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Supplementary Materials

Hairy CRISPR: Genome Editing in Plants Using Hairy Root Transformation

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Table S1. List of Agrobacterium strains and CRISPR/Cas vector components from studies using hairy root transformation

Plant species	Reference	Year	Agrobacterium strain	Vector name or type of cloning system	Promoters for gRNA expression	Promoters for Cas9 expression	Cas9 type	Gene chosen for genome editing (name or ID)	Marker of transgenic hairy roots
Arachis hypogaea **(peanut)**	[1]	2019	K599	p201B/Cas9 pUC-gRNA [2]	pMtU6.6	2xp35S	hCas9	*ahFAD2a*	Bar
	[3]	2020		p201G/Cas9 pUC-gRNA [2]				*NFR1A1*; *A2*; *B1*; *B2*; *NFR5A*; *B*	EGFP
Atropa belladonna **(belladonna)**	[4]	2021	ATCC15834	pMgP237-2A-GFP [5]	pAtU6-26	2xp35SΩ	AtCas9 fused with GFP via P2A peptide	*pyrrolidine ketide synthase* (*PYKS*)	GFP; **Km**^{PC}
Brassica carinata **(Abyssinian mustard)**	[6]	2017	C58 (pRiARqua1)*	pB-CRISPR+35S:: GFP (V112)	pAtU6-26	pPcUbi4-2	hCas9 with translational enhancers from the Cowpea Mosaic Virus 5′ and 3′ UTRs	*FASCICLIN-LIKE ARABINOGALACTAN PROTEIN 1*	**3xGFP**; PPT
Brassica napus **(rapeseed)**	[7]	2020	AR15834	pGWB401 based vectors	pAtU6	p35S	BnCas9	*HVA22 homologue C*; *AGO1*(*miR168*); *TAO1*(*miR1885a*)	Km
Catharanthus roseus **(Madagascar periwinkle)**	[8]	2021	R1000*^{PC}	MoClo system with custom modifications	pAtU6-26	pAtUbi10	izCas9	*Jasmonate-associated MYC2*; *Jasmonate-associated MYC3*; *Repressor of MYC2 targets 1*	Hygr
Cichorium intybus **(chicory)**	[9]	2019	15834	pYLCRISPR/Cas9 P35S-B [10]	pCiU6-1	2xp35S	pCas9	*PDS*	Bar

Plant species	Reference	Year	Agrobacterium strain	Vector name or type of cloning system	Promoters for gRNA expression	Promoters for Cas9 expression	Cas9 type	Gene chosen for genome editing (name or ID)	Marker of transgenic hairy roots
Eucalyptus grandis **(flodded gum)**	[11]	2020	A4RS	GoldenGate cloning system with custom components	pAtU6	2xp35S	hCas9	*Cinnamoyl-CoA reductase1*; *IAA9A*	DsRed
Glycine max **(soybean)**	[12]	2015	K599	custom vector	pAtU6	pZmUbi	dpCas9	*bar* gene in *bar* soybean line; *GmFEI1*; *GmFEI2*; *GmSHR*	GFP
	[2]			p201N/Cas9 pUC-gRNA [2]	pMtU6.6	2xp35S	hCas9	*gfp* gene in *gfp* soybean line; *Glyma07g14530*; *01gDDM1*; *11gDDM1*; *miR1509*; *miR1514*; *Glyma04g36150* (*MET1*); *Glyma06g18790* (*MET1*)	Km
	[13]		ARqua1*	pMDC32/GUS/ GmCas9	pAtU6-26	2xp35S	GmCas9	*glutamine synthase 1*; *chalcone-flavanone isomerase 20*	**GUS**; Hygr
	[14]	2016	K599	pCAMBIA3301 based vectors	pAtU6-26; pGmU6-10	p35S	pCas9	*Glyma06g14180*; *Glyma08g02290*; *Glyma12g37050*	Blp
	[15]			Cas9/sgRNA plasmid construction kit (VIEWSOLID Biotechnology)	pAtU6	pZmUbi	dpCas9	*PDS*	Bar
	[16]			custom modified pHSE401 [17]	pAtU6-26	2xp35S	zCas9	*Glyma.01G165800*; *Glyma.01G165800-D*	**GUS**; Hygr
	[18]	2017		pHSE401 [17]	pAtU6-26	2xp35S	zCas9	*Rg1*	Hygr; **visual detection for nodules formation**

