Plant Communications, Volume *5*

Supplemental information

Enhancing wheat regeneration and genetic transformation through overexpression of *TaLAX1*

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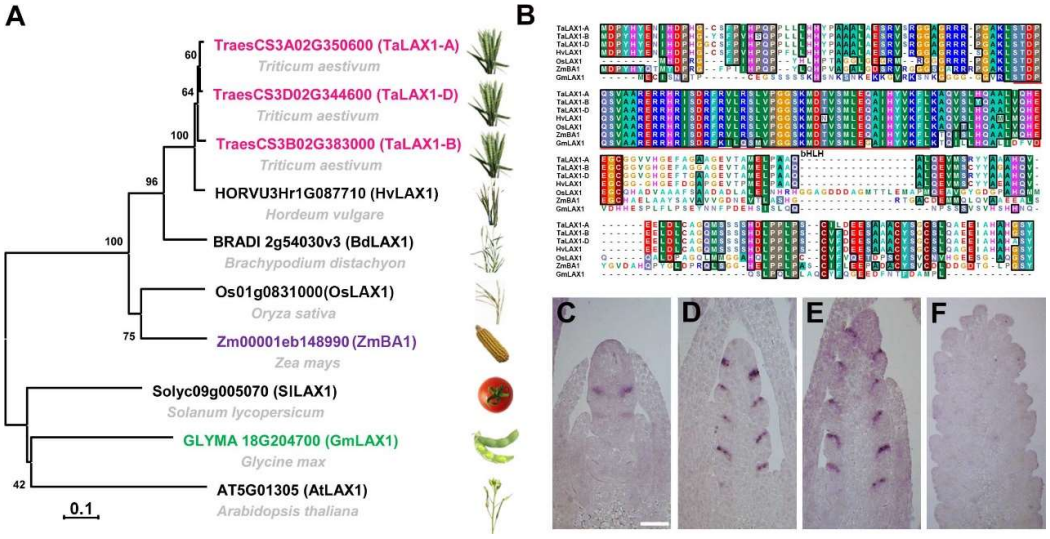
Supplemental Figures and legends

Supplemental Figure 1



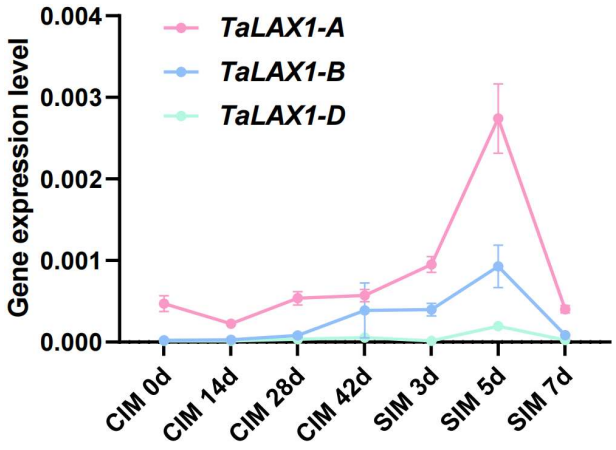
Supplemental Figure 1. Phylogenetic relationships among LAX1 homologous proteins and the expression pattern of *TaLAX1* in the development of wheat spikes. (Supports Figures 1 and 7)

(A) Phylogenetic trees of LAX1 homologous proteins for wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), Brachypodium (*Brachypodium distachyon*), rice (*Oryza sativa*), maize (*Zea mays*), tomato (*Solanum lycopersicum*), soybean (*Glycine max*) and Arabidopsis (*Arabidopsis thaliana*). The evolutionary analysis was conducted based on OsLAX1 protein sequences in MEGA 11 using the neighbor-joining method.

(B) Comparison of the predicted amino acid sequences of TaLAX1-A/B/D, HvLAX1, OsLAX1, ZmBA1, and GmLAX1 proteins. The basic helix-loop-helix (bHLH) domain is indicated by a red horizontal line.

