High efficiency gene transfer to wheat mediated by *Agrobacterium tumefaciens* and particle bombardment

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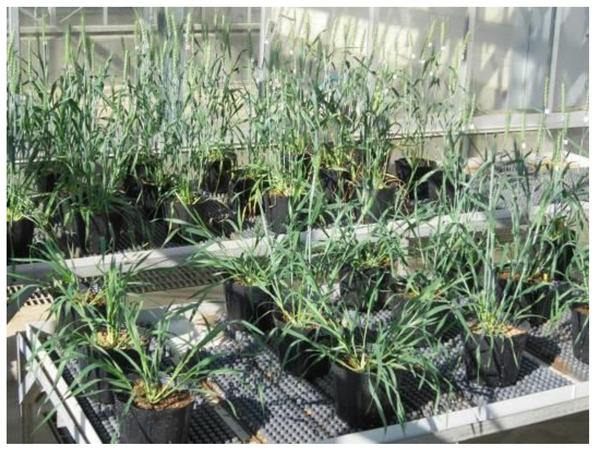
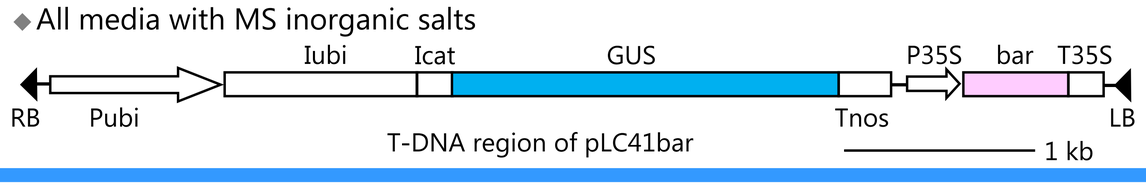
Key message

- Gene transfer methods for immature embryos were optimized further. Diverse cultivars are now transformed efficiently mediated by *Agrobacterium tumefaciens*, and gene transfer by particle bombardment is also an efficient process.

