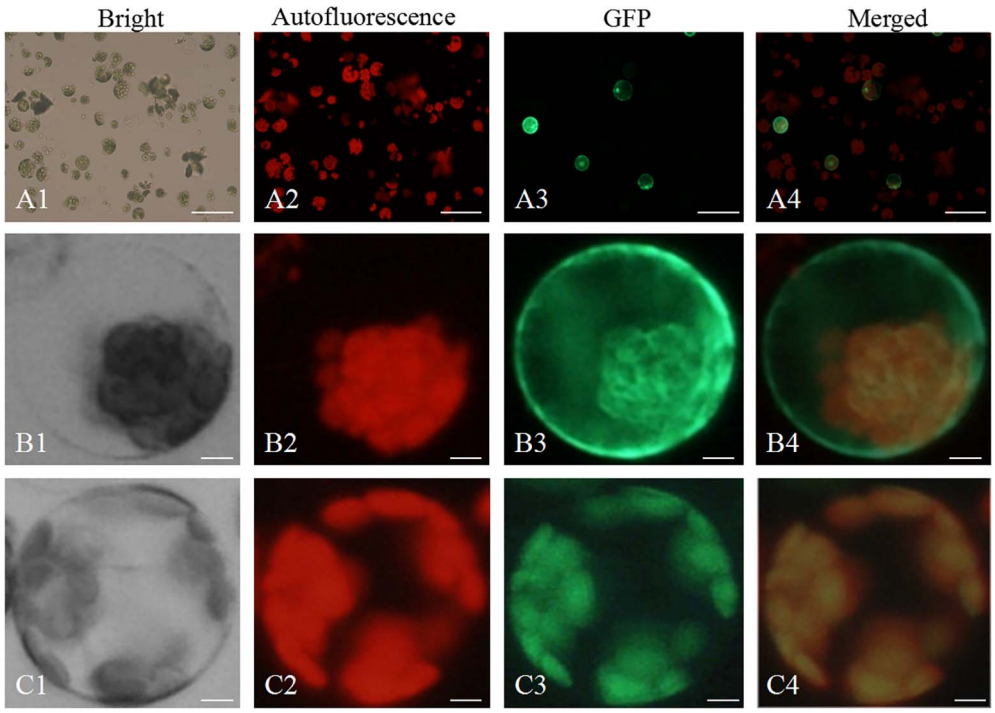

Fig. 3. Application of the optimal system to implement the subcellular localization in castor-oil plant mesophyll protoplasts. A1–A4: Efficiency transformation of castor-oil plant mesophyll protoplasts with pGFP1-GFP plasmid (bars = 100 μm); B1–B4: Empty vector (pGFP1-GFP) acted as controls was found all over the cytomembrane and cytoplasm; C1–C4: The transient expression of RcFATA:GFP fusion protein in castor-oil plant mesophyll protoplasts, showing that the RcFATA:GFP fusion protein was definitely localized in chloroplasts. Bright: bright field image of protoplasts; Autofluorescence: chloroplast autofluorescence; GFP: green fluorescent protein; Merged: GFP merged with chloroplast autofluorescence (bars = 5 μm)

order to enhance the yield and vitality of protoplasts in castor-oil plant, the leaf materials from aseptic seedling (20 days) have been used as the source for protoplasts isolation in this study.

The process of protoplasts isolation is also affected by the morphology of plant materials (Patat-Ochatt et al., 1988). In this study, the yield of protoplasts was obtained the highest when the leaves of castor-oil plant were cut into 2-mm filaments. It might be that because the thinner leaves were cut, the larger surface area of leaf would be acquired, resulting in the enzymolysis reaction with enzymes that might be more sufficient, and finally the maximum yield of protoplasts could be gained.

A large number of good quality castor-oil plant leaf protoplasts were obtained using the self-prepared simple filter device, which had the advantages of novel design, simple structure, simple manufacture, and low cost.

Several studies reported that appropriate enzyme digestion time and enzyme combination are crucial in the process of enzymolysis for protoplast isolation (Raikar et al., 2008; Yao et al., 2016). For example, a modified method for cassava protoplast isolation had been established with the combination of Cellulase R-10 and 0.8% Macerozyme R-10 for 16 hr of digestion (Wu et al., 2017). In this study, the optimization of the enzyme digestion time and enzymolysis composition was carried out smoothly. Furthermore, the compositions of cellulase and macerozyme, which have been usually applied for protoplast isolation in plants, were verified to acquire protoplasts with high yield and viability (Raikar et al., 2008; Wu et al., 2017; Yao et al., 2016). As the content of cellulase and macerozyme enhanced, the yield of protoplasts was found to be improved but the viability of protoplasts to be reduced obviously. It might be because the intact of cell membrane and the physical activity of protoplasts are affected by enzymes (Raikar et al., 2008; Zhu et al., 2005).

In addition, if the centrifugal speed is too fast, it will produce a strong pressing force on the protoplasts, which will easily cause the severely damaged of protoplasts. However, when the centrifugal force is too low, the protoplasts cannot be fully collected, resulting in lower yield. Therefore, the appropriate centrifugal speed will also seriously affect the yield and quality of the protoplasts.

Although the methods of heterogenous transient gene expression with utilizing the protoplasts from *Arabidopsis* or tobacco are usually applied to study the function of specific gene in other plants, those heterologous expression protocols may create inaccurate results because of various genetic basis (Wu et al., 2017). In this study, a valid castor-oil plant protoplast transient expression protocol was established successfully. Later, this system may become a notably useful method for discovering gene functions and regulatory mechanisms in castor-oil plant. The established available method in this study was utilized to verify the subcellular localization of RcFATA. Castor-oil plant RcFATA is a homologous gene of fatty acyl-acyl carrier thioesterase A in *Arabidopsis thaliana* and *Jatropha curcas*, which encodes an fatty acyl acyl carrier thioesterase regulating the synthesis and metabolism of fatty acid and is a predicted plastid protein (Liu et al., 2017; Salas & Ohlrogge 2002).

CONCLUSION FOR FUTURE BIOLOGY

The results indicated that RcFATA might be positioned on the chloroplasts in castor-oil plant. This result verified that the castor-oil plant mesophyll protoplast expression is a credible protocol for protein subcellular localization. Furthermore, this efficient protoplast isolation and protoplast transient expression system can be used to facilitate to analyze the function of specific gene in castor.

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Data Accessibility: All data generated or analyzed during this study are included in this published article.

Competing Interests: The authors declare no competing interests.

Authors' Contributions: YL, JC, and MC conceived and designed the experiment, contributed regents/materials/analysis tools, and participated in the drafting or revising the article. YL performed the experiments. YL, YX, and JT analyzed the data.

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