(C-F) RNA *in situ* hybridizations analyses indicate the expression pattern of *TaLAX1* at the single ridge stage **(C)**, double ridge stage **(D)**, glume primordium differentiation stage **(E)** and floret differentiation stage **(F)** of the Chinese Spring wheat spikes. An antisense probe derived from the whole coding region of *TaLAX1-A* was used. Scale bar = 200 μm.

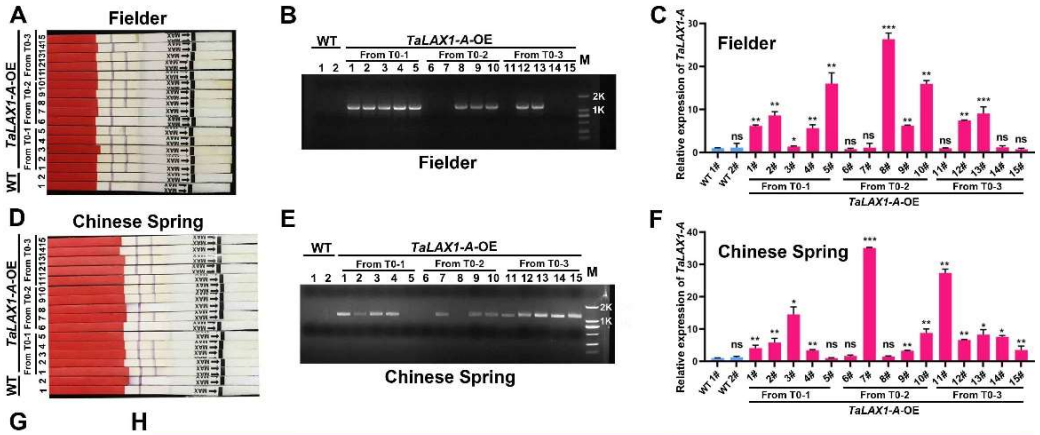
Supplemental Figure 2



Supplemental Figure 2. Expression level of *TaLAX1-A*/*B*/*D* in the regeneration process of Chinese Spring. (Supports Figure 1)

Values are means ± SD, calculated from three individual experiments.

Supplemental Figure 3



G

H

Fielder	Effective tiller number	Spike length (cm)	Grain number per spike	Thousand grain weight (g)	Grain yield per plant (g)	Plant height (cm)
Control	14.59±1.25	10.10±0.15	60.06±2.24	34.65±0.88	21.84±1.64	74.85±1.20
TaLAX1-A-OE	20.40±1.57	11.24±0.13	63.17±1.47	27.94±0.51	27.64±3.09	78.68±1.29
P-value	0.0064	<0.0001	0.2462	0.0009	0.0737	0.0436

I

J

Chinese Spring	Effective tiller number	Spike length (cm)	Grain number per spike	Thousand grain weight (g)	Grain yield per plant (g)	Plant height (cm)
Control	31.74±3.39	8.32±0.10	47.56±2.15	30.80±0.36	41.13±4.62	112.10±0.89
TaLAX1-A-OE	42.13±3.75	11.68±0.13	47.74±2.28	26.65±0.64	51.04±8.49	116.28±1.33
P-value	0.0476	<0.0001	0.9569	0.0026	0.2799	0.0200

Supplemental Figure 3. T1 progeny of *TaLAX1-A*-OE transgenic lines in Fielder and Chinese Spring. (Supports Figure 3)

(A-C) Identification of putative T1 progeny of *TaLAX1-A*-OE transgenic lines from 3 T0 lines in Fielder by QuickStix strips for the Bar protein **(A)**, amplification of transgenic-specific PCR product **(B)** and RT-qPCR analysis **(C)**. 2K: 2000 bp, 1K: 1000 bp, M: DNA marker.

(D-F) Identification of putative T1 progeny of *TaLAX1-A*-OE transgenic lines from 3 T0 lines in Chinese Spring by QuickStix strips for the Bar protein **(D)**, amplification of transgenic-specific PCR product **(E)** and RT-qPCR analysis **(F)**. 2K: 2000 bp, 1K: 1000 bp, M: DNA marker.

