Supplementary Table S1. Primer and probe sequences for PCR, qPCR, and qRT-PCR

Assay	Type	Gene	Primer and probe sequences 5’ 3’	Concentration in the reaction	Amplicon length
Photosynthesis enhancement - qPCR	Reference	PtaRP	Forward: ATCAAGAAGGACTCAGCCAAA	250nM	111bp
			/5HEX/AGAAATCCC/ZEN/GGGTCAAGTGCTTCA/3IABkFQ/	125nM	
			Reverse: GTCCACATCCAGCGTGTAA	250nM	
	GOI	NptII	Forward: CGTTGGCTACCCGTGATATT	250nM	122bp
			/56-FAM/CCGCTTCCT/ZEN/CGTGCTTTACGGTAT/3IABkFQ/	125nM	
			Reverse: CTCGTCAAGAAGGCGATAGAAG	250nM	
	GOI	CrGDH	Forward: AAGGCGTACGAGCTGATG	250nM	116bp
			/5Cy5/AACCCGGGC/TAO/GTCATCCTCAA/3IAbRQSp/	125nM	
			Reverse: GAGGGCTTCAGGAACTTGAT	250nM	
	GOI	ChMS	Forward: ATGGAATGCCGCAGAGAAG	250nM	104bp
			/5TexRd-XN/ATACTTGGTCGCCGGATCAAACCC/3IAbRQSp/	125nM	
			Reverse: GATGCACATGTCCACTCAGAT	250nM	
Photosynthesis enhancement - qRT-PCR	Reference	PtaRP	Forward: ATCAAGAAGGACTCAGCCAAA	250nM	111bp
			/5HEX/AGAAATCCC/ZEN/GGGTCAAGTGCTTCA/3IABkFQ/	125nM	
			Reverse: GTCCACATCCAGCGTGTAA	250nM	
	Reference	PtaAct	Forward: CTGCAATGTATGTTGCCATCC	250nM	89bp
			/5HEX/ACTGGCATA/ZEN/CAGGGAAAGGACAGC/3IABkFQ/	125nM	
			Reverse: CACACCATCACCAGAATCCA	250nM	
	Reference	PtaPP2A-2	Forward: GAACCCTAAATAGCAACCTCTAAAT	250nM	141bp
			/5HEX/CCGGACGAT/ZEN/GACCTGAATCATTCCC/3IABkFQ/	125nM	
			Reverse: CTTCAAAGAGACTTTCGATTGATTG	250nM	
	Reference	PtaEF1B-1	Forward: CATCACTACCACTCCACACAA	125nM	85bp
			/5Quasar 705/TCCTCTTTC/Nova/AATCACACTGTTTATCGCTCT/3BHQ-3/	125nM	
			Reverse: TTTCAATTACTTGGAAGATGGGATG	250nM	
	GOI	PtaPLGG1	Forward: CAGGTGTCAAGATTTGCTTCATC	125nM	116bp
			/56-FAM/CTTTGCGTG/ZEN/GCGGGTTTCACAG/3IABkFQ/	125nM	
			Reverse: AGCGTCAGTCATTTCTGTCTT	250nM	
	GOI	CrGDH	Forward: AAGGCGTACGAGCTGATG	250nM	116bp
			/5Cy5/AACCCGGGC/TAO/GTCATCCTCAA/3IAbRQSp/	125nM	
			Reverse: GAGGGCTTCAGGAACTTGAT	250nM	
	GOI	ChMS	Forward: ATGGAATGCCGCAGAGAAG	125nM	104bp
			/5TexRd-XN/ATACTTGGTCGCCGGATCAAACCC/3IAbRQSp/	125nM	
			Reverse: GATGCACATGTCCACTCAGAT	250nM	
PCR validation of transgenic status	GOI	NptII	Forward: CTCTCAATTCTCTCTACCGTGATC	500nM	932bp
			Reverse: TTTTCCACCATGATATTCGGCAAG	500nM	
	GOI	CrGDH	Forward: TGCTGGATCTCGATCTTGTTTTCTC	500nM	1662bp
			Reverse: TTGATGATCTCAATGGGGTTGTCC	500nM	
	GOI	ChMS	Forward: CCGACACTCTTAATGGAAGTAAAAGTG	500nM	1037bp
			Reverse: GGAGTGCTCCATTTTGGGAAG	500nM	

Supplementary Table S2. Growth rate and copy number estimation of transgenes in transgenic events and controls. * indicates non-transgenic control, ** indicates an event that has been through transformation but lacks transgenes so acts as a transformation control.