Plant species	Reference	Year	Agrobacterium strain	Vector name or type of cloning system	Promoters for gRNA expression	Promoters for Cas9 expression	Cas9 type	Gene chosen for genome editing (name or ID)	Marker of transgenic hairy roots
***Glycine max* (soybean)**	[19]	2018	K599	pCAMBIA3301 based vectors	pGmU6	p35S	pCas9	*GmMYB118*	Blp
	[20]			custom modified pKSE401 [17]	pAtU6-26; pAtU6-29	2xp35S	zCas9	*Nod Factor Receptor 1a*	**GFP**; Km
	[21]	2019		pYLCRISPR/Cas9 P35s-B [10]	pAtU3b; pAtU3d; pAtU6-26; pAtU6-1	2xp35S	pCas9	*LATE ELONGATED HYPOCOTYL 1a*; *1b*; *2a*; *2b*	Bar
	[22]			pFGC5941	pAtU6-26	2xp35S	hCas9	*GmFAD2-1A*; *GmFAD2-1B*	Bar; **PCR**
	[23]			pZG23C05 (ZGene Biotechnology Inc.)	pAtU6	pUbi	dpCas9	*Glyma.03g163500*; *Glyma.10g037100*; *Glyma.10g246300*; *Glyma.13g123500*; *Glyma.19g164800*; *Glyma.19g164900*; *Glyma.20g146200*; *Glyma.20g148200*; *Glyma.20g148400*	Bar
	[24]			pRI201-AN (TaKaRa Bio)	pGmU6	p35S	GmCas9 with AtADH 5′UTR	*Isoflavonoid syntase*	Bar
	[25]			custom modified pCAMBIA1300 based vectors	pGmU6	pM4	Cas9	74 nodulation genes; 27 seed genes	Bar
	[26]			custom modified pHSE401 [17]	pAtU6-26	2xp35S	zCas9	*Rg1*	AtMYB75/ PAP1
	[27]	2020		HBT-pcoCas9 [28] pBlu-gRNA [13]	pAtU6-26	2xp35S	pCas9	*galactinol synthase* (*GOLS*) genes: *GOLS1A*; *GOLS1B*	Bar
	[29]			pHairyRed [30]	pGmU6	p35S	pCas9	*GmPHR1*; *GmPHR4*	Hygr; **DsRed2**
	[31]			Tri-KO1, Tri-KO2, Tri-KO3	pGmU3; pGmU6	pGmUbi3	dpCas9	*GmF3H1*; *GmF3H2*; *GmFNSII-1*	Bar

Plant species	Reference	Year	Agrobacterium strain	Vector name or type of cloning system	Promoters for gRNA expression	Promoters for Cas9 expression	Cas9 type	Gene chosen for genome editing (name or ID)	Marker of transgenic hairy roots
	[32]			custom modified pCAMBIA3300 based vectors	pAtU6-26; pAtU3b1; pAtU6-1; pAtU3d; pAtU6-29; pAtU3b2	2xp35S	Cas9	*GmAGO7a*; *b*	**Bar**; **EGFP**
	[33]		ARqua1*	p201G/Cas9; pUC-gRNA [2]	pMtU6.6	2xp35S	hCas9	*Glyma15G191200*	EGFP
	[34]			p201-EGFP-C9	pGmUbi3; pMtU6	pGmUbi3	hCas9	*GmIPK1*; *GmIPK2*	EGFP
	[35]			pFGC5941	pU6	p35S	Cas9	*Glyma06g14180*	Bar; **GUS/GFP**
	[36]			p201G/Cas9; pUC-gRNA [2]	pMtU6.6	2xp35S	hCas9	*WI12*$_{Rhg1}$; *DELLA11* and *18*	EGFP
	[37]			pFGC5941	pGmU6	pGmUbi; p35S	LbCpf1; ttLbCpf1	*FAD2-A* (*Glyma10g42470*); *MCT* (*Glyma10G276600*); *RPL15* (*Glyma10G273900*); *PDCD6IP* (*Glyma10G266600*)	Bar
***Glycine max* (soybean)**	[38]	2021	K599	pCAMBIA3301 based vectors	pGmU6-10	p35S	pCas9	*GmNAC06*	Blp
	[39]			GoldenGate GATEWAY	p35S	p35S	Cas9 fused with Csy4 via P2A peptide	*GW2*	Bar
	[40]			pCAMBIA3301 based vectors	pGmU6	p35S	pCas9	*GmCRY1a-1d; 2a-2b*	Blp
	[41]			pCAMBIA3301 based vector^{PC}	pGmU6-6^{PC}	2xp35S^{PC}	Cas9^{PC}	*lncRNA77580*	Blp
	[42]			pBSE401 [17]	pAtU6-26; pAtU6-29	p35S	zCas9	*GmNHX5*	Blp; **PCR**
	[43]			not reported	not reported	not reported	Cas9	*galactinol synthase* genes (G03 and 19)	Bar
	[44]			pCAMBIA3301 based vectors	pAtU6-26; pGmU6-10	p35S	Cas9	*GmpPLA-IIε*; *GmpPLA-IIζ*	Blp; **PCR**

Plant species	Reference	Year	Agrobacterium strain	Vector name or type of cloning system	Promoters for gRNA expression	Promoters for Cas9 expression	Cas9 type	Gene chosen for genome editing (name or ID)	Marker of transgenic hairy roots
	[45]			JRH0951 (gRNA); JRH0945 (Cas9)	pGmU6-10	2xp35S	Cas9	*GmCONSTANS*	Bar
	[46]			custom modified pCAMBIA1300 based vectors	pGmU6	pM4	Cas9	*miR169c*	Bar
	[47]			pSoy10	not reported	not reported	Cas9	*GmDDR1*	DsRed2
***Glycine soja* (wild soybean)**	[48]	2020	K599	pCAMBIA3301 based vectors	pGmU6-10	p35S	Cas9	*SOS1* (plasma membrane Na^+/H^+ antiporter); nonselective cation channels *(NSCC)*	Blp
***Glycyrrhiza glabra* (liquorice)**	[49]	2021	ATCC15834	pHSE401 [17]	pAtU6-26	2xp35S	zCas9	*F5H*	Hygr
***Glycyrrhiza uralensis* (Chinese liquorice)**	[50]	2021	ATCC15834	pHSE401 [17]	pAtU6-26	2xp35S	zCas9	*β-AS*	Hygr
***Gossypium hirsutum* (cotton)**	[51]	2021	K599	custom modified pCAMBIA2301	pAtU6-26	2xp35S	Cas9	*GhMYB25-like A* and *D*	Km
Lotus japonicus	[52]	2016	LBA1334*	custom modified pCAMBIA1300	pLjU6-1	2xp35S; pLjLb2	Cas9	*Leghemoglobins* (*Lb*) *b1*; *b2* and *b3*	sGFP
	[53]	2019	ATCC15834	pMgP237-2A-GFP [5]	pAtU6-26	2xp35SΩ	AtCas9 fused with GFP via T2A peptide	*CYP716A51*	**GFP**; Km
***Lupinus albus* (white lupin)**	[54,55]	2020	A4T	pCas9::ALMT-sgRNA1/2	pAtU6-6^{PC}	pPcUbi4-2^{PC}	pCas9	*LaALMT1* (*(Al)-activated malate transporter 1*)	Bar; **GFP**
	[54,56]	2021		pCas9::MATEPS1; pCas9::MATEPS2				*LaMATE*; *LaMATE3* (*Multidrug and Toxic Compound Extrusion*)	
Medicago truncatula	[13]	2015	ARqua1*	pMDC32/GmCas9	pAtU6-26	2xp35S	GmCas9	*GUS* gene in *GUS* barrelclover line	Hygr

