Supporting information

Cowpea transformation protocol

Unless otherwise specified, all the chemicals used for media preparation are from Sigma-Aldrich.

Agrobacterium preparation

1. **Master plate preparation:** Streak *Agrobacterium* from glycerol stock on the master plate medium (Table S1) containing different antibiotics based on the *Agrobacterium* strains and the constructs that bacterium carries to make master plates. Incubate the master plates at 28°C for 3-4 days. The master plates can be kept in the fridge to make working plates and last for a month.
2. **Working plate preparation:** Streak a working plate on the working plate medium (Table S2) using a loop of bacteria from the master plate prepared above and incubate the working plate at 28°C for overnight or over 20 hrs for LBA4404 Thy- and AGL1, respectively.
3. **Inoculum preparation:** Collect 5-7 full loops of bacteria from the working plate using a sterile loop, suspend bacteria in 30 mL infection medium (IM) (Table S3) with acetosyringone (AS, 1M stock in DMSO protected from light, final 200 µM) and dithiothreitol (DTT, 1 M stock, final 1 mM) freshly added in a sterile 50 mL centrifuge tube, adjust OD to 0.5.

Cowpea EA explant preparation

1. **Seed sterilization:** Cowpea seeds were surface sterilized using chlorine gas made by mixing in 3.5 mL of 12N HCl and 100 mL bleach (5.25% sodium hypochlorite) for 16 hrs.

2. **Seed pretreatment:** Soak sterilized cowpea IT89D-1010 seeds in the Bean Germination Medium (BGM) (Table S4) with ~45mL of water added for ~16 hrs. For other cowpea varieties transformed with AGL1 30 mL 0MS medium (Table S8) was used to replace BGM.
3. **EA explants isolation:** Isolate embryo axis (EA) explants by removing the seed coats, cotyledons and plumules and put them into sterile water in a petri dish until infection.

Cowpea transformation

1. **Infection:** Remove water from petri dish (as much as possible), add 15 mL inoculum, and 50 µL sterile Poloxamer 188 10% solution. Wrap the plate with parafilm and sonicate (VWR, Motel 50T, 120Volts, 1 A or FS30H, Fisher Scientific) for 3 sec. After sonication, add additional 10 mL inoculum (total 25 mL in petri dish) to the mix and gently shake on a at ~60 rpm for 1.5 hrs at room temperature.
2. **Co-cultivation:** Remove bacterium and transfer EAs to filter paper (VWR Cat No. 28320-020) blotted with 700 µL IM in a 100 x 25mm petri dish. Thirty EAs can be piled up on the paper (2-4 piles per plate). Seal plates with micropore tape and keep plates in 21^{o}C, 45% RH, 7.7 lums/ft^{2} chamber for 2 days.

Cowpea regeneration

1. **Regeneration with selection:** Insert the roots of EAs vertically into SIM (Table S5) with cot-node and SAM above the medium. Incubate the EAs on SIM at 26°C under 24 hrs light conditions. Remove the SAM by cutting through the middle of epicotyl after 4-5 days culturing on SIM to promote axillary shoots formation at the cotyledon node region.

2. **Rooting:** After 3-5 weeks regeneration, harvest shoots bigger than 3 cm by cutting at base of shoot, and place into root induction medium (RIM) (Table S6).
3. **Shoot elongation:** After 3-5 weeks regeneration, if shoots did not reach to 3 cm, they were transferred to the shoot elongation medium (SEM) (Table S7) for 2-4 weeks culture before transfer to RIM.

