

Figure S1

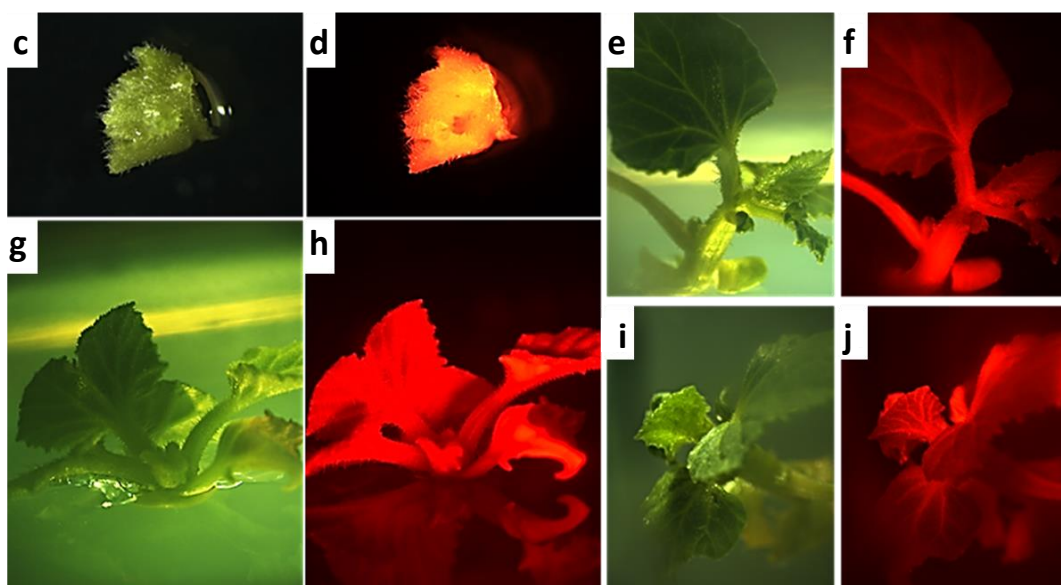
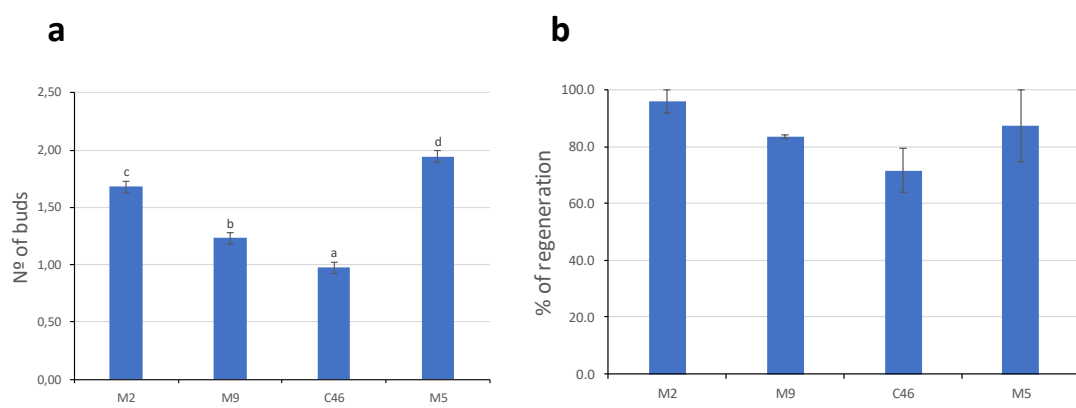
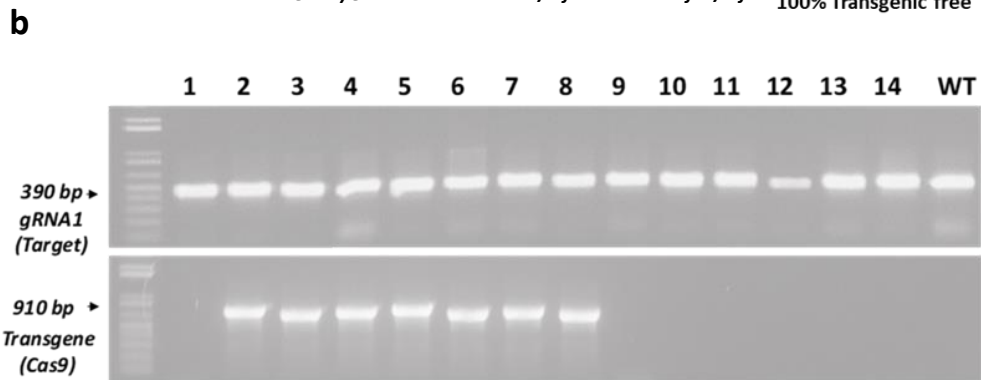
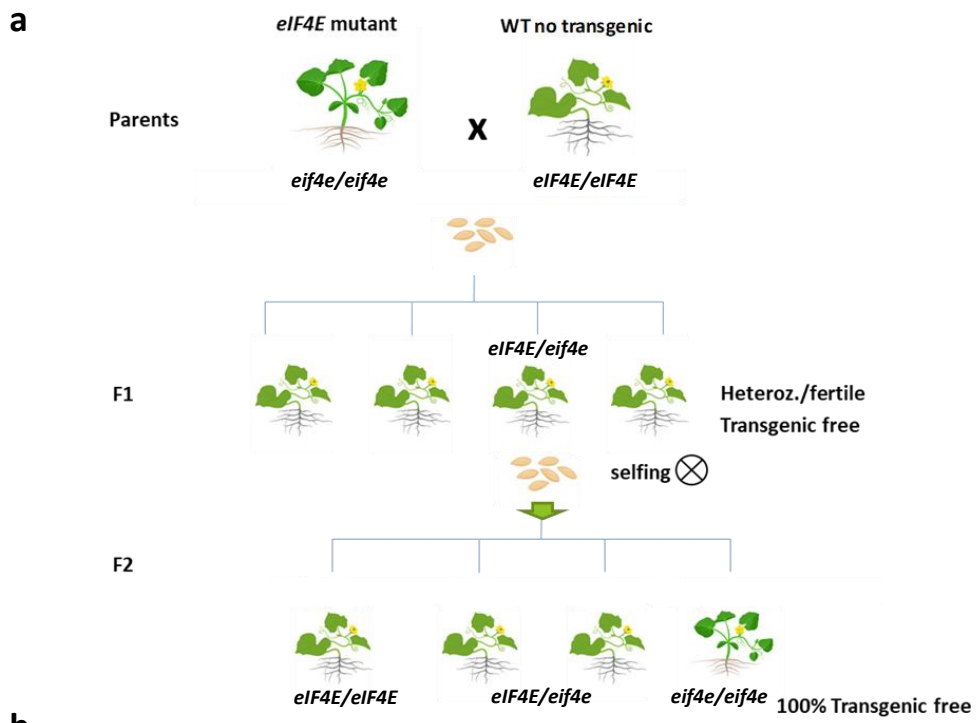