			Presence or copy number of transgene						
T0 event name	**C1 Group**	**Growth rate (GR, cm^3/day)**	**GR increase over the best in-group control (%)**	**nptII**	**CrGDH**	**ChMS**	**Field trial selection**	**Extended GH evaluation**	**Notes**
CT717-2*	1	0.79	0	no	no	no			
CT717-1*	1	0.71	-10	no	no	no			
1	1	0.80	1	2	2	2	yes		
2	1	0.52	-34	yes	yes	yes			
3	1	0.62	-22	yes	yes	yes			
4	1	0.64	-19	yes	yes	yes			
5	1	0.77	-3	1	1	1	yes		
6	1	0.68	-14	yes	yes	yes			
7	1	1.03	30	3	3	4	yes		
CT717-4*	2	0.86	0	no	no	no			
8-9	2	0.25	-71	yes	yes	yes			
13-15B	2	0.99	15	1	1	1	yes		
16-20**	2	0.69	-19	0	0	0	yes		
1C	2	0.70	-18	3	4	4	yes		
2G	2	0.68	-20	yes	yes	yes			
2H	2	0.82	-5	1	1	1	yes		
3D	2	0.79	-8	yes	yes	yes			Early branching

4A	2	0.77	-10	3	3	4	yes		
4B	2	0.82	-4	3	3	4	yes		
4G	2	0.57	-33	yes	yes	yes			
5C	2	0.82	-5	1	1	1	yes		
5E	2	0.76	-11	yes	yes	yes			Early branching
8-9D**	2	0.83	-3	0	0	0	yes		
CT717-9*	3	0.92	0	no	no	no		yes	
CT717-3*	3	0.83	-9	no	no	no		yes	
13-15E	3	1.26	38	1	1	1	yes	yes	
1A	3	0.64	-30	yes	yes	yes			
3G	3	0.94	2	yes	yes	yes			
5A	3	0.95	4	1	1	1	yes	yes	
6D	3	0.77	-16	yes	yes	yes			
7F	3	0.98	6	yes	yes	yes		yes	
8-9A	3	1.01	10	yes	yes	yes		yes	

Supplementary Table S3. Relative expression level of transgenes in various transgenic events

Event Name	Relative Expression		
	nptll	**ChMS**	**CrGDH**
LC0102.2C	++++	+++++	+++
LC0102.2G	+++++	+++++	++
LC0102.2A	+++	++++	++
LC0102.3C	+++	++++	++
LC0102.5F	+++	++++	+++
LC0102.21-25A	+++	+++	+++
LC0102.5E	++++	+++++	+++
LC0102.3H	+++	++++	++
LC0102.13-15 B	++	++++	+
LC0102.2H	++++	++++	+++
LC0102.5A	++++	+++++	++
LC0102.21-25E	++++	+++++	+++
LC0102.68	+++	++++	+++
LC0102.5C	+++++	+++++	++++
LC0102.6	++	++	++
LC01022	+++	+++	+++
LC0102.1	+++++	+++	+++
LC0102.3	++++	+++	++++
LC0102.5	+++	++	+++
LC0102.4G	+++	+++++	++
LC0102.2F	++	+++++	+
LC0102.1B	+++	++++	+
LC0102.1C	+++++	+++	++++
LC0102.13-15	++	+++	+++
LC0102.4	+++	++	++
LC0102.16-20B**	-	-	-
CT717-1*	-	-	-
LC0102.4A	+++	++++	+
LC0102.7	++++	++	+
LC0102.4B	++++	+++++	++
LC0102.8-9A	+++	++++	+++
CT717-2*	-	-	-
LC0102.3F	+++	+++++	+++
LC0102.3B	+++	++++	+
LC0102.3D	++	+++++	+++
LC0102.1A	+++++	++++	+++++
LC0102.6D	++++	+++++	++++
LC0102.1D	++++	+++++	+++
LC0102.16-20**	-	-	-
LC0102.7F	+++	++++	++
LC0102.8-9	-	-	-
LC0102.13-15E	+++	++++	+++
LC0102.8-9D**	-	-	-