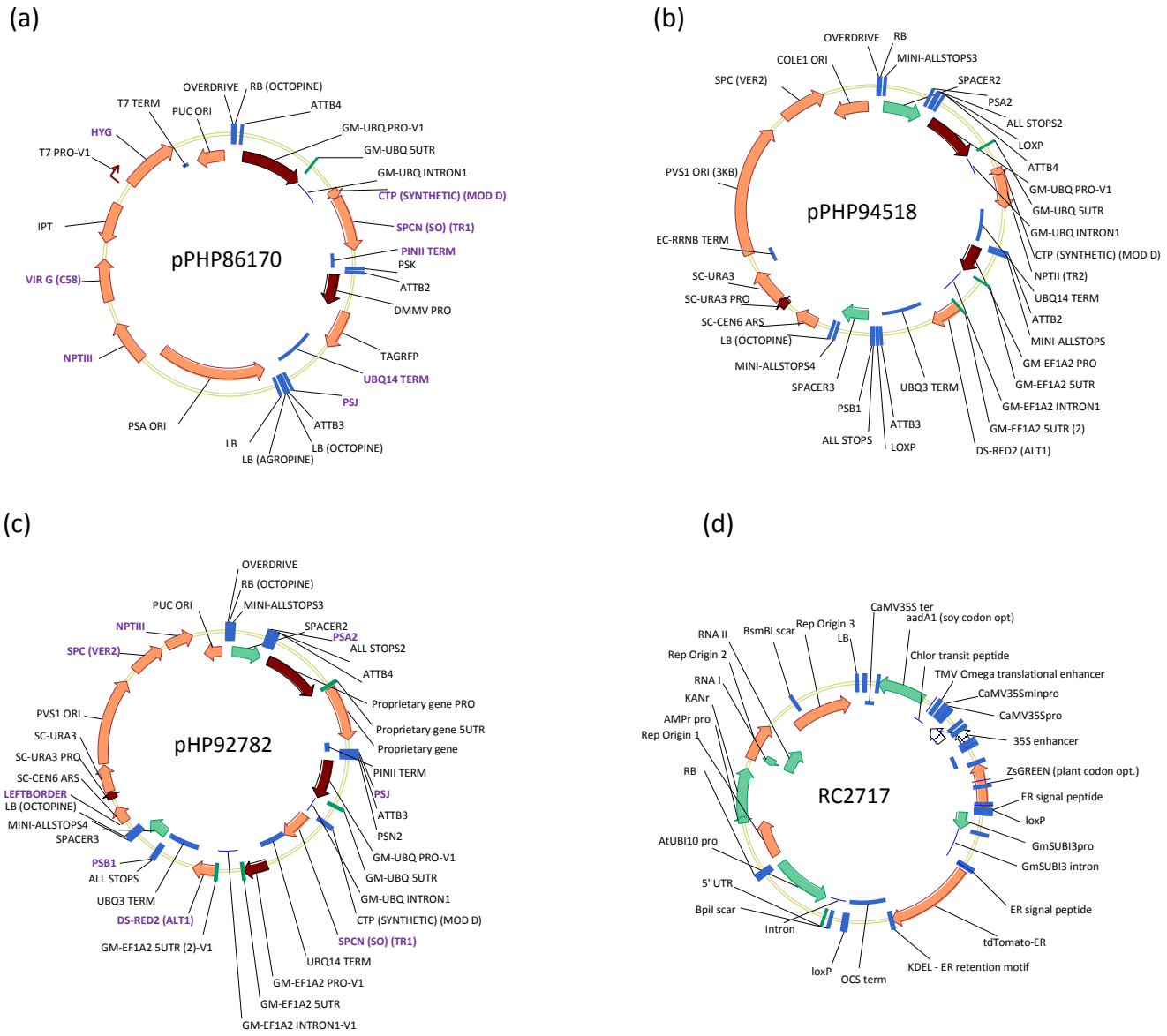

Figure S1 Schematic representation of the molecular components of constructs used in this study. (a) pPHP86170 (b) pPHP84518 and (c) pPHP92782 were transformed with ternary vector system using pPHP71539 as helper in *Agrobacterium* strain LBA4404 Thy-. The genes and elements highlighted in purple are covered by event quality assays (Table S9). (d) RC2717 is a modified pCAMBIA vector transformed into *Agrobacterium* AGL1 strain.

