Paper S1

Extraction method of DNA:

The CTAB method was used to extract DNA from the seedling leaves of the maize introgression lines IB030, IB021 and 301 $F_{2:3}$ population materials. The specific operation steps are as follows:

(1) Preheat 2% CTAB extraction buffer in a 65°C water bath for 10 min;

(2) Weigh 2g of seedling leaves and put them in a cleaned mortar, cut them into pieces, pour in an appropriate amount of liquid nitrogen and quickly grind to powder, and quickly pour the powder into a sterilized and pre-frozen 5ml centrifuge tube , the loaded volume is about a quarter of the centrifuge tube;

(3) Then add 2ml of preheated 2% CTAB buffer, and shake gently; place the centrifuge tube in a constant temperature water bath at 65°C for about 45 minutes, and shake it every 5 minutes during this period;

(4) Take out the centrifuge tube and let it cool to room temperature, then add 2ml of chloroform-isoamyl alcohol (24:1), put it on a shaker and shake it for 10 minutes to fully mix the two;

(5) Put it in a centrifuge at 10,000 rpm for 10 min;

(6) After the centrifugation is completed, gently suck up the supernatant with a pipette and transfer it to another 5ml sterilized centrifuge tube; then add an equal volume of isopropanol that has been frozen at -20°C in advance, and slowly rotate the centrifuge tube. Shake up and down to fully mix isopropanol and supernatant until white DNA flocs appear, and place in a constant temperature refrigerator at 4°C for 1 hour;

(7) Use a capillary to pick the DNA white flocs into a 2ml sterile centrifuge tube prepared in advance, wash with 1000ul of 100% and 75% ethanol, and slowly pour out the ethanol to wash the waste liquid;

(8) Put the centrifuge tube upright on the ultra-clean workbench and let it air dry naturally;

(9) Add 100ulddH2O and place it in a constant temperature refrigerator at 4°C to fully dissolve it;

(10) Store the samples at -20°C for later use.

The reagent formulation is as follows:

(1) 2% CTAB: Dissolve 124g NaCl, 25g Tris base, 15g EDTA·Na2, and 40g CTAB in 1.6 L of distilled water, respectively, and stir with a magnetic stirrer to fully dissolve and set the volume to 2 L. PH=8.O;

(2) Trichloromethane-isoamyl alcohol (24:1): V trichloromethane: V isoamyl alcohol=480 ml: 20ml;

(3) 75% ethanol: 1500ml absolute ethanol + 500ml distilled water;

(4) 0.2% β-mercaptoethanol (v/v, added to CTAB when used).

PCR amplification of DNA:

The PCR reaction system selected in the process of DNA amplification was 15ul (as shown in Table 1); the PCR amplification program was a common program (as shown in Table 2).

Table 1 PCR amplification system

Groupe	Volume/μL
DNA（200ng/μL）	1
2 x 3G Taq Master Mix for PAGE	7.5
PCR Forward Primer (10μmol/L)	1
PCR Reverse Primer (10μmol/L)	1
ddH_2O	4.5

Table 2 PCR procedure

Steps	Temperature/℃	Time
1	94	5min
2	94	30s
3	-	30s
Cycle 32 times in 2-3 steps		
4	72	40s
5	72	5min
6	4	Forever

Paper S2

Polyacrylamide gel electrophoresis:

Preparation of PAGE preparation gel and related reagents:

10×TBE buffer: 216g Tris Base, 14.88g EDTA·Na2, 110g boric acid and distilled water to

make up to 2L.

6% polyacrylamide glue: 840.84g urea, 120g acrylamide, 6.32g methylidene, 100ml 10×TBE and dilute to 2L.

Fixative solution: add 450ml of glacial acetic acid to distilled water and make up to 3L.

Silver staining solution: 8g AgNO3 was dissolved in 3L of distilled water.

Developer: dissolve 80g NaOH in 2L of distilled water until it is completely dissolved, then add 22ml of formaldehyde solution and distilled water to make the volume to 3L.

15% ammonium persulfate APS: put 7.5g of ammonium persulfate into a 50ml EP tube, and make up to 50ml with distilled water.

