Efficient and Genotype Independent Maize pollen Transfection Mediated by Magnetic Nanoparticles

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Research article

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Abstract

Background: Biotechnological engineering of maize to introduce favorable new traits relies on delivery of foreign DNA into its cells. Current gene delivery methods for maize is limited to specific genotypes, and depend on time-consuming and labor-intensive processes of tissue culture.

Results: Here, we report a new method to transfect maize that is culture-free and genotype independent. Enhanced green fluorescent protein gene (*EGFP*) or bialaphos resistance gene (*Bar*) bound with magnetic nanoparticles (MNPs) was delivered into maize pollens, and female florets of five maize varieties were pollinated. Green fluorescence was detected in 92% transfected pollens and 70% immature embryos. *EGFP* gene detected by PCR ranged from 29 to 68% in T1 generation of these five transfected varieties, and 7-16% of the T1 seedlings showed immunologically active EGFP protein. Moreover, 1.41% of the *Bar* transfected T1 plants were glufosinate resistant, and heritable *Bar* gene was integrated into the maize genome effectively (verified by Southern blot), expressed normally and inherited stably in their progenies.

Conclusion: These results demonstrate that exogenous DNA could be delivered into maize efficiently and expressed normally through our genotype-independent pollen transfection system, providing a reliable, fast and large-scale gene delivery choice for most elite maize varieties recalcitrant to tissue culture.

Background

Maize (*Zea mays* L.) is a major crop cultivated globally to meet the increasing food, feed, and fuel demand. Although traditional breeding has significantly improved maize yield and quality during the past two hundred years, it still faces severe challenges, from various biotic and abiotic stresses [1]. To conquer these challenges, genetically-modified maize cultivars have been developed to introduce desired traits, such as insect and herbicide resistance, drought and cold tolerance, and increased nutritional quality [2].

Efficient genetic transformation is crucial for the development of genetic modified maize. Since the first report of successful maize transformation based on protoplast electroporation [3], several DNA delivery methods have been developed to generate genetic modified maize plants, including particle bombardment, *Agrobacterium*-mediation, PEG-mediation, liposome-meditation, silicon carbide-mediation, microinjection [4, 5]. These methods rely mainly on tissue culture system, which is high-cost, labor-intensive, time-consuming, and genotype-dependent especially. There are only few maize varieties such as Hi-II, B104 [6] and A188 [7] with relatively high gene transformation efficiencies. And most elite maize inbred lines have technical hurdle, thus limiting their commercial applications especially for the development of advanced breeding techniques like precise genome editing in elite crop germplasm directly. Although, transcription factors *Baby boom* (*Bbm*) and *Wuschel2* (*Wus2*) can increase transformation efficiency in several maize varieties [8], the strenuous tissue culture process to introduce *Bbm* and *Wus2* into desired recipient material is still inevitable. Therefore, it is very important to establish an efficient gene delivery system capable of direct and precise molecular improvement in all maize varieties.

With the development of nanobiotechnology, nanoparticles are being exploited as DNA carriers for gene delivery [2, 9]. Exogenous DNA carried by nanoparticles were delivered into rice, *Leucaena leucocephala* [10], mustard [11], tobacco, maize [12], *etc.*, based on the genotype dependent tissue culture procedure. Recently, a novel tissue culture independent gene delivery method using ferroferric oxide (Fe_3O_4) magnetic nanoparticles (MNPs)-mediated pollen transfection was developed. Transgenic cotton, pumpkin and pepper plants were generated efficiently [13]. To our knowledge, pollen transfection application in various maize genotypes growing in the field through MNPs has not been reported. In this study, we established a maize pollen transfection system applicable for large-scale, fast and efficient transfection in the field, independent of maize genotype.

Results

MNP-DNA complexes were formed and delivered into maize pollens efficiently

Electric negative plasmid DNA was bound by positively charged MNPs to form MNP-DNA complexes. According to the results of gel retardation assay (Fig. 1A), DNA was completely coated on MNPs at the 4:1 and 2:1 mass ratio (DNA/MNP), the amount of free DNA decreased with the decline of DNA/MNP ratios (DNA/MNP ≧ 10:1). For DNA protection analysis (Fig. 1B), MNPs thoroughly bounded plasmid DNA and protected DNA against endonuclease digestion at the 10:1, 4:1 and 2:1 mass ratio (DNA/MNP), uncoated DNA were digested at the DNA/MNP mass ratio more than 10:1. These results indicated that the optimum ratio between plasmid DNA and MNPs was 4:1 (DNA/MNP). And the complex size of DNA/MNP = 4:1 measured by dynamic light scattering (DLS) was 212.4 nm (Fig. 1C).

Meanwhile, we confirmed the aperture structure of maize pollen under scanning electron microscopy (SEM). Maize pollen possessed only one aperture with a diameter of about 6 μm on its relatively smooth surface (Fig. 2A), from where the MNP-DNA complexes (diameter 0.2 μm) were able to permeate into the pollen. Then we tracked MNPs spatially via transmission electron microscopy (TEM) to investigate whether magnetofection can transfer MNP-DNA complexes into maize pollen. As shown in Fig. 2B, plenty of MNP-DNA complexes were shown on the internal side of the transfected pollen wall, but none MNP-DNA complex was appeared within the untransfected pollen (Fig. 2C). These results were similar to the cotton pollen magnetofection practice, proving that MNP-DNA complexes could be transferred into pollen through aperture by magnetofection [13].