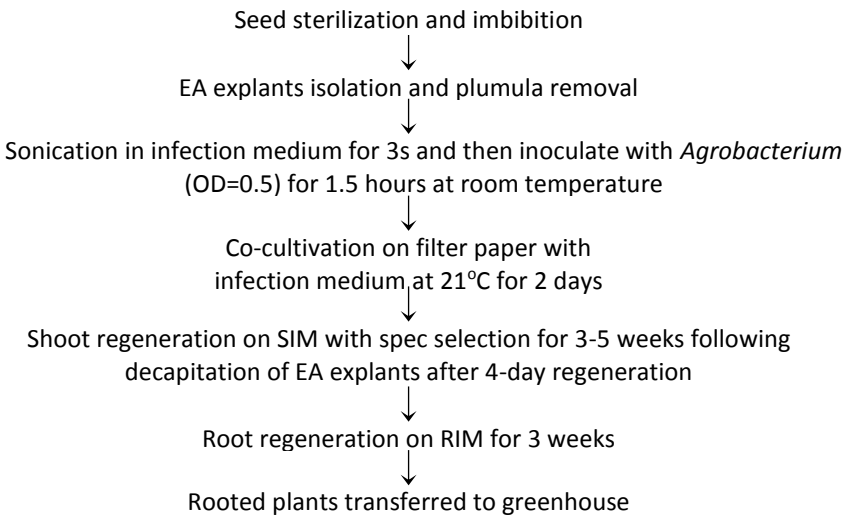

Figure S2 Flow diagram of the cowpea EA-based *Agrobacterium*-mediated transformation process.