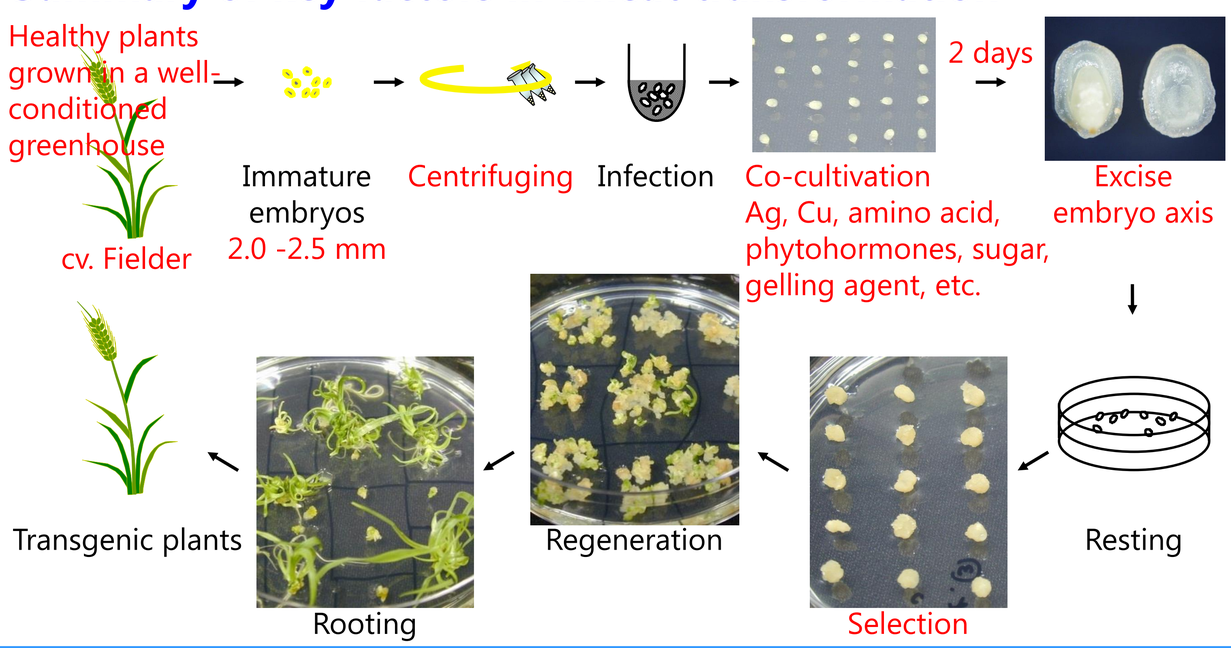
Materials

- Plant
  - Spring wheat cv. Fielder, Bobwhite
  - Immature embryos 14 - 16 days after anthesis
- *Agrobacterium* strains and vectors
  - EHA101, EHA105, LBA4404
  - pIG121Hm, pIG121Ubi-bar, pLC41Hm, pLC41bar
  - Selection marker gene: bar, hpt
  - Reporter gene: gus
- Media
  - All media with MS inorganic salts





Summary of key factors in wheat transformation



Size of immature embryo

- The developmental stage of embryo is critical, and days to reach the right stage vary seasonally.
- The size is a good indicator.
- Optimal
  - Rice 1.3 - 1.8 mm
  - Maize 1.2 - 1.5 mm
  - Wheat 2.0 - 2.5 mm

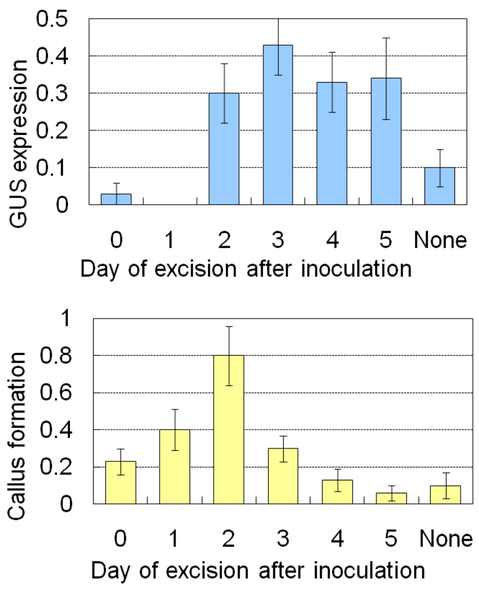
Plant	Genotype	Size of immature embryo (mm)	Independent events/piece
Rice	Japonica	< 1.3	Difficult to isolate
		1.3 - 1.8	15.0
		1.8 <	Difficult to isolate
Maize	A188	< 1.2	Difficult to handle
		1.2 - 1.5	0.50
		1.5 - 2.0	0.10
		2.0 - 2.5	0.01
Wheat	Fielder	< 1.5	0.0
		1.5 - 2.0	0.2
		2.0 - 2.5	0.7
		2.5 - 3.0	0.5

1.0 - 1.4 mm 1.5 - 2.0 mm > 2.0 mm

Excision of embryo axis

- Wheat
  - Excision 2 days after inoculation caused very high GUS expression and vigorous callus growth.
  - Excision of embryo axis early or late caused poor responses.
- Other cereals
  - This step is not necessary
  - Elongation of shoot from embryo was severely suppressed by centrifugation in rice and maize.

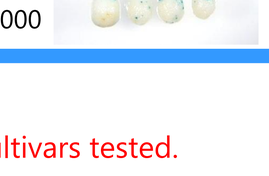
Excised before inoculation Excised 2 days after inoculation



Centrifuging before infection

- Effective range
  - 800 - 20,000 x g
  - 1 second - 2 hours
- Centrifuging elevated transient expression and frequency of transformation in cereals.
- Heating before infection is also effective in other cereals but not in wheat.