EGFP reporter was successfully expressed in transfected Jing92 pollens and plants

Pollens from maize variety, Jing92, were transfected with MNP, DNA and MNP-DNA (carrying the p35S::*EGFP* expression cassette, Figure S1), respectively. After cultured in vitro for 24 h at 25°C, green fluorescence was detected in 92% (491/536) of MNP-DNA transfected pollens (Fig. 3A), but none of MNP

(Fig. 3B) or DNA (Fig. 3C) transfected pollens, indicating that MNP was necessary and efficient to deliver DNA into maize pollen for transient expression. Furthermore MNP-DNA transfected pollens were applied to female florets of Jing92, and 70% (21/30) immature embryos (2 day after pollination, 2 DAP) showed green fluorescence (Fig. 3D). Green fluorescence was also detected in both leaves (Fig. 3E) and roots (Fig. 3F) of 18% (14/79) Jing92 T1 seedlings (at the three-leaf stage). These results demonstrate that, functional EGFP protein was successfully expressed in *EGFP*-transfected maize plants and the transgene was recovered in seeds and their T1 seedlings.

Then, the *EGFP* gene in Jing92 T1 seedlings at the three-leaf stage was analyzed. The gene encoding EGFP was detected in 46% (36/79) of T1 seedlings using PCR (Fig. 4A). RT-PCR and Western blot were used to verify whether the gene was transcribed and translated. RT-PCR results showed that the *EGFP* gene was transcribed normally in 32% (25/79) seedlings (Fig. 4B, with *ZmActin1* as the reference gene), and EGFP protein was detected in only 16% (13/79) seedlings (Fig. 4C, with ZmACTIN1 as the reference protein). These results demonstrated that the foreign *EGFP* gene was delivered into maize efficiently through pollen transfection and expressed successfully.

EGFP reporter was efficiently delivered into different maize varieties

In order to further test the efficiency of pollen transfection in different maize varieties, *EGFP* (Fig. S1) was delivered into five maize varieties 178, B73, HZ178, Jing92 and Zheng58. At the three-leaf stage, T1 seedlings from different transfected varieties were examined to ensure timely detection of transient and stable products of target genes. As shown in Table 1, the efficiency of target gene delivery calculated by PCR positive rate was quite high, ranging from 29–68%. The transfection efficiency analyzed by RNA recovery dropped to 18–32% possibly due to exogenous DNA degradation by the host nuclease and the incomplete introduction of expression cassette. The transfection efficiency scored by recovery of protein based on immunostaining reached 7–16%. Detailed data were shown in Fig. S3-S6. These results proved that target genes were delivered efficiently into different maize varieties through pollen transfection and expressed functional products.

Table 1
Transfection and expression of EGFP reporter in different maize varieties.

Maize variety	Total plants	Relative recovery of marker gene, RNA and protein in maize T1 seedlings		
		Gene	RNA	Protein
178	30	17/30 (57%)	8/30 (27%)	2/30 (7%)
B73	41	28/41 (68%)	12/41 (29%)	5/41 (12%)
HZ178	89	51/89 (57%)	16/89 (18%)	7/89 (8%)
Jing92	79	36/79 (46%)	25/79 (32%)	13/79 (16%)
Zheng58	42	12/42 (29%)	9/42 (21%)	4/42 (10%)
Average		51.4%	25.4%	10.8%

Plants were transfected using pollens carrying *EGFP*. Seeds from transfected plants were germinated and the *EGFP* gene, RNA or protein were analyzed in three-leaf stage T1 seedlings (detailed data were shown in Fig. S3-S6).

Bar selective marker was heritable in transfected maize progenies

For the convenience of selecting stable integrated progenies, the selective marker gene *Bar* (Fig. S7) was transferred into maize variety Zheng58. After glufosinate screening, 1.41% (5/355) of T1 seedlings were survived (Fig. 5A). These 5 glufosinate resistant plants showed the positive BAR band (test line) during the quick strip test (Fig. 5B), indicating that *Bar* gene was delivered into maize successfully through pollen transfection and expressed normally. Furthermore, Southern blot indicated that these T1 plants had at least 2–3 integrations in their genomes (Fig. 5C), and their T2 progenies showed genetic segregation (Fig. 5D). The above results demonstrated that, through our maize pollen transfection, exogenous gene was integrated into the maize genome effectively, expressed normally and inherited stably in their progenies.

Discussion

Maize is an ideal plant for pollen transfection, as it has large panicles, concentrated blossom and abundant pollen. It is possible to collect sufficient maize pollen and carry out large-scale transfection in the field. We expanded the pollen treatment system capable of handling 5 g maize pollen in each transfection. As maize pollen grains were mainly 60–100 μm in diameter (Fig. 2A, 3A-C), the 150 μm aperture sifter was used to purify and separate the pollen grains, and the 25 μm aperture nylon fabric was applied to collect maize pollen and filter liquid, thus transfected pollen grains were mainly reserved. After transfection, maize pollen was mixed with corn starch to accelerate the dry process. Through these

optimizations, the MNP-mediated maize pollen transfection system became efficient. Maize varieties that are recalcitrant to tissue culture were successfully transfected with *EGFP* or *Bar*. Transient EGFP signal can be observed in 92% transfected pollens and 70% immature embryos. The *EGFP* gene was detected in part or whole in as high as 68% T1 seedlings germinated from transfected seeds, and the functional protein was detected in around 11% of T1 seedlings (Table 1). Moreover, 1.41% of the *Bar* transfected T1 plants were glufosinate resistant, and heritable *Bar* gene was integrated into the maize genome effectively (verified by Southern blot), expressed normally and inherited stably in their progenies. Taken together, the maize pollen transfection system is genotype independent and efficient, we envision this system will benefit gene delivery for all maize varieties growing in field.