Figure S2



**c**

Cas9 (-) / *eIF4E-eif4e*: Plants N<sup>o</sup>. 1-9-10-11-12-13-14

TAATTCCATTGCTAATCAAAAACCTAGAGGACGTGGCGGTGACGAAAATGAGGAACTTGA WT  
 TAATTCCATTGCTAATCAAAAACCTAGAGGACGTGGCGGTGACGAAAATGAGGAACTTGA WT  
 TAATTCCATTGCTAATCAAAAACCTAGAGGAC-TGGCGGTGACGAAGATGAGGAACTTGA -1

Cas9 (+) / *eIF4E-eif4e*: Plants N<sup>o</sup>. 2-4-5-6-8

TAATTCCATTGCTAATCAAAAACCTAGAGGACGTGGCGGTGACGAAAATGAGGAACTTGA WT  
 TAATTCCATTGCTAATCAAAAACCTAGAGGACGTGGCGGTGACGAAAATGAGGAACTTGA WT  
 TAATTCCATTGCTAATCAAAAACCTAGAGGAC-TGGCGGTGACGAAGATGAGGAACTTGA -1

Cas9 (+) / *eif4e-eif4e*: Plants N<sup>o</sup>. 3-7

TAATTCCATTGCTAATCAAAAACCTAGAGGACGTGGCGGTGACGAAAATGAGGAACTTGA WT  
 TAATTCCATTGCTAATCAAAAACCTAGAGGAC-TGGCGGTGACGAAAATGAGGAACTTGA -1  
 TAATTCCATTGCTAATCAAAAACCTAGAGGAC-TGGCGGTGACGAAGATGAGGAACTTGA -1