(G) Spike phenotypes the T1 progeny of non-transgenic lines (control) or *TaLAX1-A*-OE transgenic lines in Fielder. Scale bar = 1 cm.

(H) Main agronomic characters of the T1 progeny of control or *TaLAX1-A*-OE transgenic lines in Fielder.

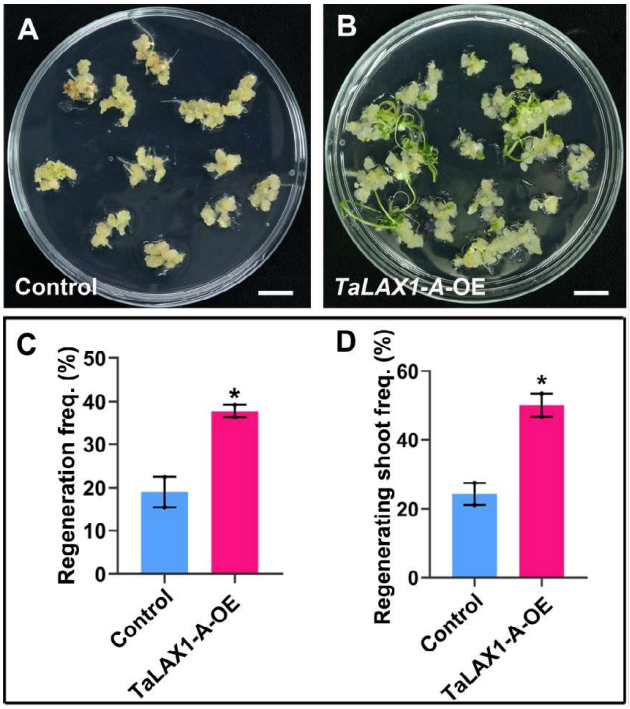
(I) Spike phenotypes of the T1 progeny of control or *TaLAX1-A*-OE transgenic lines in Chinese Spring. Scale bar = 1 cm.

(J) Main agronomic characters of the T1 progeny of control or *TaLAX1-A*-OE transgenic lines in Chinese Spring.

Values in **(C**, **F)** are means ± SD, values in **(H**, **J)** are means ± SEM. All experiments

48 in **(C**, **F)** were performed at least three times. The data in **(H**, **J)** presents a count of the
49 main agronomic characteristics for 15 positive transgenic lines of the T1 generation in
50 Fielder and 16 positive transgenic lines of the T1 generation in Chinese Spring,
51 respectively. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant (Student's *t*-test,
52 two-tailed).

Supplemental Figure 4



Supplemental Figure 4. Overexpression of *TaLAX1-A* promotes shoot regeneration after particle bombardment. (Supports Figures 1, 2 and 3)

(A) Shoot regeneration phenotypes of Fielder immature embryos infected with empty vector (control) by particle bombardment. Scale bar = 1 cm.

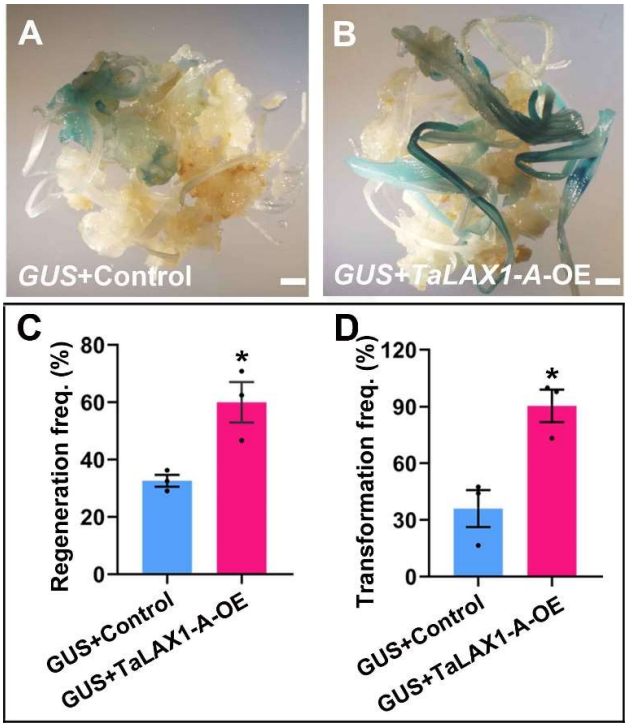
(B) Shoot regeneration phenotypes of Fielder immature embryos infected with *TaLAX1-A*-OE by particle bombardment. Scale bar = 1 cm.