The key limiting factor of the maize pollen transfection system is the relative seed set, which is directly dependent on pollen viability. Maize pollen viability is mainly affected by the incubation time of pollen with MNP-DNA solution and the duration of pollen operation in vitro. High concentration of sucrose is necessary in the MNP-DNA solution to maintain the osmotic pressure of pollen, resulting in difficulty to dry transfected pollen naturally. Following measures could be taken to increase seed set : a) collect maize pollen grains as fresh as possible; b) shorten the incubation time of pollen with MNP-DNA solution properly, to keep the balance between DNA delivery rate and pollen viability; c) further reduce the pollen drying time, even pollinate transfected pollen directly after mixing with corn starch; d) increase the pollen amount of pollination for each ear; or e) pollinate the same ear repeatedly for 2–3 times in different days.

Using the same or other nanomaterials as DNA carriers, several inheritable transgenic plants were generated [11, 13], but most transfections are transient [12, 14, 15]. The nanomaterial-based plant transient transformation methods are also beneficial for plant genome editing technology where gene expression without transgene integration is desired [16]. Similar technology has been developed and proven effective, termed as DNA-free genome editing, in which protein, RNA, or ribonucleoprotein are directly delivered, from a regulatory perspective, to eliminate all risk of transgene integration [17–19]. Our maize pollen transfection system, in which efficient and genotype independent introduction and transient expression could be achieved, providing a reliable, fast and large-scale gene delivery choice for most elite maize varieties recalcitrant to tissue culture, especially for the development of the advanced breeding techniques like precise genome editing in elite crop germplasm directly [20].

Conclusions

The results of this practical-based study clearly demonstrated that exogenous DNA could be delivered into maize efficiently and expressed normally through our genotype-independent pollen transfection system, providing a reliable, fast and large-scale gene delivery choice for most elite maize varieties recalcitrant to tissue culture, especially for the development of the advanced breeding techniques like precise genome editing in elite crop germplasm directly.

Methods

Materials

Maize varieties (major inbred lines, tissue culture recalcitrant): 178, B73, HZ178, Jing92 and Zheng58, were kindly provided by Corn Research Center, Beijing Academy of Agriculture and Forestry Sciences. Two plasmids, pYBA1132 (Fig. S1) and pYBA1132-Bar (Fig. S7), were constructed and preserved by our research center. The pYBA1132 plasmid, whose NCBI accession number was KF876796, harboring *EGFP* gene [21] under the control of cauliflower mosaic virus (CaMV) 35S promoter and CaMV 35S terminator. While pYBA1132-Bar plasmid carried the *Bar* gene [22] under the control of CaMV 35S promoter and NOS terminator. Polyethyleneimine (PEI) modified Fe_3O_4 magnetic nanoparticles (MNPs, PolyMag1000, catalogue No. 9003) and MagnetoFACTOR-96 plates (catalogue No. 9008-96) applicable for large-scale transfection were purchased from Chemicell GmbH (Berlin, Germany).

DNA solution preparation and MNP-DNA complexation

Plasmid DNA of pYBA1132 and pYBA1132-Bar were extracted by alkaline lysis with sodium dodecyl sulfate (SDS) maxipreparation method, respectively. DNA concentration was determined by spectrophotometry and DNA was diluted to 1 μg/μL with double distilled water (ddH_2O) for the following experiments. Gel retardation assay and DNA protection analysis were carried out to identify the optimal DNA/MNP binding ratio. For gel retardation assay, 0.5 μg DNA was mixed with MNPs at the ratios of 2:1, 4:1 10:1, 20:1, 50:1 and 100:1, and settled at room temperature in dark for 30 min, respectively. For DNA protection analysis, 0.5 μg pure plasmid DNA and MNP-DNA complexes were digested for 16 h with 0.5 U *Xba*Ⅰ (New England Biolabs, Ipswich, UK) at 37 °C. Pure plasmid DNA, MNP-DNA complexes, plasmid DNA digested product and MNP/DNA complexes digested products were electrophorized in 1% (w/v) agarose gel (Biowest) at 6 V/cm for 30 min. DNA was stained with 0.01% (v/v, final concentration in gel) Gelstain (Transgene Biotech Co., Ltd., Beijing, China). The gel image was analyzed by Tanon 1600 Scanner (Tanon Science & Technology Co., Ltd., Beijing, China). The sizes of MNPs and MNP-DNA complexes were measured by dynamic light scattering (DLS) on Dynapro Titan TC (Wyatt Technology Co., Ltd., USA).