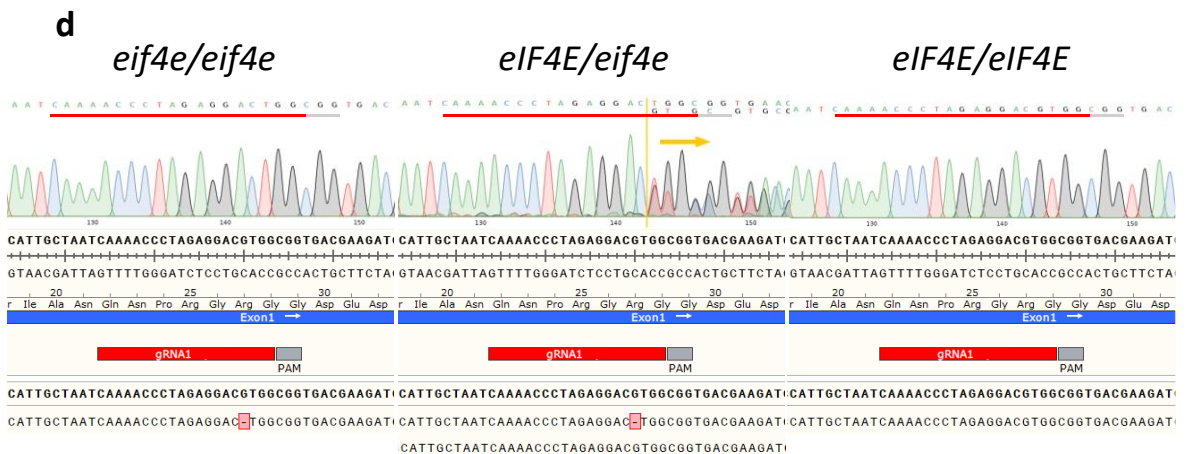


Figure S3

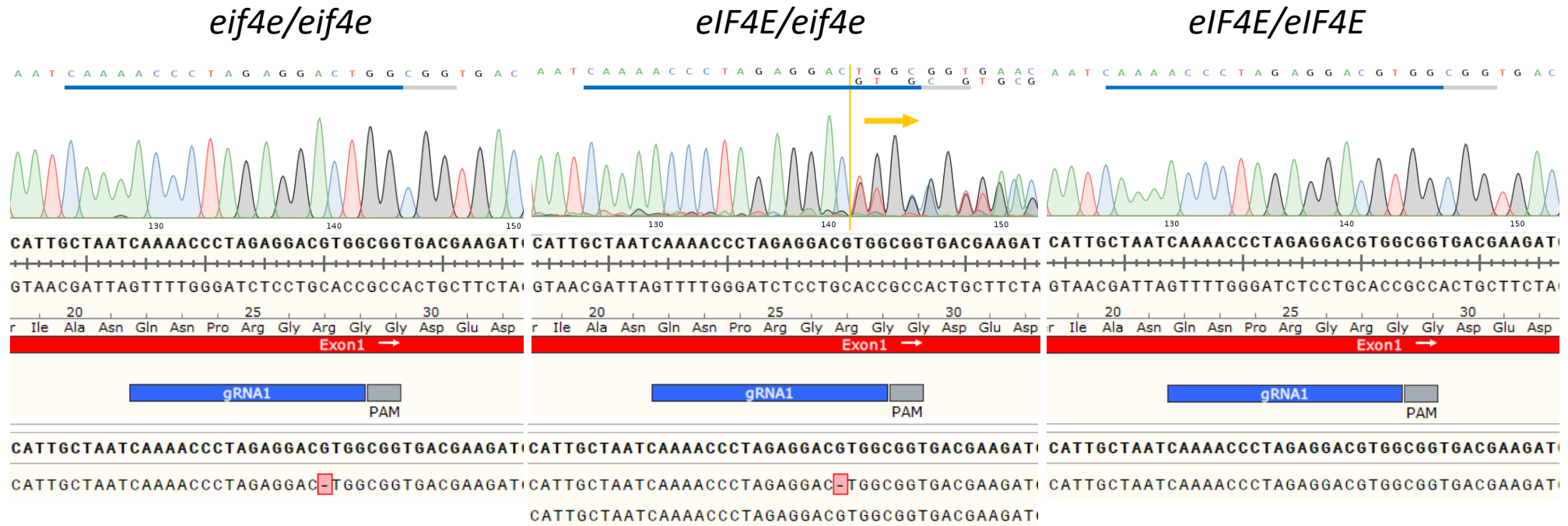


Figure S4

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CVYV      -----GKVGYRRDKRVGRFVFDGPDEDIIEENFGVEYSHDVTKKMSKAQKLIKQAK  50
PVY       GKNKSKRIQALKFRHARDKRAGFEID-NNDDTIEEFFGSAYRKKGKGG-----  48
MWMV_SQ10_1.1 -GFSARQRQKLKFKDARIAKLGREYV-GDDGTIEHFFGEAYTKKGKGG-----  47
MWMV_RB    -GFSARQRQKLKFKDARIAKLGREYV-GDDGTIEHFFGEAYTKKGKGG-----  47
ZYMV      --SKKRRSQKLKFRDAYDRKVGREIF-GDDDTIGRTFGEAYTKRGKVKGN-----  47
WMV       --GKKRQIQKLKFRDAFDRKVGREYV-ADDYTMEHTFGEAYTKKGKQKGS-----  47
          *      : * : * : . ** * : *

CVYV      EKGWKIGKVDPRPKKIFRQLYGVNPLEFDEVYLTVGDFKGEVW-ETKDM---DIDEMYSDL 106
PVY       ----TTVGMGKSSRRFINMYGFDPT EYSFIQFV-DPLTGRQIEENVYADIRDIQERFSEV 103
MWMV_SQ10_1.1 ----KMHGMGVKTRKFVSTYGFKPEDYSYVRYL-DPLTGETVDENVNTDVSLSVQEHFGEL 102
MWMV_RB    ----KMHGMGVKTRKFVSTYGFKPEDYSYVRYL-DPLTGETVDENVNTDVSLSVQEHFGEL 102
ZYMV      ---NSTKGMGRKTRNFVHLYGVEPENYSFIRFV-DPLTGHTLDESTHTDISLSVQEEFGNI 103
WMV       ---TRTKGMGRKSRNFIMYGVPEPENYSMIRFV-DPLTGHTMDESTRVDIRLVQQEFGEI 103
          :.  : *  **..*  :. :  . :.*  *  : : : :