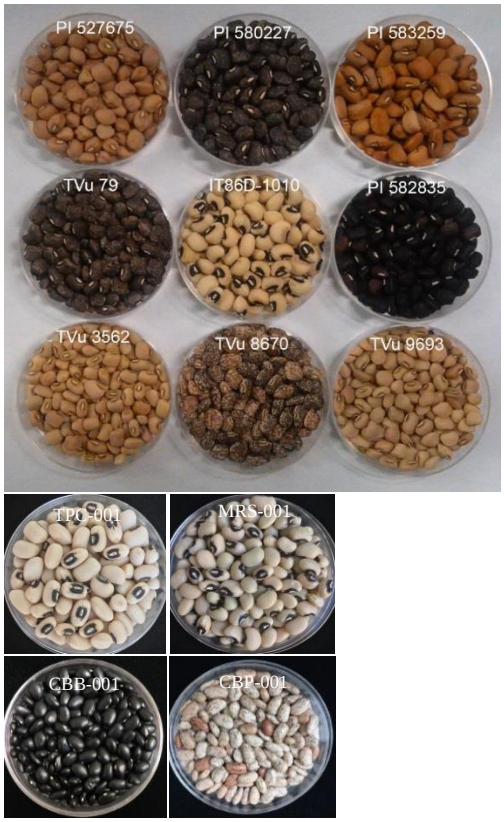

Figure S3 Dry mature seeds of selected accessions of cowpea and common bean**.**

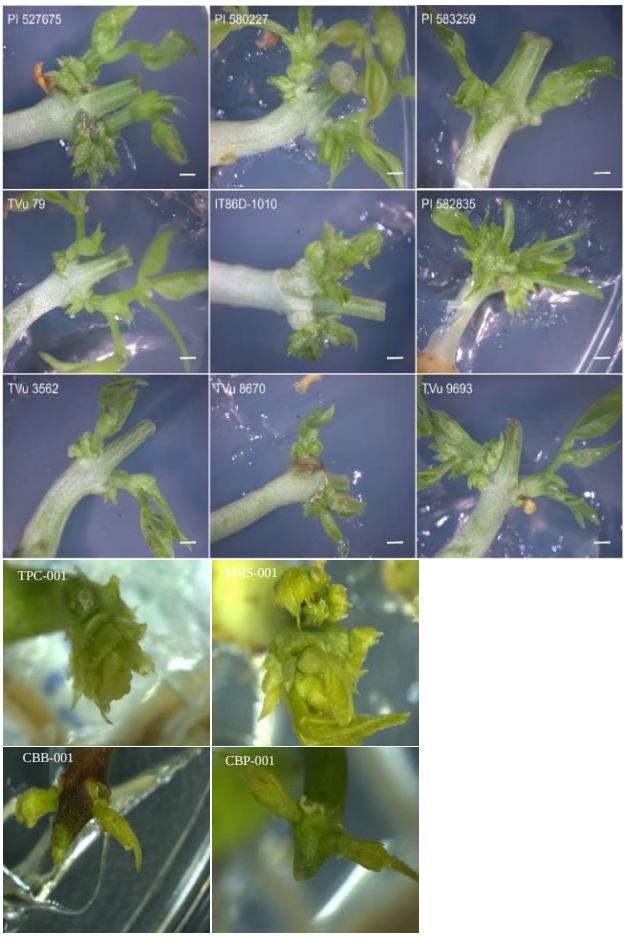

Figure S4 Shoot organogenesis of selected accessions of cowpea and common bean**.**

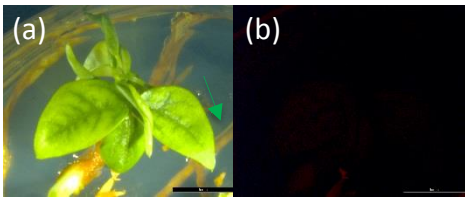

Figure S5 Autofluorescence evaluation. (a) The bright field image and (b) fluorescence image under RFP filter of regenerated wild-type cowpea IT86D-1010. The arrow indicates the regenerated roots.

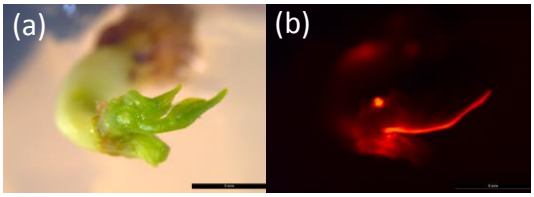

Figure S6 Development of chimeric event using *CTP-NPTII*/G418 selection system. (a) Bright field image. (b) Fluorescence image under RFP filter.

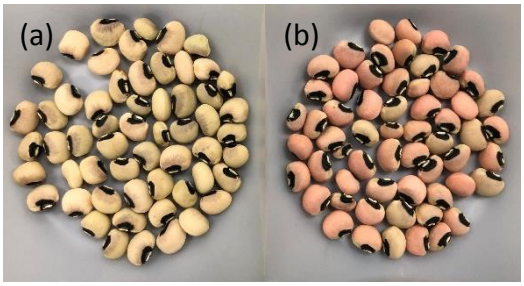

Figure S7 Transgene segregation in the progeny. (a) Mature wild-type cowpea IT86D-1010 seeds. (b) Segregated T1 seeds (Event ID 125739950) in IT86D-1010 background harvested from T0 plant containing the *proGM-EF1A2:Ds-RED* as visual marker.