Plant species	Reference	Year	Agrobacterium strain	Vector name or type of cloning system	Promoters for gRNA expression	Promoters for Cas9 expression	Cas9 type	Gene chosen for genome editing (name or ID)	Marker of transgenic hairy roots
	[57]	2017		custom modified pKSE401 [17]	pAtU6-26	2xp35S	zCas9	*DZA315(Fix⁺) allele of Medtr8g465280* (*NFS2*)	**GUS**; Km
	[58]							*NCR-α*; *NCR-β*	
	[59]	2018						*NFS*$^{1-}$; *NFS*$^{2-}$ alleles	
	[60]	2020	LBA9402	pYLCRISPR/Cas9 P35s-B [10]	pAtU3b	2xp35S	pCas9 with highest GC content at the 5′ terminal region for Gramineae genes	*PDS*	Bar
Medicago truncatula	[61]	2020	ARqua1*	pHSN401 [17]	pAtU6-26; pMtU6-1	2xp35S	zCas9	*MtCRA2*; *MtEIN2*	Hygr
Nicotiana tabacum **(tobacco)**	[62]	2020	ATCC15834	GoldenBraid 4.0	*snoRNA* promoters	p35S	hCas9	*N-methylputrescine oxidase* (*MPO*) gene family	**Km**; **DsRed**
Ophiorrhiza pumila	[63]	2020	C58C1*	pCAMBIA1300 based vectors	pAtU6	pAtUbi	hCas9	*secologanin synthetase*; *geraniol-10-hydroxylase*	Hygr
Phtheirospermum japonicum	[64]	2021	AR1193*	GoldenGate	pAtU6	2xp35S	hCas9	*PjIPT1a*	DsRed
Populus tremula* x *alba **(hybrid polar)**	[65]	2020	ARqua1*	p201G/Cas9; pUC-gRNA [2]	pMtU6.6	2xp35S	hCas9	*UDP-dependent glycosyltransferases* (*UGT71L1*, *UGT78M1*)	EGFP
	[66]	2021		MoClo system	pAtU6-26; pAtU6-29	pAtAct2	Cas9	*SHORTROOT*; *YUC4*; *PLETHORA1*; *LBD4*; *LBD12*	tdTomato
Punica granatum **(pomegranate)**	[67]	2019	MSU440*	custom modified pCAMBIA1300	pAtU6	pAtUbi1	Cas9	*UDP-dependent glycosyltransferases* (*UDP84A23*; *UDP84A24*)	GFP
Salvia miltiorrhiza **(red sage)**	[68]	2017	ACCC10060	pCAMBIA1300 based vectors	pAtU6-26	2xp35S	Cas9	*committed diterpene synthase 1*	Hygr

Plant species	Reference	Year	Agrobacterium strain	Vector name or type of cloning system	Promoters for gRNA expression	Promoters for Cas9 expression	Cas9 type	Gene chosen for genome editing (name or ID)	Marker of transgenic hairy roots
	[69]	2018	C58C1*		pAtU6-26; pOsU3	pAtUbi; pOsUbi	hCas9	*rosmarinic acid synthase*	
	[70] [71]	2020	C58C1 (pRiA4)*		pAtU6	pAtUbi	hCas9	*bZIP1* *MYB98*	
	[72]		ATCC15834	pICSL002218A (TSL SynBio, Norwich, UK)	pAtU6-26^{PC}	pAtUbi10^{PC}	hCas9^{PC}	*SmKFB5*	**Km**; **PCR**^{PC}
	[73]	2021	C58C1*	pCAMBIA1300 based vectors	pAtU6	pAtUbi	Cas9	23 *LACCASE* genes (*1-4*; *8-13*; *15-24*; *26-28*)	Hygr
***Solanum lycopersicum* (tomato)**	[74]	2014	ATCC15834	pMR093	pAtU6	p35S	*Nicotiana* sp. codon optimized Cas9	*GFP* and *SHR* genes in *SlSCRpro:modified (m) mGFP5* tomato line	BASTA
	[75]	2016	ARqua1*	p201N/Cas9 pUC-gRNA [2]	pMtU6.6	2xp35S	hCas9	no genes were reported	Km
***Solanum lycopersicum* (tomato)**	[76]	2019	ATCC15834	pMgP237-2A-GFP [5]	pAtU6-26	2xp35SΩ	AtCas9 fused with GFP via T2A peptide	*Sl5αR1*; *R2*	GFP; Km; **PCR**
	[77]	2020	ATCC15834	GoldenGate; pDe-Cas9(VQR)-Km; pDe-Cas9-Km	pAtU6	pPcUbi4-2	Cas9; Cas9(VQR)	*MYC1*; *MYC2*; *COI1*; *C5-SD2*	Km
***Solanum tuberosum* (potato)**	[5]	2018	ATCC15834	pEgP237-2A-GFP [78]	pAtU6-26	2xp35SΩ	AtCas9 fused with GFP via T2A peptide	*St16DOX* (*2-oxoglutarate-dependent dioxygenase*)	GFP; Km; **PCR**
	[79]	2020	MSU440*	GoldenGate cloning system and vectors developed by Čermák et al., 2017 [80]	pAtU6; pAt7SL	p35S	AtCas9; AtCas9 or AtCas9D10A both fused with dpCsy4 via P2A peptide	*PDS*	Hygr
	[81]		15834 or 43056*	pBIN-HcoCas9: mGFP; pChimera (for gRNA construction)	pAtU6-26	2xp35S	hCas9	*GFP* gene in *GFP* potato line; *nonexpresser of pathogenesis-related 3*	mGFP