CVYV      YSDFN----LGNR-KGYSKDVYLVFSKKDSIEAVVDLQPHRSKMASSMSLNPMGFPEEE 161
PVY       RKKMVENDDIEMQALGSNTTIHAYFRKDWCDKALKIDLMPHNPLKVKCDKTINGIAKFPERE 163
MWMV_SQ10_1.1 RNKYIENDMMGKQKIASAPGIKAYYVRNAAKTALEVDLTPHNPLKFCDRHIAIAGFPERE 162
MWMV_RB    RNKYIENDMMDKQKIASAPGIKAYYVRNAAKTALEVDLTPHNPLKFCDRHIAIAGFPERE 162
ZYMV      REKFLENDLISRQSIINKPGIQAYFMGKGT EALKVDLTPHVPLLLCKNTNAIAGYPERE 163
WMV       REEMIGADELDPQRVYHNPGIQAYFIGKNAKEALKVDLTPHVPTLLCQNSNAIAGFPERE 163
          ..      : :      : : . .      : ** **      ..      : **.*

CVYV      GRWRQSGDVKMRKRI-----EEVEVQ- 183
PVY       LELRQTGPAVEVDVKDIPAQVEHE--- 188
MWMV_SQ10_1.1 NDLRQTGMAKEIPIISKVPAKNEDVTHE 190
MWMV_RB    YDLRQTGMAKEIPIGKVPKSEDVTHE 190
ZYMV      NELRQTGTPVKVSFKDVPEKNEHVELE- 190
WMV       GELRQTGLPQIVPKVDVPRAKERVEVE- 190
          **.*
    