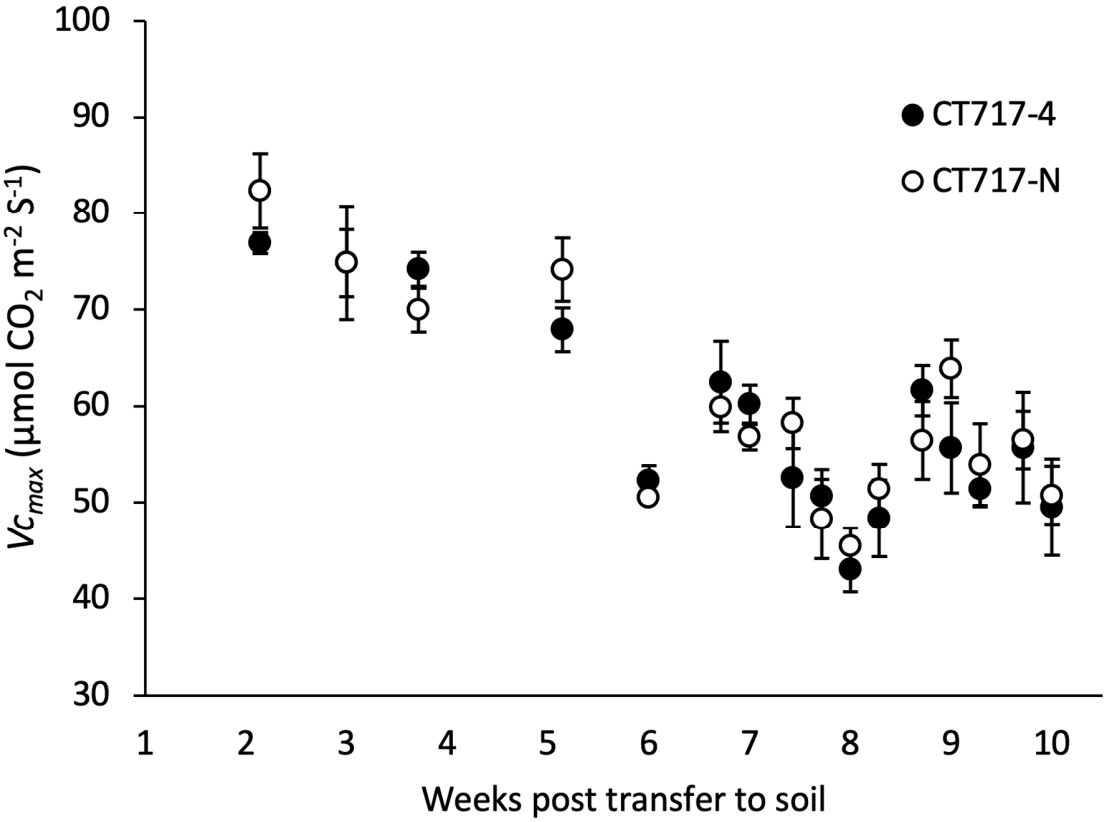
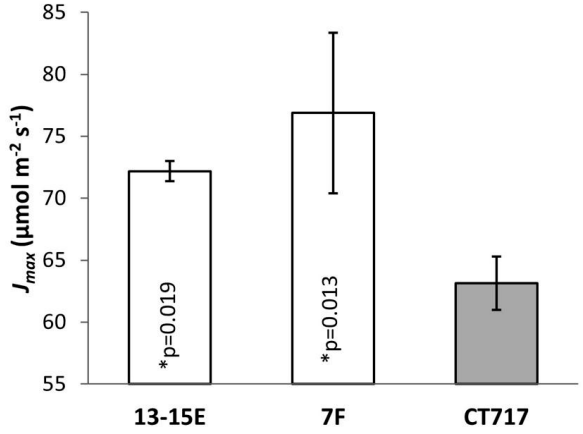
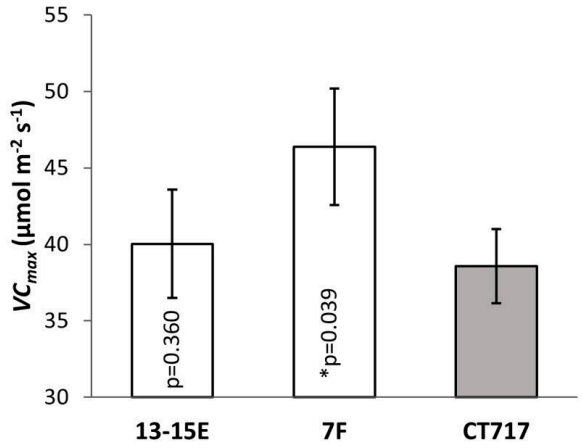