PAGE gel accelerator (TEMED): N, N, N', N'-tetramethyldiethylamine, the molecular formula is: $(CH_3)_2NCH_2CH_2N(CH_3)_2$。

Hydrophilic Silane (PlusOne Bind-Silane): Molecular formula: $C_{10}H_{20}O_5Si$

Repel-Silane: Dichlorodimethylsilane, $(CH_3)_2Cl_2Si$

Electrophoresis step:

(1) Scrub the glass plate: Wash the hydrophilic glass plate and the hydrophobic glass plate (concave plate) repeatedly with detergent mixed with tap water, and then use an alcohol watering can to spray 75% alcohol to wipe both sides, and let it dry.

(2) Glass plate assembly: spread the hydrophilic and peeling silane prepared in advance evenly on the hydrophilic plate and the hydrophobic plate, respectively, and lay the hydrophilic plate flat on the backing plate (the coating side is facing up), and the left and right sides of the Put the seal on, place the hydrophobic plate on it (the coating side is facing down), clamp 3 fixing clips symmetrically on the left and right sides to fix the glass plate, and then use a level to balance.

(3) Glue preparation: Add 45ml of pre-prepared 6% PAGE gel to the plastic gluing soft bottle, then add 50μL of 15% APS and 5μL of TEMED with a pipette, shake gently, and quickly pour it into the assembled gel. In the glass plate, gently tap the glass plate with your hands during the glue filling process to remove air bubbles. After the glue filling is completed, insert the comb into the glue surface in reverse, the depth is 4ml, and let it stand for 30 minutes.

(4) Sample loading: install the prepared glass plate on the electrophoresis apparatus, pour 600ml of 1×TBE buffer into the electrophoresis tank, let it cover the metal wire of the electrophoresis apparatus, and then put the 96-well intact comb into the electrophoresis tank. To the glue surface inserted between the two glass plates, 3 μl of sample was dispensed into each well. The voltage of the electrophoresis apparatus was set to 1500V, the current was 75A, the power was 75W, and the electrophoresis time was 45 minutes. After electrophoresis is complete, cut off the power, remove the wash and split the glass plate.

(5) Fixation: Put the hydrophilic glass plate with the film glue side up and put it into the pre-configured fixative solution for 8 minutes. During this period, you can slowly shake it once or twice. After the fixation is completed, rinse it with distilled water 2-3 times. , stand upright for 1 minute and wait for the water to dry, then put it into the silver dye solution for silver dyeing.

(6) Silver staining: continue to place the fixed glass plate in silver staining solution for 12 minutes, repeat the fixing steps and wait for development.

(7) Development: Use distilled water to clean the glass plate 2-3 times. The cleaning speed should be fast and the cleaned glass plate must wait for no water droplets to flow down, so as not to cause the developer to be contaminated and turn black. The development time is 3-5 minutes until until the film turns yellow and clear bands appear.

(8) Observing strips and taking pictures: place the developed glass plate on a light table for observation, and take pictures for preservation.

Paper S3 Cloning primers for candidate genes

Gene	Forward primer	Reverse primer
Zm00001d012321	CAGTCCACCATCGAACGACA	AAAAGGCCCCAACGAAATGC
Zm00001d037590	ATGGCGTTCCCTCAGATCTCC	TCACGAAGAGAATATCGAAAG

Paper S4 Preparation of PCR reaction solution

Reagent	Usage amount/μl
PrimeSTAR Max Premix（2×）	25
Primer 1	1.5
Primer 2	1.5
Template	2
ddH_2O_2	20

Paper S5 Components of reaction

Remove genomic contamination system in RNA		Reverse transcription system	
Reagent	Volume (μL)	Reagent	Volume (μL)
10X Reaction Buffer	1	5X Reaction Buffer	4
with $MgCl_2$	1	Oligo (dT) 18 primer	1
total RNA	1	10 mM dNTP Mix	2
DNase I	1	total RNA	1
Water, nuclease-free	7	RiboLock Rnase Inhibitor	1
		RevertAid M-MuLV RT	1
		Water, nuclease-free	10
Total	10	Total	20