Plant species	Reference	Year	Agrobacterium strain	Vector name or type of cloning system	Promoters for gRNA expression	Promoters for Cas9 expression	Cas9 type	Gene chosen for genome editing (name or ID)	Marker of transgenic hairy roots
Symphytum officinale **(comfrey)**	[82]	2021	ATCC15834	pEn-Chimera (for sgRNA); modified pDe-Cas9 [83]	pAtU6-26	pPcUbi4-2	AtCas9	*homospermidine synthase*	Hygr
Taraxacum kok-saghyz **(rubber dandelion)**	[84]	2016	K599	pFGC-pcoCas9 (AG # 52256); pICH86966 (gRNA) [85]	pAtU6	p35SPPDK	pCas9	*fructan:fructan 1-fructosyltransferase*	BASTA; **PCR**
Vigna unguiculata **(cowpea)**	[86]	2019	K599	custom modified pCAMBIA1300	pLjU6-1	2xp35S	Cas9	*VuSYMRK*	sGFP

***Agrobacterium* strains: *** — *Agrobacterium rhizogenes* strains with the chromosomal background of *Agrobacterium tumefaciens* C58.

Cas9 types: AtCas9 — Arabidopsis codon optimized Cas9; **AtCas9D10A** — Arabidopsis codon optimized nickase Cas9D10A; **BnCas9** — rapeseed codon optimized Cas9; **Cas9(VQR)** — mutant Cas9 recognizing the 5′—NGA—3′ protospacer adjacent motif (PAM) instead of the 5′—NGG—3′ PAM; **dpCas9** — Cas9 codon optimized for dicots; **GmCas9** — soybean codon optimized Cas9; **hCas9** — human codon optimized Cas9; **Cpf1** — Cas nuclease from CRISPR system of *Prevotella* and *Francisella* 1 (also known as Cas12); **LbCpf1** —Cpf1 nuclease of *Lachnospiraceae* bacterium (recognizing the 5′—TTTN—3′ PAM instead of the NGG PAM); **ttLbCpf1** — temperature-tolerant LbCpf1 variant; **pCas9** — plant codon optimized Cas9; **zCas9** — maize codon optimized Cas9; izCas9 — zCas9 with multiple introns.

Promoters: **pAct2** — promoter of the *actin2* gene; **pLb** — leghemoglobin promoter**; p35S** — 35S promoter from the Cauliflower Mosaic Virus; **2xp35S** — double p35S; **2xp35SΩ** — 2xp35S with the omega translational enhancer from the Cauliflower Mosaic Virus 5′ UnTranslated Region (5′-UTR); **p35SPPDK** — constitutive *35S* enhancer fused to the maize C4 pyruvate orthophosphate dikinase (C4PPDK) basal promoter**; pM4** — promoter of the soybean *SCREAM M4* gene; **pUbi** — promoter of a *ubiquitin* gene; **pU6**, **pU3** or **p7SL** — promoters of the small nucleolar RNA (*snoRNA*) genes.

Markers for transgenic hairy root detection: Bar (synonyms **BASTA, Blp** or **PPT**) — gene encoding resistance to the phosphinothricin (PPT) or to the PPT-containing compounds (e.g. tripeptide bialaphos (Blp), herbicide BASTA®); **DsRed** — Red Fluorescent Protein from *Discosoma* sp.; **GFP** — green fluorescent protein from *Aequorea victoria* and it variants: **3xGFP** — triple GFP, **EGFP** — enhanced GFP, **mGFP** — monomeric GFP, **sGFP** — synthetic GFP; **GUS** — *β*-glucuronidase; **GUS/GFP** — bifunctional fusion between GUS and GFP; **Hygr** — hygromycin resistance; **Km** — kanamycin resistance; **MYB75/PAP1** — anthocyanin pigmentation screenable marker encoded by the *MYELOBLASTOSIS75*/*PRODUCTION OF ANTHOCYANIN PIGMENTS1* (*MYB75*/*PAP1*) gene; **PCR** — genotyping by the polymerase chain reaction (PCR); **tdTOMATO** — tandem linked dimers of the Red Fluorescent Protein TOMATO (DsRed1 derivative). If more than one selectable/screenable marker was present in the CRISPR/Cas9 plasmid, the marker preferred by investigators is listed in underlined bold print.