Figure S1. Photosynthesis activity in the form of Vc_{max} in poplar plants after transplanting to soil pots. Post transplanting, light intensity was gradually increased from 50 µmol $m^{-2}s^{-1}$ at crown height to 300 µmol $m^{-2}s^{-1}$ at 50 µmol $m^{-2}s^{-1}$ per day. Growth conditions: RH, 60%, 16h/8h light/dark cycle, with temperature at 25ºC during the day and 22ºC at night. Gas-exchange measurement was performed weekly starting from 2 weeks post soil. More frequent measurement was performed after 6 weeks post transplanting. Vc_{max} values were derived from the A-Ci curves. CT717-4, 10 ramets propagated from a soil grown CT717 mother. CT717-N, 10 ramets propagated in tissue culture and directly transferred to soil.

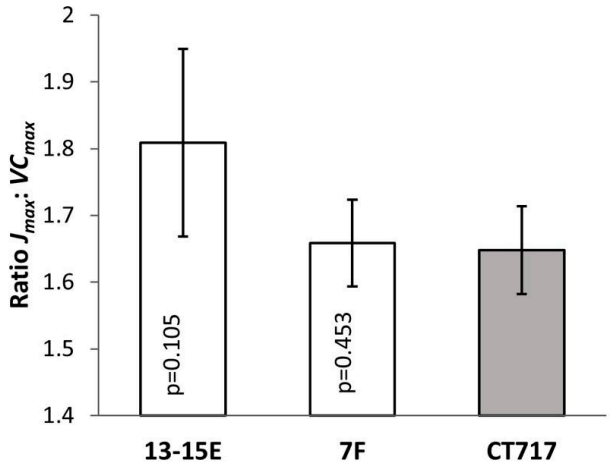
a


85
80
75
70
65
60
55
J_{max} (µmol m^{-2} s^{-1})
*p=0.019
*p=0.013
13-15E
7F
CT717

b


55
50
45
40
35
30
VC_{max} (µmol m^{-2} s^{-1})
p=0.360
*p=0.039
13-15E
7F
CT717

c



d

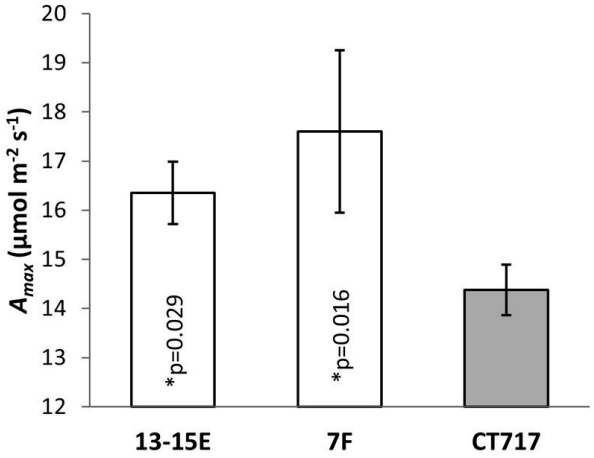


Figure S2. Determination of photosynthetic parameters J_{max} (a), Vc_{max} (b), J_{max}:Vc_{max} ratio (c), and A_{max} (d) in 18 weeks old C1 experimental plants. Number of ramets: CT717 non-transgenic control, 5; Event 13-15E, 2; Event 7F, 3. Error bars indicate SEM. All *p*-values were calculated using t-test and represent the difference compared to the non-transgenic CT717 control plants.