Magnetic nanoparticles mediated pollen transfection of maize

All maize transfection procedures were handled gently. Firstly, pYBA1132 or pYBA1132-Bar plasmid DNA was mixed with MNPs at the optimum mass ratio, and incubated for 0.5 h at room temperature to form MNP-DNA complexes via electrostatic interaction. The complexes were diluted with 20 mL pollen medium (200 g/L sucrose, 103 mg/L H_3BO_3, 53 mg/L KNO_3, 103 mg/L $Ca(NO_3)_2$, 517 mg/L $MnSO_4$, 103 mg/L $MgSO_4 \cdot 7H_2O$ and 30 mg/L GA_3, [13]), and mixed with 5 g sieved pollens (collected from 5 maize varieties: 178, B73, HZ178, Jing92 and Zheng58, Fig. 6A, 6B) to prepare the pollen and MNP-DNA suspension. Then, the suspension was placed on top of the MagnetoFACTOR-96 plate and kept still at room temperature for 0.5 h to transfect pollens (Fig. 6C). After transfection, the supernatant was carefully removed and the transfected pollens were spread on a 20 cm × 20 cm nylon fabric (25 μm aperture, which

could filter liquid while reserve maize pollens) and the fabric was folded in half, extra liquid was absorbed completely by 3 layers of filter paper (18 cm in diameter, Fig. 6D), then the wet sticky pollens were dried with 3 g corn starch (Fig. 1E), and pollinated artificially to 20 ears of the same maize variety (Fig. 6F) to generate transfected seeds (Fig. 6G).

Electron microscopy of maize pollen

In order to confirm their aperture structures, fresh maize pollen grains were collected and spread onto the silicon slice surface. After gold spray, pollen grains were observed under the scanning electron microscope (S3400N, Hitachi Co., Ltd., Japan) at 5.0 kV. On the other hand, we tracked MNP-DNA complexes spatially to investigate whether magnetofection can transfer MNPs into maize pollen. Transfected and untransfected pollen grains were fixed and cut to prepare for ultrathin sections. The sections were mounted on copper grids and checked by transmission electron microscopy (JEM-1400, JEOL Ltd., Japan) at 80 kV.

Observation of green fluorescence in transfected maize pollens and plants

In order to observe the expression of *EGFP* reporter (in plasmid pYBA1132, Fig. S1) in the transfected maize pollens and plants, the following materials from maize variety, Jing92, with or without transfection were collected for green fluorescence observation. Pollens transfected with MNP, DNA and MNP-DNA (cultured in dark for 24 h at 25℃, on pollen medium containing 15 g/L agar, brushed and disperse in the pollen medium before observation), immature embryos (2 day after pollination, 2 DAP), roots and leaves of T1 seedlings (at three-leaf stage) germinated from *EGFP* transfected seeds were observed for GFP signal under Confocal laser scanning microscopy (A1+, Nikon, Japan), using 488 nm wavelength light for excitation.

Molecular analysis of EGFP reporter in T1 transfected seedlings

Molecular analysis was carried out at the three-leaf stage to ensure timely detection of *EGFP* products. Genomic DNA and total RNA in leaves from maize seedlings were extracted via CTAB (Hexadecyl trimethyl ammonium Bromide) and TRIzol method, respectively. Gene specific primer EGFP-F/R (Table S1) were used in PCR and reverse transcription PCR (RT-PCR) to validate the delivery and expression of *EGFP*, with the maize *ZmActin1* (NCBI Gene ID: 100282267) as the internal RT-PCR control. Total protein in leaves of T1 seedlings germinated from transfected seeds was extracted in protein extraction buffer (50 mM PBS (pH7.0), 10 mM EDTA, 0.1% SDS, 0.1% Triton X-100) [23], protein concentration was determined by micro bicinchoninic acid (BCA) protein assay. Simple Western Size Assay was carried out on Wes (ProteinSimple, anti-mouse/rabbit detection kits, catalogue No. DM-001/DM-002, SM-W004 and PS-ST01EZ-8), with the maize ZmACTIN1 (NCBI accession: NP_001148651) as a reference protein. As 0.5 μg total protein was sampled, and 0.05 μg anti-EGFP mouse monoclonal antibody/anti-ACTIN

(Plants) rabbit polyclonal antibody (catalogue No. D199989/D110007, Sangon Biotech Co., Ltd., Shanghai, China) were used as EGFP/ZmACTIN1 primary antibody, then 0.05 μg horseradish peroxidase (HRP)-conjugated goat anti-mouse/rabbit IgG were used as secondary antibody (included in the Wes anti-mouse detection kits). EGFP and ZmACTIN1 positive bands were 27 and 42 kDa, respectively. EGFP recombinant protein (catalogue No. C600323, Sangon Biotech Co., Ltd., Shanghai, China) was used as positive control.

Bar selective marker transfection, selection and heredity

The selective marker gene *Bar* (in plasmid pYBA1132-Bar, Fig. S7), encoding a glufosinate resistance protein, was transferred into maize variety Zheng58, to promote the efficiency of selecting stable integrated progenies. Transfected seeds were sowed in rows of the field and germinated. At the three-leaf stage, seedlings were spread drenched with 200 mg/L glufosinate solution. After one week, survived and total seedlings number were checked to calculate the resistant rate. Then, the leaves of survived seedlings were used for BAR quick strip test (catalogue No. AS-013-LS, ENVIROLOGIX Inc., USA) according to the manufacturer's protocol, those seedlings with both test line (below) and control line (upper) were considered positive. At the shooting stage, 5 g leaves of positive maize plants at T1 or T2 generation were collected for genomic DNA extraction and Southern blot analysis. About 100 μg genomic DNA was digested with *Hind* Ⅲ and *Pst*Ⅰ, respectively, overnight. Then digested DNA was separated on a 1% agarose gel and transferred onto a positively charged nylon membrane (catalogue No. 11417240001, Roche, USA). The PCR product of *Bar* gene was used as the template DNA for probes preparing. The DIG-labeled probe was prepared and hybridized according to the DIG (Digoxigenin) High Prime DNA Labeling and Detection Starter Kit Ⅱ (catalogue No. 11745832910, Roche, USA) manufacturer's protocols.