(C) Regeneration frequencies of Fielder immature embryos infected with control or *TaLAX1-A*-OE vector by particle bombardment.

(D) Regenerating shoot frequencies of Fielder immature embryos infected with control or *TaLAX1-A*-OE vector by particle bombardment.

Values in **(C**, **D)** are means ± SEM from two independent experiments. Black points are the results from individual experiments. * $P < 0.05$ (Student's *t*-test, two-tailed).

Supplemental Figure 5



Supplemental Figure 5. Overexpression of *TaLAX1-A* promotes *GUS* transformation by two *Agrobacterium*-mediated co-transformation methods. (Supports Figure 4)

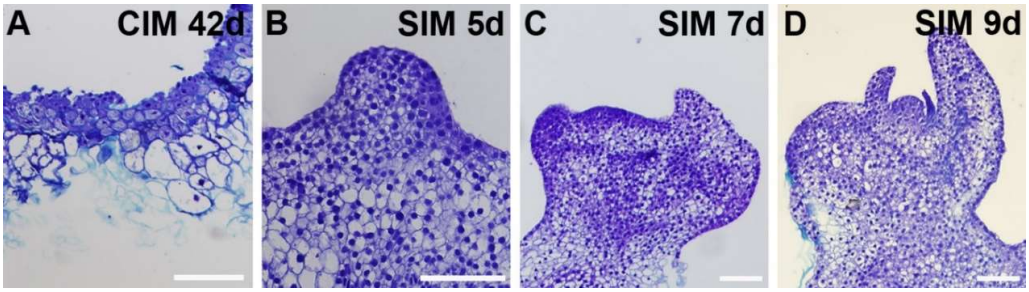
(A) and **(B)** show the shoot regeneration and transformation phenotypes of Fielder immature embryos infected with a mixture of *Agrobacterium* strains. *Agrobacterium* suspensions containing *GUS* and empty vector (control) were mixed together in a 1:1 ratio **(A)**, *GUS* and *TaLAX1-A*-OE were mixed together in a 1:1 ratio **(B)**. Scale bar = 2mm.

(C) Regeneration frequencies of Fielder immature embryos infected with mixtures of different Agrobacterium strains.

(D) Transformation frequencies of Fielder immature embryos infected with mixtures of different Agrobacterium strains.

Values in **(C**, **D)** are means ± SEM from three independent experiments. Black points are the results from individual experiments. * $P < 0.05$ (Student's *t*-test, two-tailed).

Supplemental Figure 6

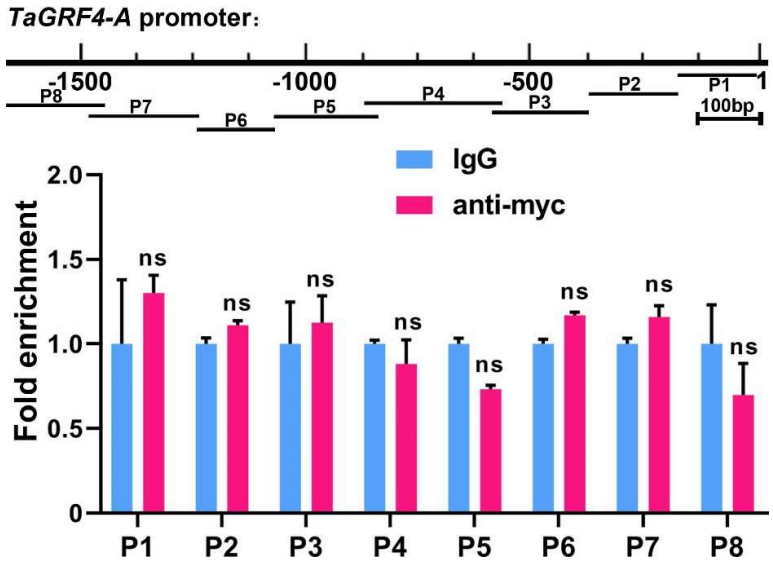


Supplemental Figure 6. Paraffin sections of the shoot regeneration process of Chinese Spring. (Supports Figure 5)

(A) The immature embryos of Chinese Spring were cultured on CIM for 42 days. Scale bar = 200 µm.