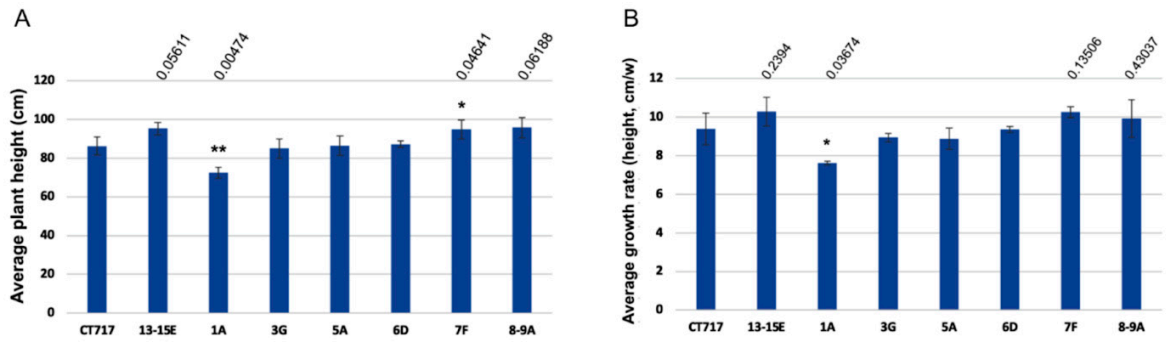


Figure S3. Comparison of plant growth between transgenic events and non-transgenic control 9 weeks post transplanting. Number of ramets: CT717, 5; 13-15E, 2; All others, 3 per event. **a.** Average plant height at week 9. **b.** Average plant height growth rate from week 1 post transplanting (cm/week).

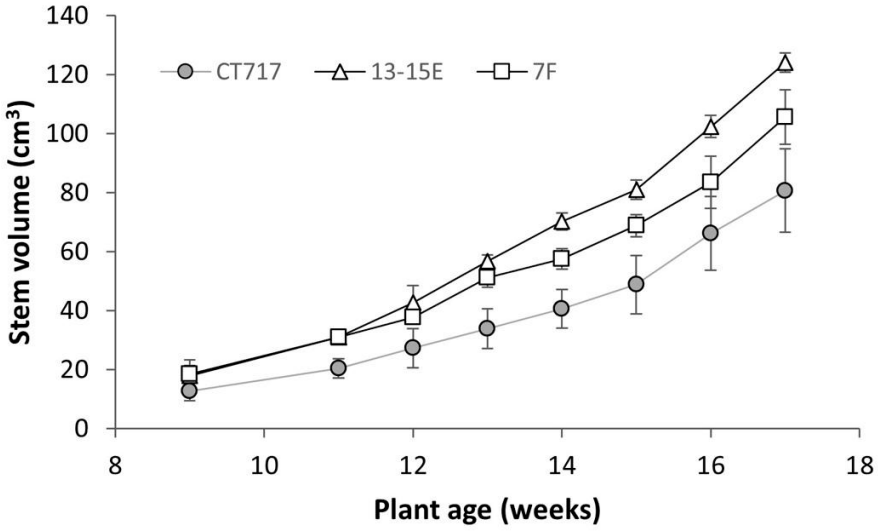


Figure S4. Weekly stem volume growth curves of lead transgenic events 13-15E and 7F plotted against non-transgenic control CT717 from week 9 to 17. C1 experimental plants. Number of ramets: Event 13-15E, 2; Event 7F, 3; Non-transgenic control CT717, 5.