Abbreviations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

Z-PW, Z-BZ and Z-YW have applied for a patent in China (patent number 201910623296.5). The authors declare that they have no other conflict of interest.

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Authors' contributions

Z-YW, J-HW and Z-BZ designed and supervised the experiments; Z-PW, L-FY, X-LL and CZ performed the experiments; Z-PW, Z-BZ, RY and Z-YW analyzed the results; Z-PW and LW prepared the first draft and Z-PW, Z-BZ, J-HW, RY and Z-YW contributed to the final editing of manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Figures

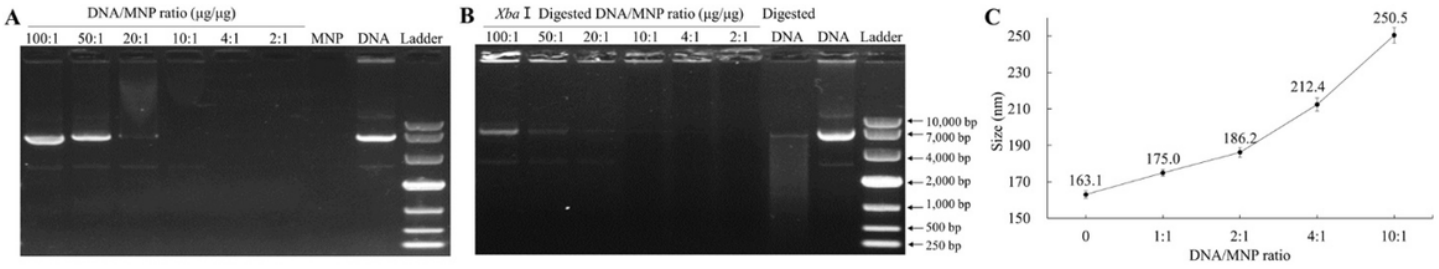

Figure 1

MNP-DNA complexation. (A) Gel retardation assay of MNP-DNA complexes. (B) DNA protection analysis of MNP-DNA complexes. Each sample contains 0.5 μg DNA. DNA was completely bound and protected against endonuclease digestion by MNPs when DNA/MNP ratio≤4:1. (C) Size of MNPs and MNP-DNA complexes measured by dynamic light scattering (DLS). Each sample contains 2 μg MNPs. Error bars represent standard deviation from three replicates.