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Figure S5

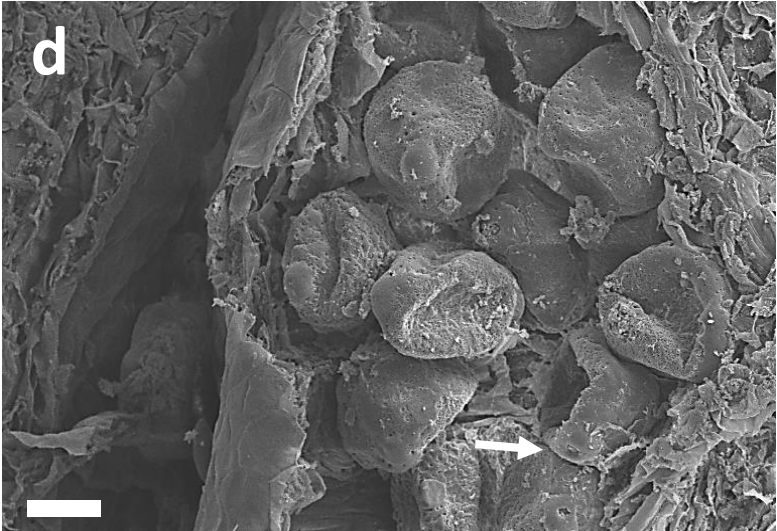
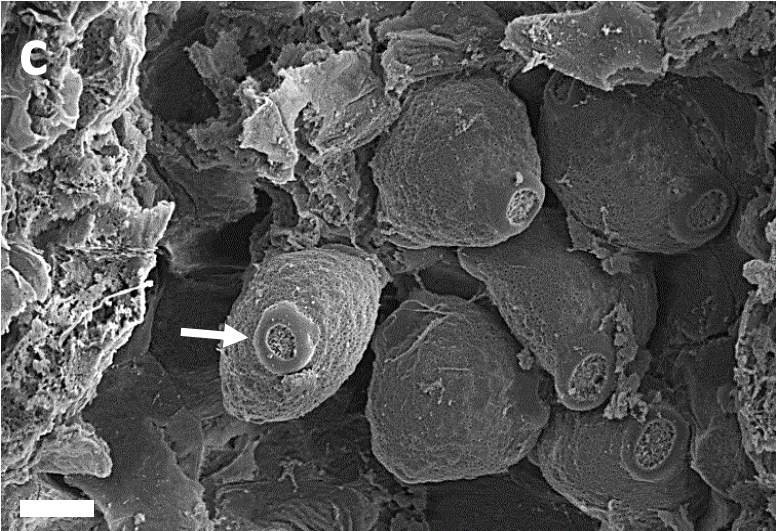
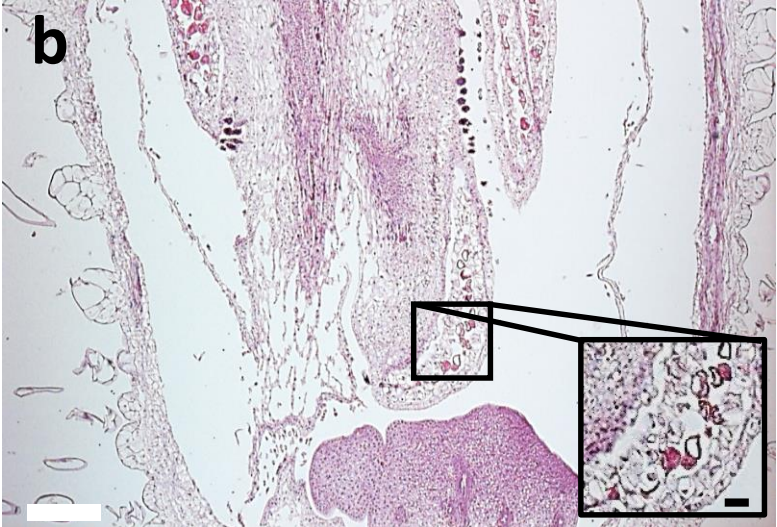