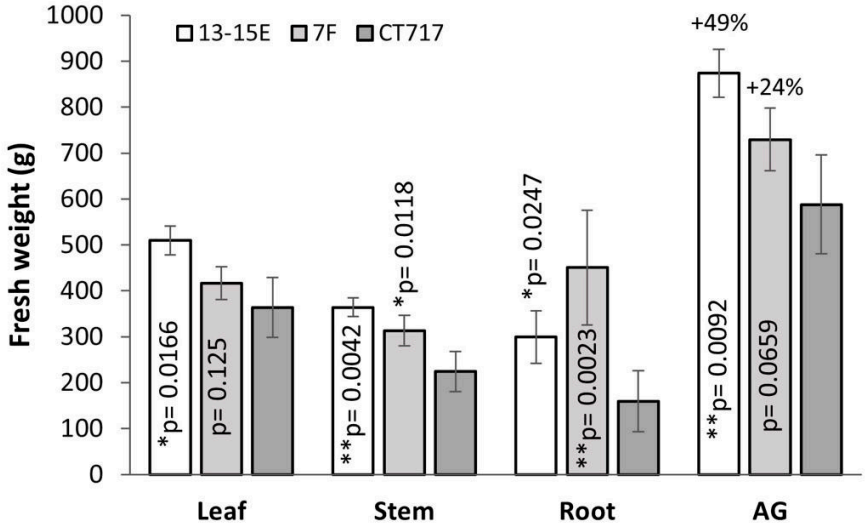
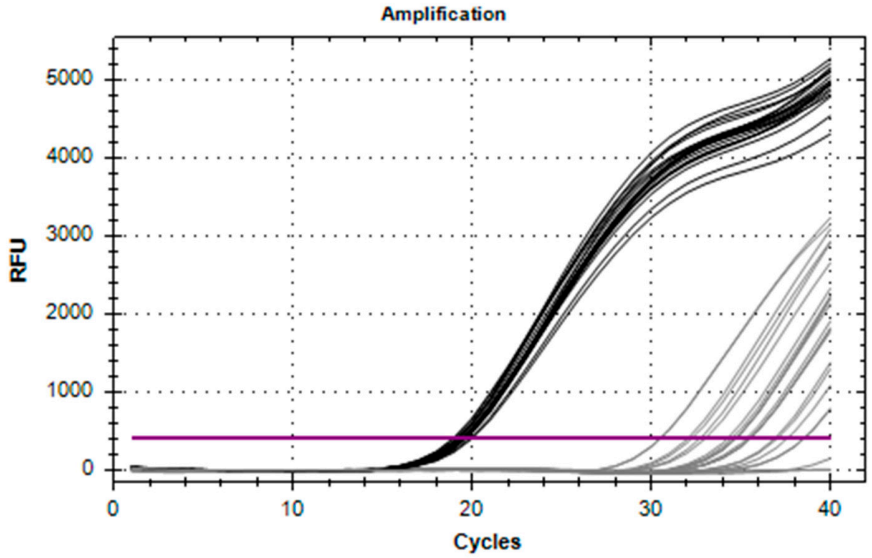


Figure S5. Fresh weight (g) of harvested 21-weeks old C1 experimental plants. Number of ramets: Event 13-15E, 2; Event 7F, 3; CT717 control plants, 5. All *p*-values were calculated using t-test and represent the difference compared to the non-transgenic CT717 control plants.

a.



b.