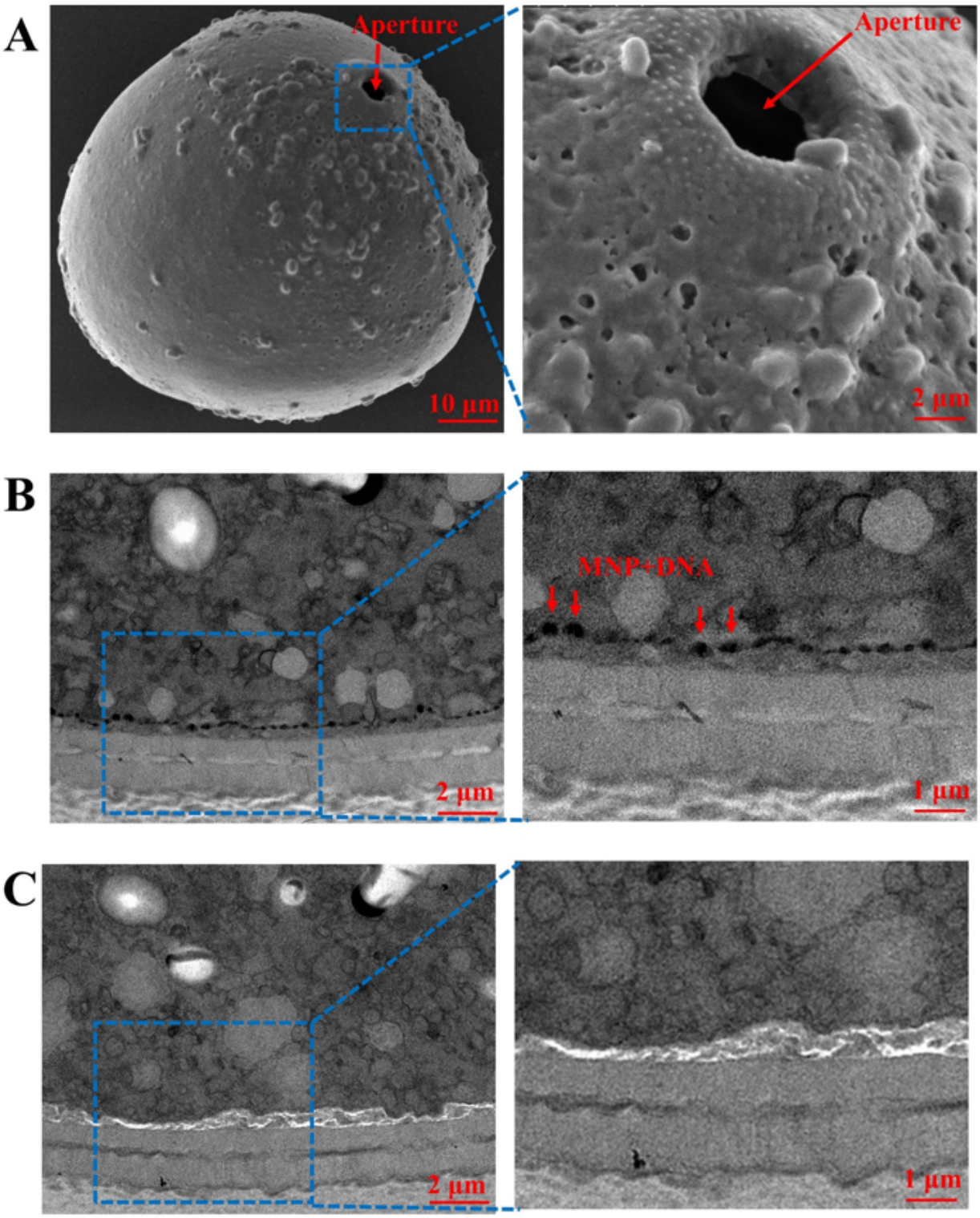

Figure 2

Electron microscopy images of maize pollen. (A) SEM images of maize pollen. Maize pollen possessed only one aperture with a diameter of about 6 μm, from where the MNP-DNA complexes (diameter 0.2 μm) were able to permeate into the pollen. (B) TEM images of MNP-DNA transfected maize pollen. Plenty of MNP-DNA complexes were shown on the internal side of the transfected pollen wall. (C) TEM images of untransfected maize pollen. None MNP-DNA complex was appeared within the untransfected pollen.

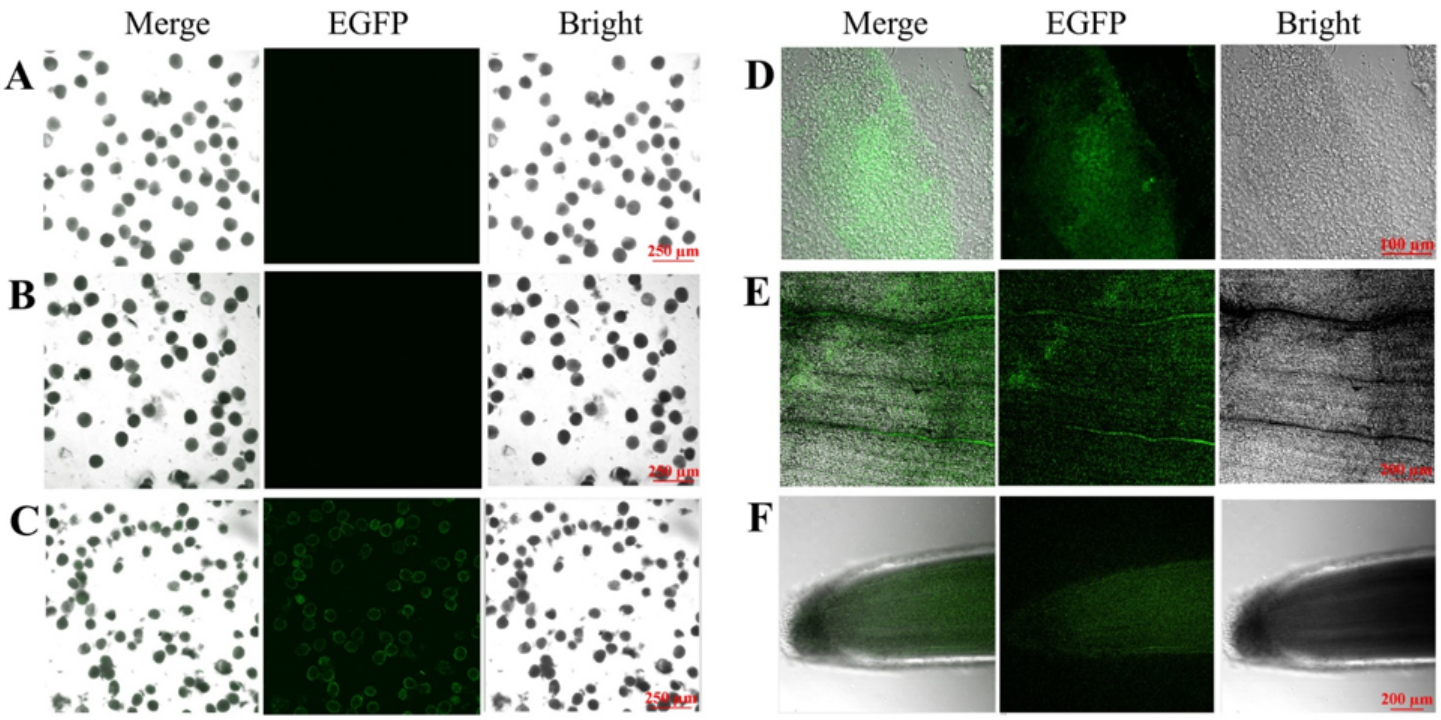

Figure 3

Green fluorescence in transfected Jing92 pollens and plants. Green fluorescence was undetectable in either MNP (A) or DNA (B) treated pollens, but can be detected in MNP-DNA complexes transfected Jing92 pollens (C), immature embryos at 2 DAP (D), leaf (E) and root (F) of three-leaf stage T1 seedlings. Merge: green fluorescent field and bright field overlay image. GFP: green fluorescent field. Bright: bright field.