Species abbreviations: At — *Arabidopsis thaliana*; **Ci** — *Cichorium intybus*; **Gm** — *Glycine max*; **La** — *Lupinus albus*; **Lj** — *Lotus japonicus*; **Mt** — *Medicago truncatula*; **Os** — *Oryza sativa*; **Pc** — *Petroselinum crispum*; **Sl** — *Solanum lycopersicum*; **Zm** — *Zea mays*.

Other vector components and abbreviations: ADH — translational enhancer from the 5′-UTR of the *alcohol dehydrogenase* gene; **AG** — AddGene (plasmid repository); **Csy4** — RNA endoribonuclease Csy4 from *Pseudomonas aeruginosa***; dpCsy4** — dicots codon optimized Csy4; **PC** — personal communication (used when the information about *A. rhizogenes* strain or vector components was not found in a published study, either in the main text or in the supplementary materials; PC was used for refs 4, 8, 39; 49-51, 67); **P2A** — self cleaving 2A peptide from porcine teschovirus-1 (PTV); **T2A** — self cleaving 2A peptide from *Thosea assigna* virus (TaV).

Table S2. Examples of the old-known conventional and nonconventional markers used for identification of transgenicity in transformed plants

Year of the first report about marker usage for identification of transgenicity	Reference	Plant species chosen for testing the marker	Common name of marker	Type of marker	Protein	Source of marker (including year and reference)
1983	[87]	*Nicotiana tabacum*	Neo/Km	antibiotic resistance (selectable)	neomycin phosphotransferase II (NPTII) or aminoglycoside 3-phosphotransferase II (APH (3′)-II)	*nptII* gene from the Tn*5* transposon (1982) [88]
			Mtx		methotrexate-insensitive dihydrofolate reductase (DHFR)	*dhfr* gene from the *Tn7* transposon (1982) [89]
1985 (March)*	[90]	*Nicotiana tabacum*	Hygr	antibiotic resistance (selectable)	hygromycin phosphotransferase (HPT)	*Escherichia coli* *aphIV* gene (1983) [91,92]
1985 (October)	[93]	*Nicotiana tabacum*	Glp	herbicide resistance (to *N*-phosphono methylglycin (glyphosate) containing herbicides, e.g. Roundup®) (selectable)	5-enolpyruvyl-shikimate 3-phosphate (EPSP) synthase	*Salmonella tiphimurium* mutant allele of the *aroA* gene (1983) [94]
1986	[95]	*Nicotiana plumbaginifolia*	Bm	antibiotic resistance (selectable)	glycopeptide interacting with DNA causes double strand breaks	*ble* gene from the Tn*5* transposon (1985) [96,97]
1987 (September)	[98]	*Nicotiana tabacum* *Solanum lycopersicum* *Solanum tuberosum*	Bar (Blp; BASTA)	herbicide resistance (to phosphinothricin (PPT) or bialaphos containing herbicides, e.g. BASTA®) (selectable)	phosphinothricin *N*-acetyltransferase (PAT)	*Streptomyces hygroscopicus* *bar* gene conferring resistance to bialaphos (the tripeptide containing PPT) (1986) [99]
1987 (November)	[100]	*Nicotiana tabacum* *Nicotiana plumbaginifolia*	Str (Sm)	antibiotic resistance (selectable)	streptomycin phosphotransferase (SPT)	*spt* gene from the Tn*5* transposon (1983/85) [97,101]
1988 (February)	[102]	*Arabidopsis thaliana*	ALS	herbicide resistance (to the sulfonylureas, e.g. chlorsulfuron) (selectable)	acetolactate synthase (ALS)	*als* gene from the chlorsulfuron resistant *Arabidopsis thaliana* mutant (1988) [102]
1988 (April)	[103]	*Arabidopsis thaliana* *Petunia hybrida*	Gm/Neo/Km	antibiotic resistance (selectable)	aminoglycoside-3-*N*-acetyl transferases AAC(3)-III and IV	*aacC3* and *C4* genes from the IS*140* mobile element (1984/85) [104,105]

Year of the first report about marker usage for identification of transgenicity	Reference	Plant species chosen for testing the marker	Common name of marker	Type of marker	Protein	Source of marker (including year and reference)
1988 (May)	[106]	*Zea mays*	GUS	colorimetric (screenable)	*β*-glucuronidase	*Escherichia coli* *uidA* gene (1973/86) [107,108]
1989 (August)	[109]	*Nicotiana tabacum*	2,4-D	2,4-dichlorophenoxyacetate (synthetic auxin) resistance (selectable)	2,4-dichlorophenoxyacetate monooxygenase (DPAM)	the soil bacterium *Alcaligenes eutrophus* *tfdA* gene (1987) [110]
1989 (October)	[111]	*Nicotiana tabacum*	Pm/Bm	antibiotic resistance (selectable)	glycopeptide interacting with DNA causes double strand breaks	*ble* gene from the Tn*3* transposon (1985) [96,97]; the actinomycete *Streptoalloteichus hindustanus* *ble* gene (1990) [112]
1990 (January)	[113]	*Zea mays*	anthocyanin pigmentation	colorimetric (screenable)	transcription factor of the basic Helix-Loop-Helix (bHLH) family	*Zea maize* *Leaf color* gene (*ZmLc*) (*bHLH*) (1989) [114]
1990 (February)	[115]	*Nicotiana tabacum*	Str/Sp	antibiotic resistance (selectable)	aminoglycoside-(3") (9)-adenylyltransferase (AadA)	*Escherichia coli* *aadA* gene (1985) [116]
1990 (September)	[117]	*Nicotiana tabacum*	Bsd	antibiotic resistance (selectable)	blasticidin S deaminase (Bsd) (aminohydrolase)	*Bacillus cereus* *bsr* gene (1987/88) [118,119]
1991	[120]	*Nicotiana tabacum*	Gm	antibiotic resistance (selectable)	aminoglycoside-3-*N*-acetyl-transferase (AAC(3)-I)	*aacC1* gene from the Tn*21*-like transposon [121]
1995	[122]	*Citrus sinensis*	GFP	fluorescent protein (screenable)	Green Fluorescent Protein	*gfp10* template obtained from the native *gfp* of the jellyfish *Aequorea victoria* (1992) [123]
1998 (April)	[124]	*Beta vulgaris*	ManA	selection for ability to metabolize mannose (selectable)	phosphomannose isomerase (PMI)	*Escherichia coli* *manA* gene (1984) [125]
1998 (May)	[126]	*Nicotiana tabacum* *Solanum lycopersicum* *Solanum tuberosum*	XylA	selection for ability to metabolize xylose (selectable)	xylose isomerase	*Clostridium thermosulfurogenes* *xylA* gene (1990) [127]
2001	[128]	*Nicotiana tabacum*	DsRed	fluorescent protein (screenable)	Orange (Red) Fluorescent Protein	*Discosoma* sp. *DsRed* gene (1999) [129]