(B-D) The immature embryos of Chinese Spring were cultured on CIM for 42 days, followed by incubation on SIM for 5 days **(B)**, 7 days **(C)** or 9 days **(D)**. Scale bar = 200 µm.

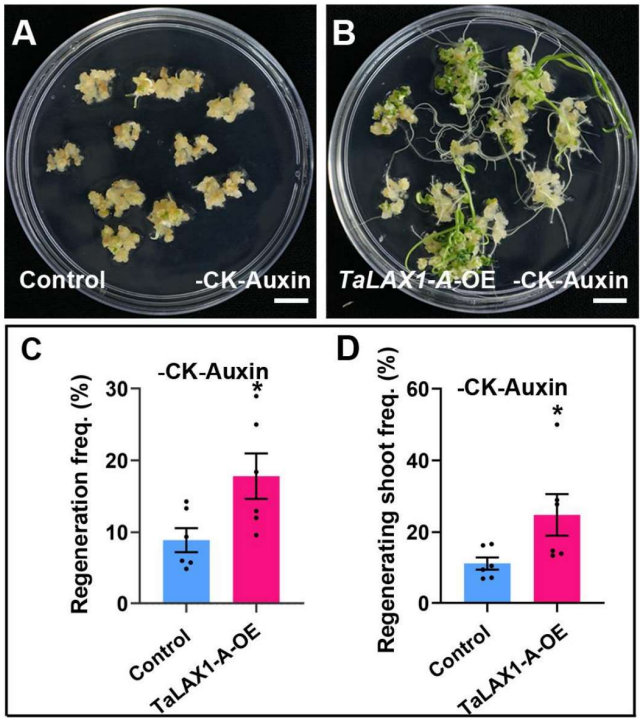
Supplemental Figure 7



Supplemental Figure 7. TaLAX1-A does not bind to the *TaGRF4-A* promoter. (Supports Figure 5)

ChIP-qPCR of the *TaGRF4-A* promoter using an anti-*myc* antibody in the *TaLAX1-A*-OE transgenic plants, negative controls used IgG antibody on *TaLAX1-A*-OE samples. There was no enrichment of fragments. Values are means ± SD from three independent experiments. ns, not significant (Student's *t*-test, two-tailed).

Supplemental Figure 8



Supplemental Figure 8. Effect of *TaLAX1-A* overexpression on shoot regeneration in the absence of exogenous cytokinin and auxin. (Supports Figure 6)

(A) Shoot regeneration phenotypes of Fielder immature embryos cultured on CIM (without exogenous auxin) for 42 days and on SIM (without exogenous cytokinin) for 20 days after infection with empty vector (control). Scale bar = 1 cm.