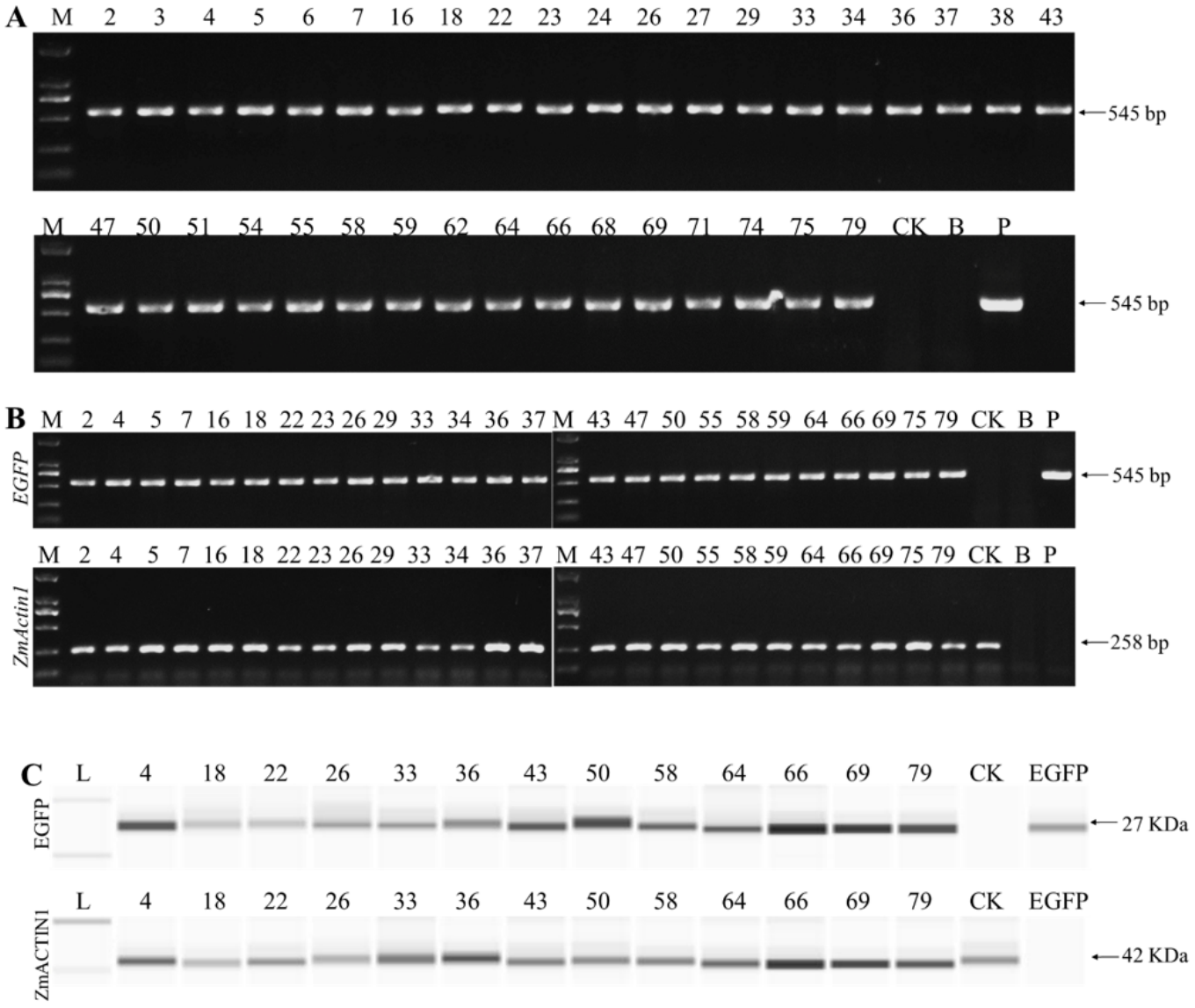

Figure 4

Molecular analysis of EGFP reporter in Jing92 seedlings. (A) Detection of EGFP gene by PCR. (B) RNA of EGFP and ZmActin1 detected by RT-PCR. (C) Immunostain of EGFP and ZmACTIN1 protein. Crude protein was separated and blot through capillary electrophoresis, full scare blot images were shown in Fig. S2. M: DL2000 DNA molecular marker. Numbers at top refer to seedling from seeds of EGFP transfected plants. CK: control seedling. B: blank, no sample DNA control. P: plasmid of pYBA1132. L: biotinylated ladder. EGFP: purified recombinant EGFP protein.