Year of the first report about marker usage for identification of transgenicity	Reference	Plant species chosen for testing the marker	Common name of marker	Type of marker	Protein	Source of marker (including year and reference)
2004	[130]	*Arabidopsis thaliana*	TPS1	selection for insensitivity to the medium containing glucose in high concenration (selectable)	trehalose-6-phophate synthase (TPS)	*Arabidopsis thaliana* *TPS1* gene (1998) [131]

*If two or more studies were published per same year, then studies were ranged by month of publication.

Table S3. List of research tasks solved using CRISPR/Cas editing of different genes in hairy roots.

Plant species	Reference	Year	Gene chosen for genome editing (name or ID)	Method of hairy roots obtaining*	Aims of studies reporting only identification of genome editing events in hairy roots	Aims of studies using both identification of genome editing events and phenotyping of edited hairy roots
Arachis hypogaea **(peanut)**	[1]	2019	*ahFAD2a*	CP	testing of genome editing capability in hairy roots	
	[3]	2020	*NFR1A1*; *A2*; *B1*; *B2*; *NFR5A*; *B*	CP		root nodule (RN) symbiosysis investigation (phenotyping for nodules formation)
Atropa belladonna **(belladonna)**	[4]	2021	*pyrrolidine ketide synthase* (*PYKS*)	LE		metabolic engineering (phenotyping for changes of tropane alcaloids)
Brassica carinata **(Abyssinian mustard)**	[6]	2017	*FASCICLIN-LIKE ARABINOGALACTAN PROTEIN 1*	CP		root development (phenotyping for root hairs formation)
Brassica napus **(rapeseed)**	[7]	2020	*HVA22 homologue C*; *AGO1*(*miR168*); *TAO1*(*miR1885a*)	CE	testing of genome editing eviciency before stable transformation	
Catharanthus roseus **(Madagascar periwinkle)**	[8]	2021	*Jasmonate-associated MYC2*; *Jasmonate-associated MYC3*; *Repressor of MYC2 targets 1*	SE	testing of genome editing capability in hairy roots	
Cichorium intybus **(chicory)**	[9]	2019	*PDS*	LE	regeneration to the whole genome edited plant	
Eucalyptus grandis **(flodded gum)**	[11]	2020	*Cinnamoyl-CoA reductase1*; *IAA9A*	CP		root development (phenotyping for lignin biosynthesis)
Glycine max **(soybean)**	[12]	2015	*bar* gene in *bar* soybean line; *GmFEI1*; *GmFEI2*; *GmSHR*	HE and CE	testing of genome editing capability in hairy roots	
	[2]		*gfp* gene in *gfp* soybean line; *Glyma07g14530*; *01gDDM1*; *11gDDM1*; *miR1509*; *miR1514*; *MET1*	HE and CE		
Glycine max **(soybean)**	[13]	2015	*glutamine synthase 1*; *chalcone-flavanone isomerase 20*	CE	testing of genome editing capability in hairy roots	
	[14]		*Glyma06g14180*; *Glyma08g02290*; *Glyma12g37050*	CP		
	[15]	2016	*PDS*	CE	testing of genome editing eviciency before stable transformation	