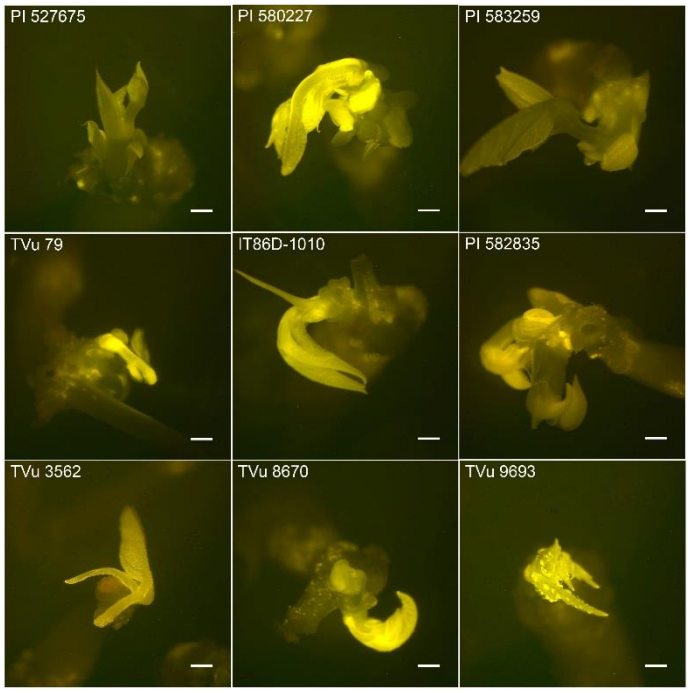

Figure S8 Formation of transgenic shoots expressing *TdTomato* on the EA explants of nine cowpea germplasm lines after 14-d culture on SIM, bar = 1 mm.

Table S1 Master plate medium

Glucose	5 g/l
Bacto agar	15 g/l
Ferrous sulfate heptahydrate	2.5 mg/l
Potassium phosphate dibasic	3 g/l
Sodium phosphate, monobasic	1 g/l
Ammonium chloride	1 g/l
Magnesium sulfate heptahydrate	0.3 g/l
Potassium chloride	0.15 g/l
Calcium chloride dihydrate	11.4 mg/l
Thymidine (only for LBA4404 THY- strain)	50 mg/l
Antibiotics*	

*****100 mg/l kanamycin, 100 mg/l carbenicillin and 25 mg/l rifampicin were used for AGL1 carrying RC2717.
50mg/l gentamycin and 50 mg/l kanamycin were used for LBA4404 Thy- carrying pPHP86170/pPHP71539.
50mg/l gentamycin and 50 mg/l spectinomycin were used for LBA4404 Thy- carrying pPHP94518/pPHP71539 and pPHP92782/ pPHP71539.

Table S2 Working plate medium

Yeast extract (BD DIFCO)	5 g/l
Peptone	10 g/l
Sodium chloride	5 g/l
Bacto agar	15 g/l
Thymidine (only for LBA4404 THY- strain)	50 mg/l
Antibiotics*	

*****100 mg/l kanamycin, 100 mg/l carbenicillin and 25 mg/l rifampicin were used for AGL1 carrying RC2717.
50mg/l gentamycin and 50 mg/l kanamycin were used for LBA4404 Thy- carrying pPHP86170/pPHP71539.
50mg/l gentamycin and 50 mg/l spectinomycin were used for LBA4404 Thy- carrying pPHP94518/pPHP71539 and pPHP92782/ pPHP71539.

Table S3 Infection medium (IM)

MS salt	1 x
MS vitamins	1 x
MES	20 Mm (3.9 g/l)
Sucrose (w/v)	30 g/l
pH	5.4
BAP	0.5 mg/l
Kinetin	0.5 mg/l
GA3	0.25 mg/l
Acetosyringone	Add fresh
L-cysteine	400 mg/l
BCDA (bathocuproinedisulfonic acid disodium salt) (filter sterilized stock)	800 µL stock
Thymidine (only for LBA4404 THY-)	50 mg/l
Polyvinylpyrrolidone (PVP40)	1 mg/l
Acetosyringone (stock 1M; final 200 µM)	0.2 mL
Dithiothrietol (DTT, stock 1M, filter sterilized aliquot and stored at -80°c; final 1Mm)	1 mL

Table S4 Bean germination medium (BGM)

Sucrose	25 g/l
Thiamine Hydrochloride	1.34 mg/l
Nicotinic acid	0.5 mg/l
Pyridoxine Hydrochloride	0.82 mg/l
EDTA disodium	3.348 mg/l

Ferrous sulfate heptahydrate	2.502 mg/l
Boric acid	1.86 mg/l
Manganese sulfate, monohydrate	5.07 mg/l
Zinc sulfate, heptahydrate	2.58 mg/l
Potassium iodide	0.249 mg/l
Sodium molybdate dihydrate	0.216 mg/l
Cupric sulfate pentahydrate	0.00075 mg/l
Cobalt chloride hexahydrate	0.00075 mg/l
Calcium chloride dihydrate	0.176 g/l
Potassium nitrate	0.505 g/l
Ammonium nitrate	0.24 g/l
Potassium phosphate monobasic anhydrous	0.027 g/l
Magnesium sulfate heptahydrate	0.493 g/l
TC agar (phytotechnology A296)	6 g/l