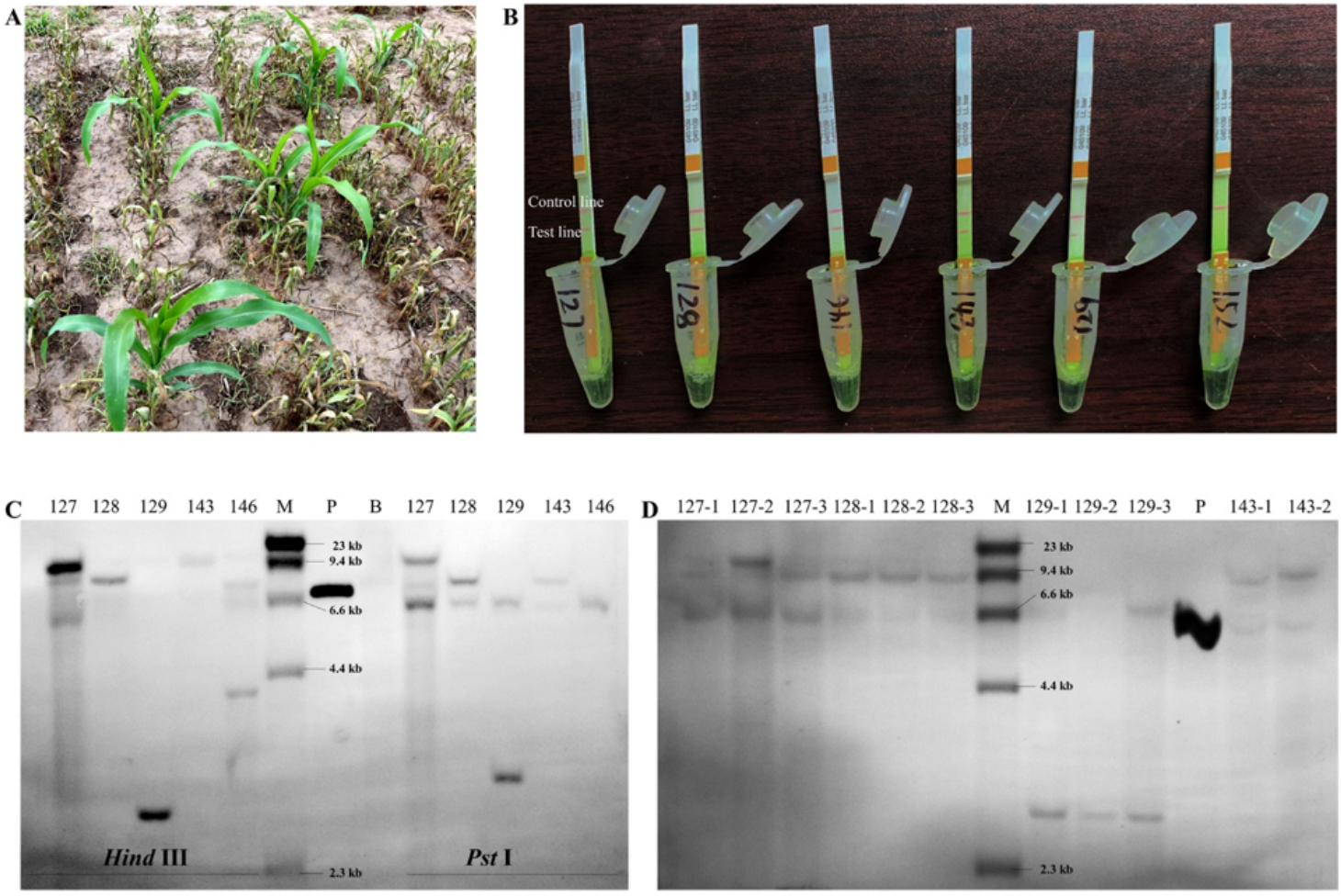

Figure 5

Bar selective marker was heritable in transfected maize progenies. (A) Glufosinate screening of T1 seedlings from Bar transfected seeds. At the three-leaf stage, seedlings were spread drenched with 200 mg/L glufosinate solution. Screening results were photographed one week later. (B) BAR quick strip test results of glufosinate resistant T1 seedlings. No. 127, 128, 129, 143, 146 seedlings with both test line (below) and control line (upper) were positive, No. 152 seedling with only control line (upper) was negative. (C) Southern blot of Bar in T1 seedlings. No. 127, 128, 129, 143, 146 seedlings possessed at least 2-3 integrations in their genomes. M: DIG-labeled lambda/Hind□ DNA Marker; P: pYBA1132-Bar plasmid digested with Hind□; B: untransfected Zheng58. (D) Southern blot of Bar in T2 seedlings. Genomic DNA of T2 seedlings was digested with Hind□. No. 127-1, 127-2, 127-3, 129-1, 129-2,129-3 seedlings showed genetic segregation, other seedlings inherited stably.

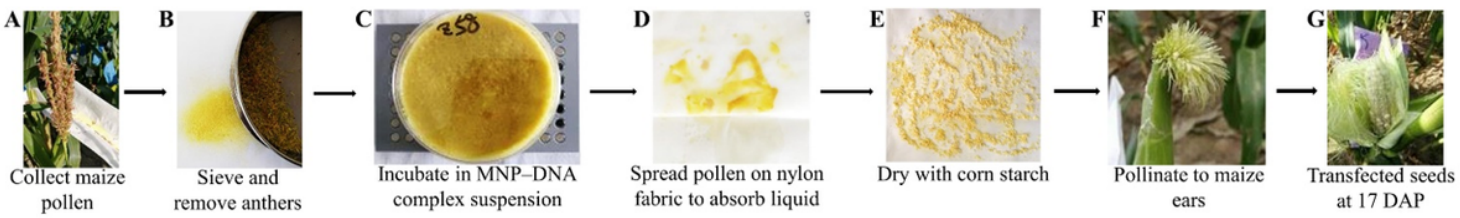

Figure 6

Maize pollen transfection experiment procedures. (A) Collect maize pollens at blossom period. (B) Sieve through 150 μm aperture sifter to remove anthers and obtain 5 g clean pollens. (C) Add MNP-DNA complex suspension and incubate for 30 min. (D) Spread pollen on 25 μm aperture nylon fabric and absorb extra liquid by clean filter paper. (E) Mix with 3 g corn starch. (F) Pollinate silks of one maize ear with about 0.4 g pollen-starch mixture. (G) Immature seeds are visible at 17 days after pollination with transfected pollens.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- figS1.png
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- figS6.png
- figS7.png
- TableS1.docx