Plant species	Reference	Year	Gene chosen for genome editing (name or ID)	Method of hairy roots obtaining*	Aims of studies reporting only identification of genome editing events in hairy roots	Aims of studies using both identification of genome editing events and phenotyping of edited hairy roots
***Glycine max* (soybean)**	[16]	2017	*Glyma.01G165800*; *Glyma.01G165800-D*	CP		RN symbiosysis investigation (phenotyping for nodules formation)
	[18]		*Rg1*	CP		
	[19]	2018	*GmMYB118*	CP		investigation of resistance to the abiotic stress conditions (phenotyping of sensitivity to the drought)
	[20]		*Nod Factor Receptor 1a*	CP		RN symbiosysis investigation (phenotyping for nodules formation)
	[21]	2019	*LATE ELONGATED HYPOCOTYL 1a*; *1b*; *2a*; *2b*	CP	testing of genome editing eviciency before stable transformation	
	[22]		*GmFAD2-1A*; *GmFAD2-1B*	CP		
	[23]		*Glyma.03g163500*; *Glyma.10g037100*; *Glyma.10g246300*; *Glyma.13g123500*; *Glyma.19g164800*; *Glyma.19g164900*; *Glyma.20g146200*; *Glyma.20g148200*; *Glyma.20g148400*	CP	testing of genome editing capability in hairy roots	
	[24]		*Isoflavonoid syntase*	CE		metabolic engineering (phenotyping for changes in isoflavone metabolic pathway)
	[25]	2020	74 nodulation genes; 27 seed genes	CP	testing of genome editing eviciency before stable transformation	
	[26]		*Rg1*	CP		RN symbiosysis investigation (phenotyping for nodules formation)
	[27]		*galactinol synthase* (*GOLS*) genes: *GOLS1A*; *GOLS1B*	CE	testing of genome editing eviciency before stable transformation	
	[29]		*GmPHR1*; *GmPHR4*	CP		RN symbiosysis investigation (phenotyping for nodules formation)
	[31]		*GmF3H1*; *GmF3H2*; *GmFNSII-1*	CE	testing of genome editing eviciency before stable transformation	
	[32]		*GmAGO7a*; *b*	CP		

Plant species	Reference	Year	Gene chosen for genome editing (name or ID)	Method of hairy roots obtaining*	Aims of studies reporting only identification of genome editing events in hairy roots	Aims of studies using both identification of genome editing events and phenotyping of edited hairy roots
***Glycine max* (soybean)**	[33]	2021	*Glyma15G191200*	CE		investigation of resistance to the biotic stress conditions (phenotyping for resistance to soybean cyst nematode)
	[34]		*GmIPK1; GmIPK2*		testing of genome editing capability in hairy roots	
	[35]		*Glyma06g14180*			
	[36]		*DELLA 11 and 18*; $WI12_{Rhg1}$	CE		investigation of resistance to the biotic stress conditions (phenotyping of interaction with root knot nematode)
	[37]		*FAD2-A (Glyma10g42470); MCT* (*Glyma10G276600*); *RPL15* (*Glyma10G273900*); *PDCD6IP* (*Glyma10G266600*)		testing of genome editing capability in hairy roots	
	[38]		*GmNAC06*	CP		investigation of resistance to the abiotic stress conditions (phenotyping of sensitivity to the salinity)
	[39]		*GW2*	CE	testing of genome editing capability in hairy roots	
	[40]		*GmCRY1a-1d; 2a-2b*	CP	testing of genome editing eviciency before stable transformation	
	[41]		*lncRNA77580*	HE		investigation of resistance to the abiotic stress conditions (phenotyping for gene expression driven by salt stress)
	[42]		*GmNHX5*	CE		investigation of resistance to the abiotic stress conditions (phenotyping of sensitivity to the salinity)
	[43]		*galactinol synthase* genes (*G03* and *G19*)	CE	testing of genome editing capability in hairy roots	
	[44]		*GmpPLA-IIε; GmpPLA-IIζ*	CE	testing of genome editing eviciency before stable transformation	
	[45]		*GmCONSTANS*	CE	testing of genome editing capability in hairy roots	
	[46]		*miR169c*	CP	testing of genome editing eviciency before stable transformation	RN symbiosysis investigation (phenotyping for nodules formation)

Plant species	Reference	Year	Gene chosen for genome editing (name or ID)	Method of hairy roots obtaining*	Aims of studies reporting only identification of genome editing events in hairy roots	Aims of studies using both identification of genome editing events and phenotyping of edited hairy roots
	[47]		*GmDDR1*	not reported		investigation of resistance to the biotic stress conditions (phenotyping for resistance to *Phytophthora sojae*)
***Glycine soja* (wild soybean)**	[48]	2020	*SOS1* (plasma membrane Na^+/H^+ antiporter); nonselective cation channels *(NSCC)*	CP		root development (phenotyping for root to shoot Na^+ and K^+ transport)
***Glycyrrhiza glabra* (liquorice)**	[49]	2021	*F5H*	HE		metabolic engineering (phenotyping for changes of glycyrrhizin (glycyrrhizic acid) content)
***Glycyrrhiza uralensis* (Chinese liquorice)**	[50]	2021	*β-AS*	HE		
***Gossypium hirsutum* (cotton)**	[51]	2021	*GhMYB25-like A* and *D*	CP	testing of genome editing capability in hairy roots	
Lotus japonicus	[52]	2016	*Leghemoglobins* (*Lb*) *b1*; *b2* and *b3*	CP		RN symbiosysis investigation (phenotyping for nodules formation)
	[53]	2019	*CYP716A51*			metabolic engineering (phenotyping of changes in C-28 oxidized triterpenoid production)
***Lupinus albus* (white lupin)**	[54,55]	2020	*LaALMT1* (*(Al)-activated malate transporter 1*)	CP		root development (phenotyping for root to shoot transport: characterization of xylem sap and shoot metal concentrations
	[54,56]	2021	*LaMATE*; *LaMATE3* (*Multidrug and Toxic Compound Extrusion*)	CP		root development (evaluation of citrate secretion from roots under low phosphorus conditions) metabolic engineering (phenotyping for changes of isoflavonoids or their glycosides content)
Medicago truncatula	[13]	2015	*GUS* gene in *GUS* barrelclover line	CP	testing of genome editing capability in hairy roots	
	[57]	2017	*DZA315(Fix⁺) allele of Medtr8g465280* (*NFS2*)	CP		RN symbiosysis investigation (phenotyping for nodules formation)
	[58]		*NCR-α*; *NCR-β*	CP		
	[59]	2018	NFS^{1-}; NFS^{2-} alleles			RN symbiosysis investigation (phenotyping for nodules formation)

