

Supplementary Table 1. Comparison among different methods for functional validation of plant genes

Method	Main application	Site-directed mutation	Sequence -dependent	Transformation -needed	Main mutation types	Mutation frequency in plants			Obvious problems	Advantages
						Mutation rate	Plant species	Referen		
Physical mutagenesis	F	No	No	No	Small deletion	0.83-2.66%	<i>Lablab purpureus</i>	[1]	High number of background mutations; Chromosomal damage; Treatments may be dangerous; Inhibition of	Desirable for large deletions and rearrangements
						2.7-4.8%	Chilli	[2]		
						~8%	Barley	[3]		
						~6.43%	Maize	[4]		
						2.34-14.4%	Soybean	[5]		
						1.8-3.75	Soybean	[6]		
Chemical mutagenesis	F	No	No	No	Point muation	0.96-2.57%	<i>Lablab purpureus</i>	[1]	High number of background mutations	Mainly point mutations occur; Lower morality compared with physical mutagenesis
						1.76-2.97%	Chilli	[2]		
						0.01%-0.18%	Rice	[5]		
						~0.47%	Maize	[6]		
						1-5%	Soybean	[7]		
						2.24-22.85%	Soybean	[8]		
TILLING for chemical and physical mutagenesis	R	No	No	No	Point mutation	0.86-5.42%	Soybean	[5]	The same to chemical or physical mutagenesis	High-throughput detection of mutations; Free of transformation;
						1/60kb	<i>Brassica rapa</i>	[9]		
						1/931kb	Peanut	[10]		
						1/526kb	<i>Sorghum bicolor</i>	[9]		
						1/51kb	<i>Arabidopsis</i>	[11]		
						1/451kb	Rice	[12]		
						1/135kb	Rice	[13]		
						1/500kb	Barley	[14]		
						1/485kb	Maize	[15]		
						1/41kb	Wheat	[16]		
Insertional mutagenesis	F	No	No	Yes	Insertion	1/140kb	Soybean	[17]	Tandem T-DNA inserts are common	Easy to detect mutation sites
						1.29 per line	<i>Arabidopsis</i>	[18]		
						~1.5 per line	<i>Arabidopsis</i>	[19]		
						1.4 per line	Rice	[20]		
						~2 per line	Rice	[21]		
						1-2 per line	Rice	[22]		
						>1 per line	Tobacco	[23]		
						2.7-28.3 per line	Maize	[24]		

RNAi	R	No	Yes	Yes	—	78%	Wheat	[25]	Off-target; Incomplete gene silencing	Can be used for silencing multigene families and homologous genes; Available for functional validation of lethal genes
						33-100%	Wheat	[26]		
						10.5-82%	<i>Arabidopsis</i>	[27]		
						66.70%	Maize	[28]		
						78-90%	Maize	[29]		
						5-60%	Potato	[26]		
						48-87%	Potato	[30]		
						53-62%	Cotton	[26]		
						60-89%	Cotton	[29]		
Over-expression	R	No	Yes	Yes	Gene addition	50%	Rice	[31]	Random insertion of target gene in genome; Unexpected target gene silencing	Without interference from functionally redundant genes
						83.30%	<i>Arabidopsis</i>	[32]		
						50%	Maize	[33]		
						100%	Maize	[34]		
						100%	Wheat	[35]		
						33%	Barley	[36]		
						100%	Tobacco	[37]		
ZFNs	R	Yes	Yes	Yes	Insertion, deletion, replacement	0.10%	<i>Arabidopsis</i>	[38]	Relatively expensive and finicky to make, compared to CRISPR/Cas	Targeted gene editing; Low chance of off-target cleavage than CRSPR/Cas
						7%, 16%	<i>Arabidopsis</i>	[39]		
						0.2-4.0%	Tobacco	[38]		
						66.70%	Soybean	[40]		
						20.80%	Maize	[41]		
TALENs	R	Yes	Yes	Yes	Insertion, deletion, replacement	~10%	Tobacco	[42]	Relatively expensive and finicky to make, compared to CRISPR/Cas	Targeted gene editing; Low chance of off-target cleavage than CRSPR/Cas
						3.4-6.0%	Wheat	[43]		
						39.10%	Maize	[44]		
						10.00%	Maize	[45]		
						40%	Rice	[46]		
						2-14%	<i>Arabidopsis</i>	[47]		
CRISPR/Cas	R	Yes	Yes	Yes	Insertion, deletion, replacement	22%	Barley	[48]	Higher chance of off-target cleavage, compared to ZFNs and TALEN	Targeted gene editing; A single RNA is required for targeted specificity; Easy and quickly; Higher efficiency than ZFNs and
						30-84%	<i>Arabidopsis</i>	[49]		
						76-89%	<i>Arabidopsis</i>	[50]		
						81.8-87.5%	Tobacco	[51]		
						6.70%	Tobacco	[52]		
						50%	Rice	[50]		
						83-91.6%	Rice	[53]		
						4-9.4%	Rice	[54]		

4.8-75%	Rice	[49]	TALEN; Can be used
5.60%	Wheat	[43]	for genome-wide
48.00%	Tomato	[55]	targeting

Note. Mutation rate for physical and chemical mutagenesis, the percentage of mutants in total plants mutagenized

Mutation rate for insertional mutagenesis, number of the insertions in each line

Mutation rate for TILLING, the average length of DNA fragment containing 1 mutation

Mutation rate for over-expression, the percentage of target gene-overexpressed transgenic plants in total transgenic plants

Mutation rate for RNAi, the percentage of target gene-suppressed or down-regulated transgenic plants in total transgenic plants

Mutation rate for ZFNs, TALEN and CRISPR/Cas, the percentage of target sequence-edited transgenic plants in total transgenic plants

F and R represent forward genetics and reverse genetics, respectively

Supplementary references

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