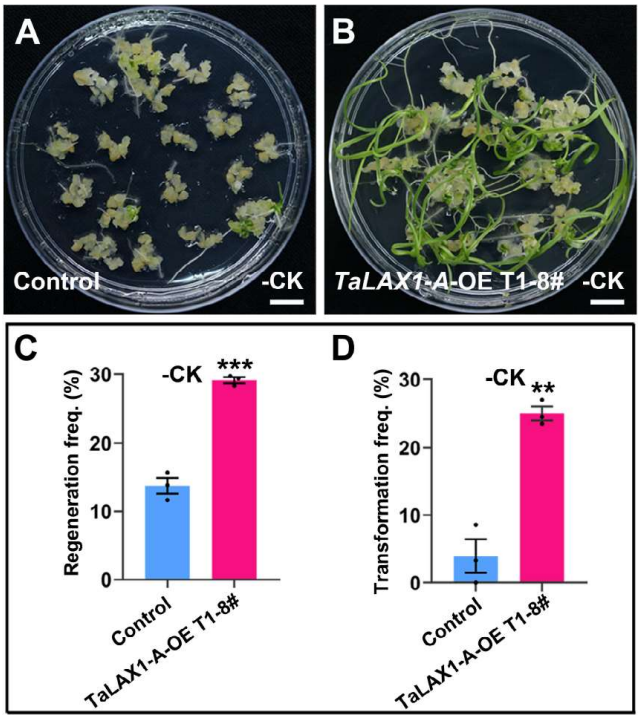
(B) Shoot regeneration phenotypes of Fielder immature embryos cultured on CIM (without exogenous auxin) for 42 days and on SIM (without exogenous cytokinin) for 20 days after infection to introduce the *TaLAX1-A*-OE vector. Scale bar = 1 cm.

(C) Regeneration frequencies of Fielder immature embryos cultured on CIM (without exogenous auxin) for 42 days and on SIM (without exogenous cytokinin) for 20 days after infection to introduce the control or *TaLAX1-A*-OE vector.

(D) Regenerating shoot frequencies of Fielder immature embryos cultured on CIM (without exogenous auxin) for 42 days and on SIM (without exogenous cytokinin) for 20 days after infection to introduce the control or *TaLAX1-A*-OE vector.

Values in **(C**, **D)** are means ± SEM from six independent experiments. Black points indicate results from individual experiments. * $P < 0.05$ (Student's *t*-test, two-tailed).

Supplemental Figure 9



Supplemental Figure 9. Regeneration and transformation of T1 progeny of *TaLAX1-A*-OE transgenic lines in the absence of exogenous cytokinin. (Supports Figure 6)

(A) Fielder immature embryos from T1 progeny of non-transgenic lines (control) were cultured on CIM for 42 days and on SIM (without exogenous cytokinin) for 20 days after infection with the Ubi_{pro}*:GUS* vector. Scale bar = 1 cm.

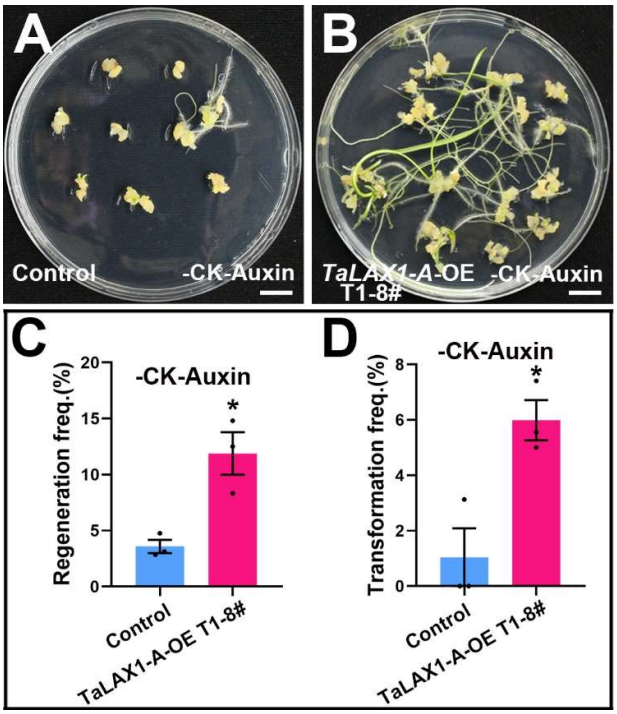
(B) Fielder immature embryos from T1 progeny of *TaLAX1-A*-OE lines were cultured on CIM for 42 days and on SIM (without exogenous cytokinin) for 20 days after infection with the Ubi_{pro}*:GUS* vector. Scale bar = 1 cm.

(C) Regeneration frequencies of immature embryos of control or *TaLAX1-A*-OE transgenic lines infected with the Ubi_{pro}*:GUS* vector in the absence of cytokinin.