Table S5 Shoot induction medium (SIM)

MS salt	1 x
MS vitamins	1 x
MES	3 Mm (0.59 g/l)
Sucrose (w/v)	30 g/l
Agar, DIFCO (w/v)	8 g/l
pH	5.6
BAP	0.5 mg/l
Kinetin	0.5 mg/l
Antibiotic selection*	
Polyvinylpyrrolidone	1 mg/l
Silver nitrate	2 mg/l

*25 mg/l spectinomycin and 15 mg/l meropenem was used for EA explants which transformation was carried out through LBA4404 Thy- strain-mediated transformation.
50 mg/l spectinomycin and 15 mg/l meropenem was used for EA explants which transformation was carried out through AGL1 strain-mediated transformation for the first 2 weeks culture and 50 mg/l spectinomycin and 30 mg/l meropenem after the first 2 weeks culture.

Table S6 Rooting induction medium (RIM)

MS salt	1 x
MS vitamins	1 x
MES	3 Mm (0.59 g/l)
Sucrose (w/v)	30 g/l
Agar, DIFCO (w/v)	8 g/l
pH	5.6
IBA	0.1 mg/l
Antibiotic selection*	
Polyvinylpyrrolidone	1 mg/l
Sliver nitrate	2 mg/l

*50 mg/l spectinomycin and 30 mg/l meropenem used for transgenic shoots which transformation were carried out through AGL1 strain-mediated transformation after the first 2 weeks culture.

Table S7 Shoot elongation medium (SEM)

MS salts	1 x
MS vitamins	1 x
MES	3 mM (0.59 g/l)
Sucrose (w/v)	30 g/l

Agar (w/v)	8 g/l
pH	5.6
GA	0.5 mg/l
Kinetin	0.1 mg/l
Asparagine	50 mg/l
Antibiotic selection*	

*25 mg/l spectinomycin and 15 mg/l meropenem used for transgenic shoots which transformation was carried through LBA4404 Thy- strain-mediated transformation.

50 mg/l spectinomycin and 30 mg/l meropenem used for transgenic shoots which transformation was carried out by AGL1 strain-mediated transformation.

Table S8 0MS

MS salts	1 x
MS vitamins	1 x
MES	3 Mm (0.59 g/l)
Sucrose (w/v)	30 g/l
Agar, DIFCO (w/v)	8 g/l
pH	5.6

Table S9 Primers used for event quality assay

Event quality assay	Assay type	Forward primer	Reverse primer
LBS	Endogenous control	CACATACCTCCAGTGAGTTCCCTTA	TCGAAGCATCTACTAACTACAGAAGAATTAA
Ds-RED	Copy number	AAGTCCATCTACATGGCCAAGAA	TGGGAGGTGATGTCCAGCTT
SPCN	Copy number	CTGCCCGCAATGCTCTTT	ATTACCACTGGACCGTCACAGA
CTP	Copy number	TGGCTGCAACTACTCTTACATCTG	TGTAAGTTGAAAGGAGCACTTGGT
UBQ14_TERM	Copy number	CAGAACCCAGAATCCCTTCATATC	TGACGGCTGGGACTTCTTTG
HYGROMYCIN	Vector backbone	CAGCGAGAGCCTGACCTATTG	CAGCGAGAGCCTGACCTATTG
VIRG	Vector backbone	TGCTCCGAGACGGTCGAT	CAGGCAGGTCTTGCAACGT
SPC	Vector backbone	GCGCTGCCATTCTCCAAAT	ATCATTCCGTGGCGTTATCC
LEFTBORDER	Vector backbone	GATCTCGCGGAGGGTAGCA	CGAGGGAGATGATATTTGATCACA
NPTIII	Vector backbone	CCGATGTGGATTGCGAAAA	GCTCGCGCGGATCTTTAA