Centrifuged	Immature embryos inoculated (A)	Independent transgenic plants (B)	Transformation frequency (B/A, %)
-	22	2	9.1
+	22	11	50.0



Transformation of other cultivars

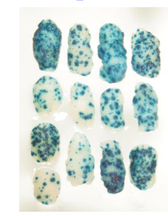
- Transgenic plants were obtained in all of the nine cultivars tested.
- Six cultivars were higher than 10% in the frequency of transformation.

Frequency of transformation in various cultivars

Cultivar	Type	Exp	Immature embryos inoculated (A)	Independent transgenic plants (B)	Transformation frequency (B/A, %)
Florida	winter	1	25	6	24.0
		2	28	6	21.4
Veery	spring	1	16	5	31.3
		2	14	1	7.1
Pavo 76	spring	1	18	1	5.6
Chinese Spring	spring	1	24	2	8.3
		2	18	1	5.6
Canon	spring	1	25	3	12.0
		2	32	3	9.4
Imp	spring	1	24	13	54.2
		2	8	2	25.0
Cadenza	facultative	1	30	3	10.0
		2	20	2	10.0
Turbo	spring	1	28	4	14.3
		2	23	2	8.7
Courtot	spring	1	34	2	5.9

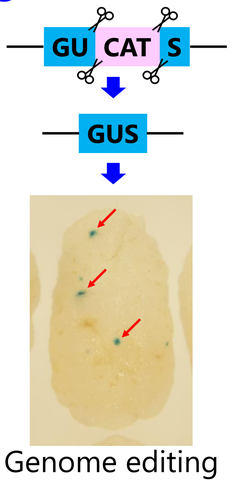
Particle bombardment is also an efficient process

- Key factors for a method of gene transfer by particle bombardment and the following regeneration were essentially the same.
- The frequency of transformation showed 10% in Fielder.
- Gene editing tools were tested successfully. For example, a disrupted GUS gene was repaired in cells in wheat immature embryos by co-bombarded Cas9 and expressed transiently.



Transient expression

Stable transformation



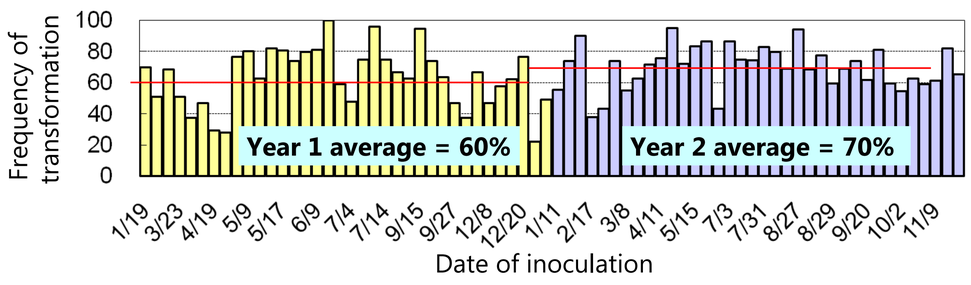
Genome editing

Results were well reproducible

- cv. Fielder was transformed very efficiently.
- No apparent seasonal difference
- The protocol has proven good at a number of laboratories in the world.

Typical wheat transformation experiments

Vector	Exp	Immature embryos inoculated (A)	Independent transgenic plants (B)	Transformation frequency (B/A, %)
pIG121 Ubi-bar	1	30	13	43.3
	2	27	14	51.9
	3	28	20	71.4
pLC41 bar	4	46	34	73.9
	5	90	67	74.4
	6	40	38	95.0
pLC41 Hm	7	12	7	58.3
	8	17	14	82.4
	9	35	24	68.4



Conclusions

- Key factors in wheat transformation mediated by *Agrobacterium* were optimized.
  - Similar to other cereals, but the details were quite different.
  - Experience in rice and maize was very useful.
- Highly efficient, typically 70%, wheat transformation protocol was developed.
- The protocol is durable and highly reproducible for routine use.
- Diverse cultivars may be transformed by the protocol.
- Key factors for gene transfer by particle bombardment were essentially the same.

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