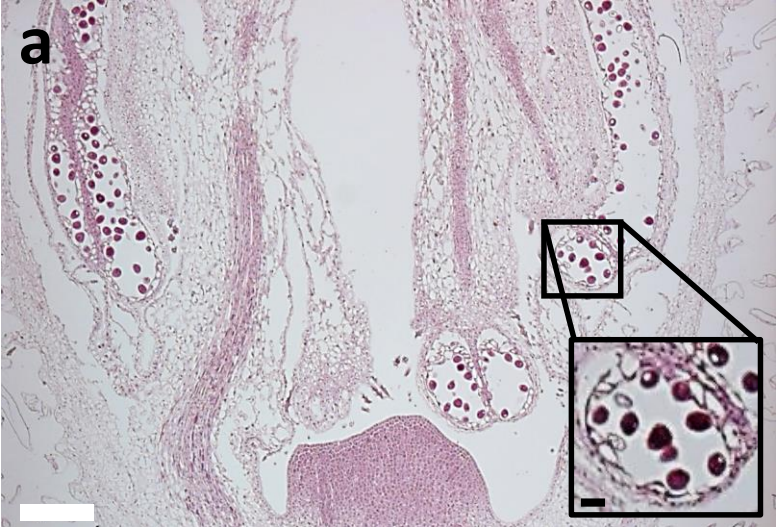
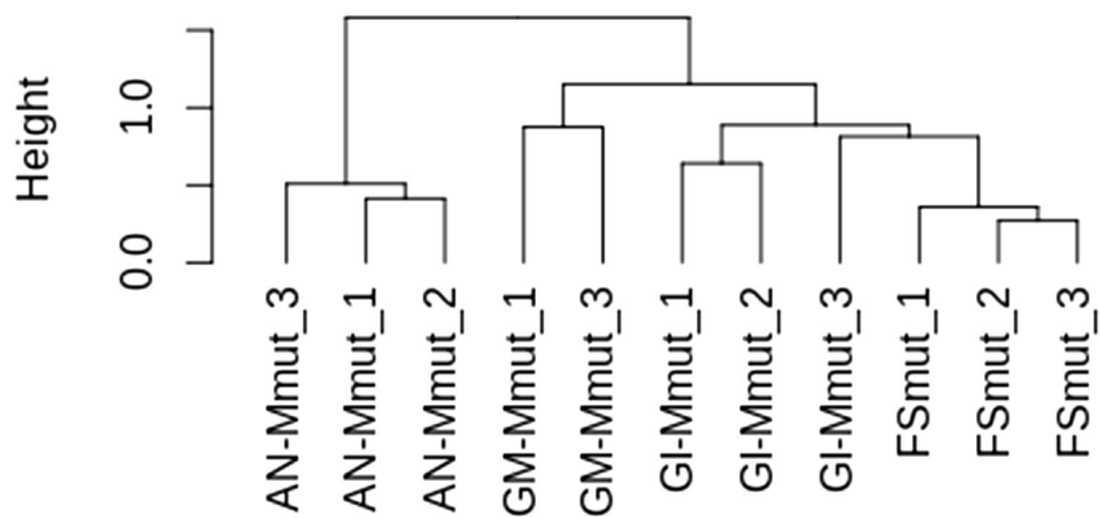
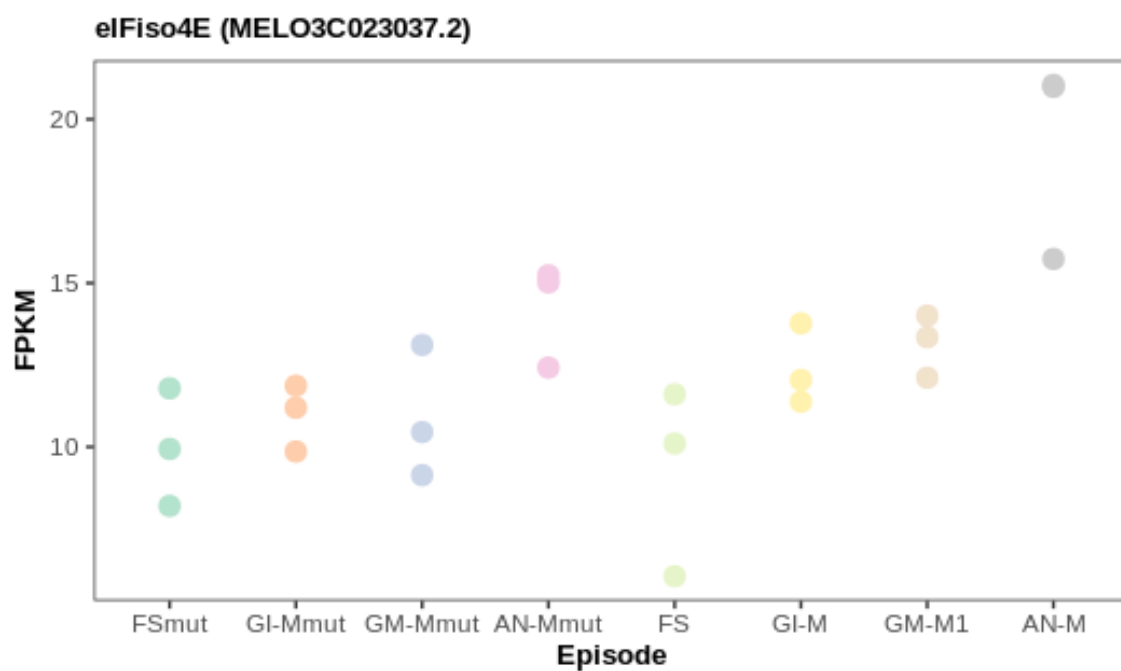
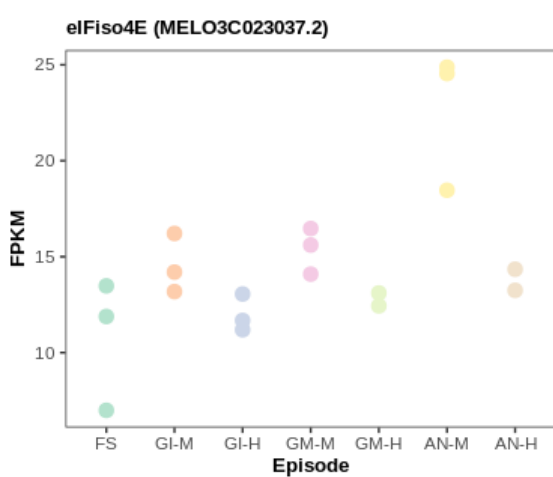
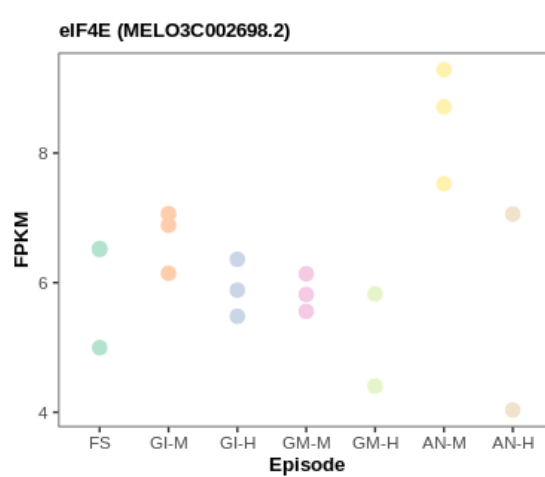