Plant species	Reference	Year	Gene chosen for genome editing (name or ID)	Method of hairy roots obtaining*	Aims of studies reporting only identification of genome editing events in hairy roots	Aims of studies using both identification of genome editing events and phenotyping of edited hairy roots
	[60]	2020	*PDS*	HE	regeneration to the whole genome edited plant	
	[61]		*MtCRA2*; *MtEIN2*	CP		RN symbiosysis investigation (phenotyping for Rhizobial early infection events)
***Nicotiana tabacum* (tobacco)**	[62]	2020	*N-methylputrescine oxidase* (*MPO*) gene family	LE	regeneration to the whole genome edited plant	
Ophiorrhiza pumila	[63]	2020	*secologanin synthetase*; *geraniol-10-hydroxylase*	SE		metabolic engineering (phenotyping for changes of camptothecin production)
Phtheirospermum japonicum	[64]	2021	*PjIPT1a*	CP		investigation of resistance to the biotic stress conditions (phenotyping of interaction between hemiparasitic plant *P. japonicum* and Arabidopsis)
***Populus tremula* x *alba* (hybrid polar)**	[65]	2020	*UDP-dependent glycosyltransferases* (*UGT71L1*, *UGT78M1*)	LE	testing of genome editing capability in hairy roots (for *YUC4*; *PLETHORA1*; *LBD4*; *LBD12*)	metabolic engineering (phenotyping for changes of salicinoid content)
	[66]	2021	*SHORTROOT*; *YUC4*; *PLETHORA1*; *LBD4*; *LBD12*	LE		root development (phenotyping for endodermis development: for *SHORTROOT*)
***Punica granatum* (pomegranate)**	[67]	2019	*UDP-dependent glycosyltransferases* (*UDP84A23*; *UDP84A24*)	HE		metabolic engineering (phenotyping for changes of gallic acid and its glicosides content)
***Salvia miltiorrhiza* (red sage)**	[68]	2017	*committed diterpene synthase 1*	LE		metabolic engineering (phenotyping for changes of tashinones (Ts) content)
	[69]	2018	*rosmarinic acid synthase*			metabolic engineering (phenotyping for changes of lithospermic acid and its precursors)
	[70]	2020	*bZIP1*	LE		metabolic engineering (phenotyping for changes in phenolic acid and Ts content)
	[71]		*MYB98*			metabolic engineering (phenotyping for changes in salvinalonic acid B (SAB) and Ts content)

Plant species	Reference	Year	Gene chosen for genome editing (name or ID)	Method of hairy roots obtaining*	Aims of studies reporting only identification of genome editing events in hairy roots	Aims of studies using both identification of genome editing events and phenotyping of edited hairy roots
	[72]	2021	*SmKFB5*			metabolic engineering (phenotyping for changes in SAB, rosmarinic (RA) and caffeic acids content)
	[73]		23 *LACCASE* genes (*1-4*; *8-13*; *15-24*; *26-28*)			metabolic engineering (phenotyping for changes in SAB, RA content; lignin biosynthesis pathway)
***Solanum lycopersicum* (tomato)**	[74]	2014	*GFP* and *SHR* genes in *SlSCRpro:modified (m) mGFP5* tomato line	CP	testing of genome editing capability in hairy roots (for *GFP*)	root development (phenotyping for meristem size: for *SHR*)
	[75]	2016	no genes were reported	CE	testing of genome editing capability in hairy roots	
	[76]	2019	*Sl5αR1*; *R2*	HE		metabolic engineering (phenotyping for changes in α-tomatine and dehydrotomatine biosynthesis pathway)
	[77]	2020	*MYC1*; *MYC2*; *COI1*; *C5-SD2*	CE		metabolic engineering (phenotyping for changes in α-tomatine and dehydrotomatine biosynthesis via supression of cholesreol and steroidal glycoalkaloid biosynthesis genes)
***Solanum tuberosum* (potato)**	[5]	2018	*St16DOX* (*2-oxoglutarate-dependent dioxygenase*)	CP		metabolic engineering (phenotyping for changes in α-solanine and α-chaconine biosynthesis pathway)
	[79]	2020	*PDS*	SE	regeneration to the whole genome edited plant	
	[81]		*GFP* gene in *GFP* potato line; *nonexpresser of pathogenesis-related 3*	CP	testing of genome editing capability in hairy roots (for *GFP*)	investigation of resistance to the biotic stress conditions (phenotyping of tolerance to *Candidatus* Liberibacter solanacearum)
***Symphytum officinale* (comfrey)**	[82]	2021	*homospermidine synthase*	LE		metabolic engineering (phenotyping for changes of honospermydine content)

Plant species	Reference	Year	Gene chosen for genome editing (name or ID)	Method of hairy roots obtaining*	Aims of studies reporting only identification of genome editing events in hairy roots	Aims of studies using both identification of genome editing events and phenotyping of edited hairy roots
***Taraxacum kok-saghyz* (rubber dandelion)**	[84]	2016	*fructan:fructan 1-fructosyltransferase*	CP	regeneration to the whole genome edited plant	
***Vigna unguiculata* (cowpea)**	[86]	2019	*VuSYMRK*	CP		RN symbiosysis investigation (phenotyping for nodules formation)

*Hairy roots were obtained from composite plants (CP); cotyledon explants (CE); hypocotyl explants (HE); leaf explants (LE) or stem explants (SE).

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