(D) Transformation frequencies of immature embryos of control or *TaLAX1-A*-OE transgenic lines infected with the Ubi_{pro}*:GUS* vector in the absence of cytokinin.

Values in **(C**, **D)** are means ± SEM from three independent experiments. Black points are the results from individual experiments. *** $P < 0.001$; ** $P < 0.01$ (Student's *t*-test, two-tailed).

Supplemental Figure 10



Supplemental Figure 10. Regeneration and transformation of T1 progeny of *TaLAX1-A*-OE transgenic lines in the absence of exogenous cytokinin and auxin. (Supports Figure 6)

(A) Fielder immature embryos from T1 progeny of non-transgenic lines (control) were cultured on CIM (without exogenous auxin) for 42 days and on SIM (without exogenous cytokinin) for 20 days after infection with the *Ubi$_{pro}$:GUS* vector. Scale bar = 1 cm.

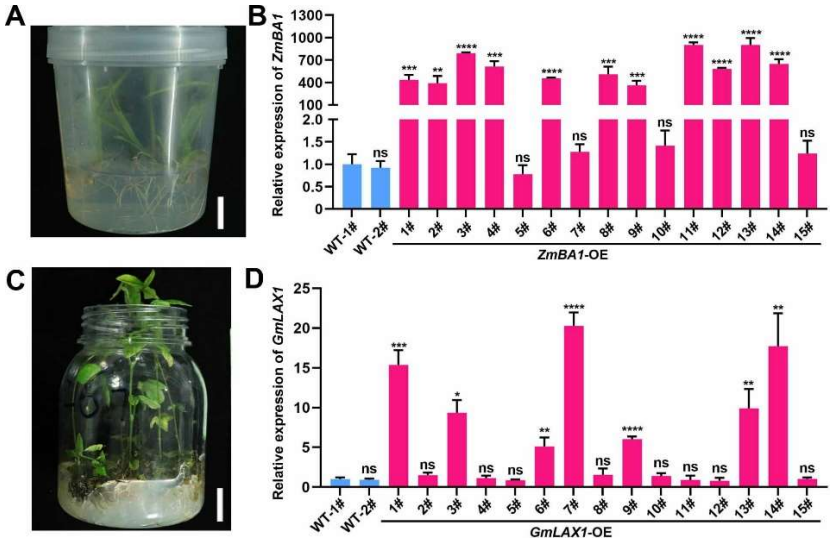
(B) Fielder immature embryos from T1 progeny of *TaLAX1-A*-OE lines were cultured on CIM (without exogenous auxin) for 42 days and on SIM (without exogenous cytokinin) for 20 days after infection with the *Ubi$_{pro}$:GUS* vector. Scale bar = 1 cm.

(C) Regeneration frequencies of immature embryos of control or *TaLAX1-A*-OE transgenic lines infected with the *Ubi$_{pro}$:GUS* vector in the absence of cytokinin and auxin.

(D) Transformation frequencies of immature embryos of control or *TaLAX1-A*-OE transgenic lines infected with the *Ubi$_{pro}$:GUS* vector in the absence of cytokinin and auxin .

Values in **(C**, **D)** are means ± SEM from three independent experiments. Black points are the results from individual experiments. * $P < 0.05$ (Student's *t*-test, two-tailed).

Supplemental Figure 11



Supplemental Figure 11. Identification of putative T0 progeny of *ZmBA1*-OE or *GmLAX1*-OE transgenic lines. (Supports Figure 7)

(A) Plants obtained from regenerated shoots induced by *ZmBA1*-OE on a rooting medium for root elongation. Scale bar = 1 cm.

(B) Identification of putative T0 progeny of *ZmBA1*-OE transgenic lines by RT-qPCR analysis.

(C) Plants obtained from regenerated shoots induced by *GmLAX1*-OE on a rooting medium for root elongation. Scale bar = 2 cm.

(D) Identification of putative T0 progeny of *GmLAX1*-OE transgenic lines by RT-qPCR analysis.

Values in **(B, D)** are means ± SD, all experiments were performed at least three times. **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant (Student's *t*-test, two-tailed).