Figure S6



**a****b****c**

**Table S1.** Putative *eIF4E* CRISPR/Cas9 gRNA1 off-target sites

Sequence of the putative off-target site	Off-targetscore	MMs	Locus	Gene ID	Gene	Region
CAAAATCGTAGAAGACGTGGAGG	0.551	3MMs	scaffold00023:-5325134			Intergenic
CAAACCTCTAAAGGACGTGAAGG	0.435	4MMs	scaffold00006:+4019441	MELO3C006540	beta-galactosidase 7-like	CDS
CAAACCGGAGAAGACGTGACGG	0.344	4MMs	scaffold00005:+401743	MELO3C005084	E3 ubiquitin-protein ligase	CDS
GAAAACGCGAGAGGACGAGCGG	0.177	4MMs	scaffold00016:+4417626	MELO3C012320	sucrose-phosphatase 2	CDS
AAAAACCTTAGAAGAGGTGGAGG	0.124	4MMs	scaffold00038:-1390329	MELO3C019462	Membralin, putative	CDS
CAAACCCCTAGAAGACGTGGGAG	0.12	2MMs	scaffold00024:-2063176	MELO3C015061	Growth inhibition and differentiation	CDS
CAAAGACGTAGAGGACATGGCAG	0.104	4MMs	scaffold00004:+7609278			Intergenic
CAAACCATGAGGTCGTTGAGG	0.076	4MMs	scaffold00012:-3766001			Intergenic
CAAACCTGGAGGAGGAGGAGG	0.05	4MMs	scaffold00053:+1762859	MELO3C022610	Formin-like protein	CDS



**Table S2.** Selected differentially expressed genes during male flower development in male-sterile *eif4e* mutants

F5mut versus F5							
GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Gene_description
MELO3C007237.2	704,2133864	1,326477173	0,364315073	3,641016443	0,000271564	0,003163174	Apoptosis inhibitor 5-like protein API5
MELO3C008417.2	3528,391737	-1,308020453	0,209546706	-6,242142772	4,32E-10	6,12E-08	Bidirectional sugar transporter SWEET
MELO3C005758.2	1467,982643	-2,469692894	0,351893805	-7,018290336	2,25E-12	7,34E-10	Bidirectional sugar transporter SWEET
MELO3C022341.2	3669,317871	-3,462272444	0,875679526	-3,953812257	7,69E-05	0,001149903	Bidirectional sugar transporter SWEET
MELO3C015961.2	1799,56506	-1,240362818	0,259830798	-4,773732853	1,81E-06	5,71E-05	Ethylene receptor
MELO3C017940.2	153,7744199	-2,760256574	0,477872466	-5,776136465	7,64E-09	6,39E-07	Ethylene-responsive transcription factor
MELO3C014441.2	411,7120135	-1,38312477	0,31047831	-4,454819312	8,40E-06	0,000199281	ethylene-responsive transcription factor 3-like
MELO3C005465.2	165,6686375	-1,969711237	0,608431462	-3,237359277	0,001206414	0,009781202	ethylene-responsive transcription factor ERF105
MELO3C021306.2	3536,602112	-1,294296722	0,308487021	-4,195627797	2,72E-05	0,000498944	ethylene-responsive transcription factor RAP2-3-like
MELO3C001947.2	902,2931563	-1,253393143	0,319039738	-3,928642713	8,54E-05	0,001254592	eukaryotic translation initiation factor 3 subunit G-like
MELO3C020380.2	7060,019315	-1,070385774	0,204173668	-5,242526053	1,58E-07	7,96E-06	Eukaryotic translation initiation factor 4G
MELO3C022717.2	849,0112378	-1,284613703	0,252873703	-5,080060468	3,77E-07	1,57E-05	FHA domain-containing protein FHA2
MELO3C007468.2	1020,437469	1,394280965	0,380864029	3,660836565	0,000251393	0,002964074	Male gametophyte defective 1
MELO3C004667.2	681,5668714	-1,628869386	0,371559249	-4,383875218	1,17E-05	0,000257038	pollen receptor-like kinase 3
MELO3C024046.2	749,3084162	1,568180352	0,335879069	4,668883825	3,03E-06	8,81E-05	Protein XRI1
MELO3C013473.2	5485,980468	-2,878672747	0,292447476	-9,8433838	7,32E-23	1,34E-18	tetraketide alpha-pyrone reductase 1
G1mut-M versus GI-M							
GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Gene_description
MELO3C020400.2	663,0962885	-1,662640113	0,446845441	-3,720839375	1,99E-04	2,90E-03	ABC transporter G family member 21
MELO3C025486.2	2230,038087	-1,476335508	0,398812406	-3,701829451	2,14E-04	3,08E-03	ABC transporter G family member 6
MELO3C007237.2	704,2133864	1,337663016	0,3652220854	3,662614011	2,50E-04	0,00346385	Apoptosis inhibitor 5-like protein API5
MELO3C005473.2	500,8277454	-1,353354402	0,262270095	-5,160155224	2,47E-07	1,32E-05	Cytochrome P450
MELO3C011413.2	323,167111	1,34207876	0,391642049	3,426799452	6,11E-04	6,81E-03	cytochrome P450 78A5
MELO3C019206.2	797,587466	-2,33213805	0,328663954	-7,095813291	1,28593E-12	4,26709E-10	cytochrome P450 81E8-like
MELO3C018259.2	1198,48083	1,133243706	0,263316062	4,303739385	1,68E-05	4,13E-04	cytochrome P450 CYP82D47-like
MELO3C026491.2	77,03835804	-3,745944237	1,025740994	-3,651939679	2,60E-04	0,003570512	cytochrome P450 CYP82D47-like
MELO3C007480.2	214,3263893	2,258246851	0,523926913	4,31023258	1,63E-05	4,02E-04	Cytochrome P450 family protein
MELO3C007481.2	89,66505357	2,956621002	0,870395236	3,396871766	6,82E-04	7,44E-03	Cytochrome P450, putative
MELO3C026759.2	1218,575927	-1,015658183	0,276107553	-3,678487505	2,35E-04	3,30E-03	Eukaryotic initiation factor 4F subunit p150 isoform 1
MELO3C035644.2	488,3204186	1,573628969	0,441137942	3,567203855	0,000360811	0,004560616	eukaryotic translation initiation factor 3 subunit A-like
MELO3C020380.2	7060,019315	-1,231358465	0,20444974	-6,022933427	1,71284E-09	2,11163E-07	Eukaryotic translation initiation factor 4G
MELO3C021195.2	280,0171795	-8,231725733	1,291353302	-6,374495436	1,84E-10	3,26E-08	LOW QUALITY PROTEIN: pollen-specific leucine-rich repeat extensin-like protein 3
MELO3C000439.2	32,60402736	2,458449412	0,625765802	3,928705284	8,54044E-05	0,001502485	MACPF domain-containing protein NSL1
MELO3C006940.2	593,67716	-1,288716306	0,290021555	-4,443519047	8,85E-06	0,000247278	MADS-box protein SOC1
MELO3C010769.2	814,6897542	-1,109187375	0,310945668	-3,567142077	3,61E-04	0,004560616	Protein EARLY FLOWERING 3
MELO3C013473.2	5485,980468	2,114765252	0,293208272	7,212502023	5,49E-13	2,11231E-10	tetraketide alpha-pyrone reductase 1
GMmut-M versus GM-M							
GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Gene_description
MELO3C004397.2	15,19993202	-8,174364274	2,399082222	-3,407288087	0,000656118	0,004703573	Alba DNA/RNA-binding protein
MELO3C023241.2	782,5566464	-5,802492331	0,849023883	-6,8343099	8,24E-12	6,00E-10	callose synthase 5-like
MELO3C015764.2	1698,234989	-1,072825018	0,255405293	-4,200480757	2,66E-05	0,000326627	F-box/LRR-repeat protein 17
MELO3C018902.2	859,8885756	-1,589810503	0,250776049	-6,339562763	2,30E-10	1,21E-08	O-fucosyltransferase family protein
MELO3C007031.2	314,47568	-2,336574137	0,457919495	-5,102587163	3,35E-07	7,46E-06	Polygalacturonase QRT3
MELO3C024046.2	749,3084162	1,442623507	0,338040752	4,267602349	1,98E-05	0,00025333	Protein XRI1
MELO3C002541.2	12,00075028	5,835825858	1,724513773	3,384041316	0,000714273	0,005033808	receptor-like serine/threonine-protein kinase SD1-8
MELO3C018777.2	317,270956	2,465954696	0,436118043	5,654328533	1,56E-08	5,12E-07	Serine/threonine-protein kinase
MELO3C018168.2	291,8477294	1,085532829	0,313230762	3,465600958	0,000529048	0,003961654	Serine/threonine-protein kinase
MELO3C016574.2	296,0353632	3,833411025	0,447799428	8,560553637	1,12E-17	2,72E-15	Serine/threonine-protein kinase
MELO3C031872.2	452,8391878	1,635217583	0,348188187	4,696361464	2,65E-06	4,49E-05	Serine/threonine-protein kinase
MELO3C016135.2	1917,036016	3,413824872	0,572641941	5,961534817	2,50E-09	1,03E-07	Serine/threonine-protein kinase
MELO3C007338.2	616,4394961	2,442796574	0,358111479	6,821329996	9,02E-12	6,47E-10	Serine/threonine-protein kinase
MELO3C002524.2	417,9389393	1,028557179	0,254874426	4,035544862	5,45E-05	0,000593225	Serine/threonine-protein kinase
MELO3C013473.2	5485,980468	1,524656182	0,29763661	5,122542492	3,01E-07	6,83E-06	tetraketide alpha-pyrone reductase 1
MELO3C010012.2	1158,509672	1,06708994	0,272805935	3,911534911	9,17E-05	0,000917833	Transmembrane protein
ANmut-M versus AN-M							
GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Gene_description
MELO3C020400.2	663,0962885	-1,365818728	0,446022444	-3,06221973	0,002197021	0,01427542	ABC transporter G family member 21
MELO3C007237.2	704,2133864	1,300945783	0,369715104	3,518779107	0,000433538	0,003778167	Apoptosis inhibitor 5-like protein API5
MELO3C005473.2	500,8277454	-1,189004515	0,252718281	-4,704861512	2,54E-06	5,04E-05	Cytochrome P450
MELO3C019206.2	797,587466	-1,413344816	0,330031895	-4,282449178	1,85E-05	0,000276837	cytochrome P450 81E8-like
MELO3C035644.2	488,3204186	1,57617869	0,444924833	3,542572974	0,000396244	0,003492468	eukaryotic translation initiation factor 3 subunit A-like
MELO3C020380.2	7060,019315	-1,086158722	0,20399723	-5,324379766	1,01E-07	3,22E-06	Eukaryotic translation initiation factor 4G
MELO3C021195.2	280,0171795	-10,92536171	0,896055537	-12,19272831	3,40E-34	4,65E-31	LOW QUALITY PROTEIN: pollen-specific leucine-rich repeat extensin-like protein 3
MELO3C013473.2	5485,980468	-1,175491161	0,295897684	-3,972627115	7,11E-05	0,000854312	tetraketide alpha-pyrone reductase 1