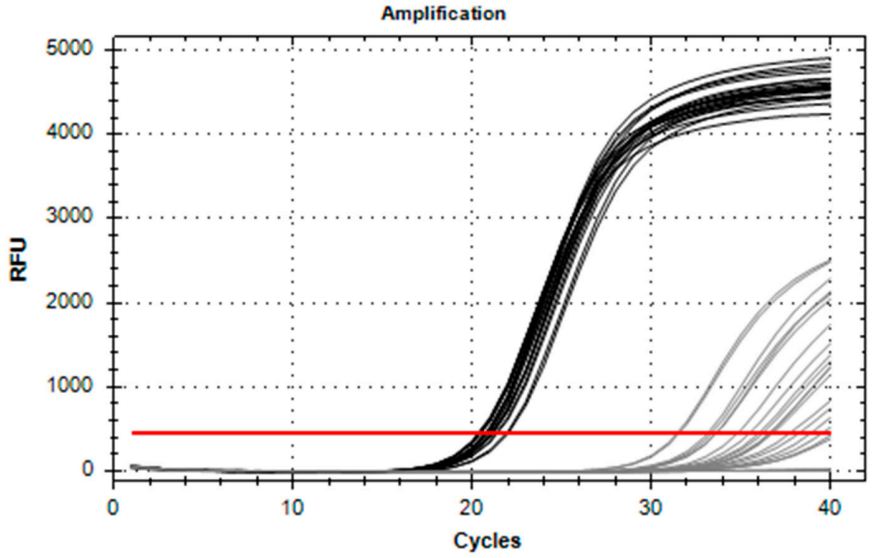
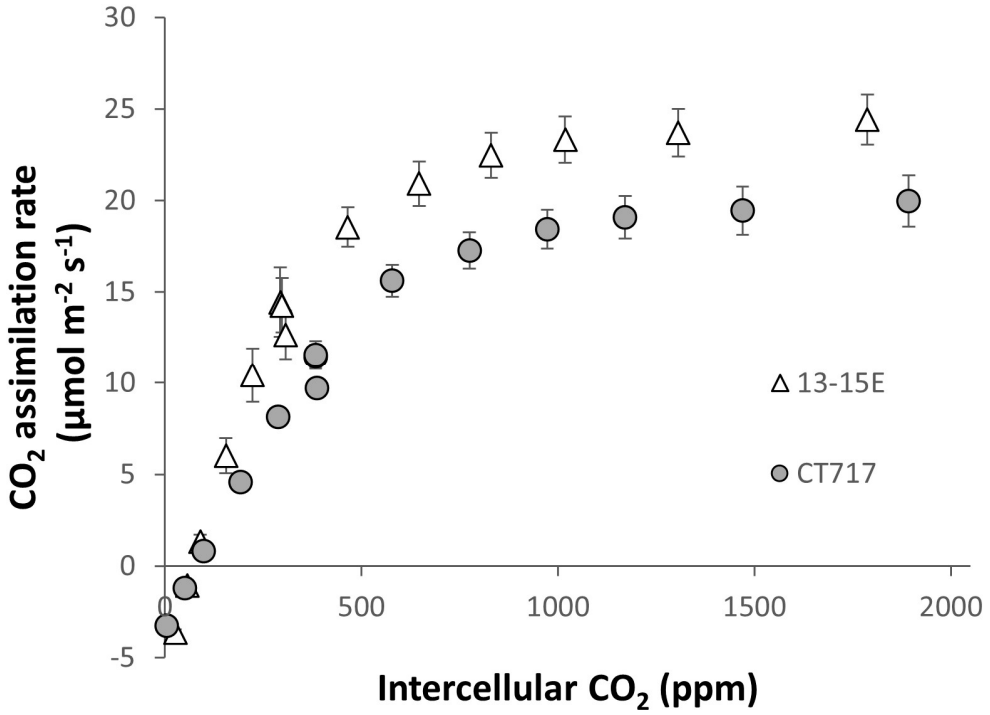
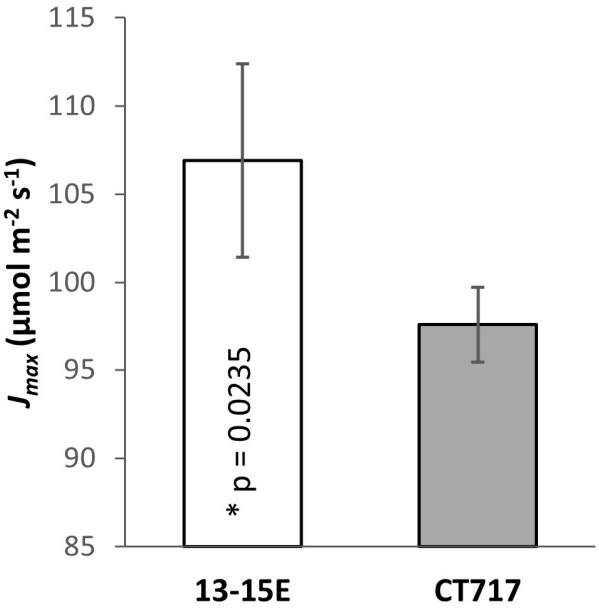


Figure S6. GDH and MS expression in 13-15E and CT717-3. qPCR of GDH (**a**) and MS (**b**) expression in 13-15E (in black) and CT717-3 (in gray). Number of ramets: Event 13-15E, 10; CT717 control plants, 11.

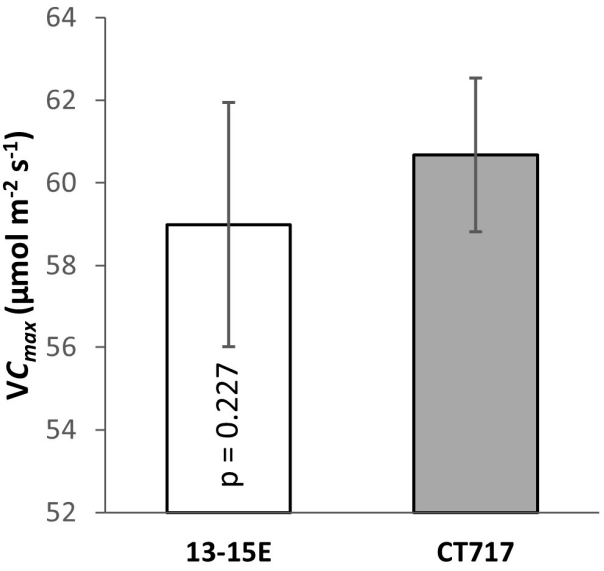
a.


30
25
20
15
10
5
0
-5
0
500
1000
1500
2000
CO2 assimilation rate (µmol m-2 s-1)
Intercellular CO2 (ppm)
13-15E
CT717

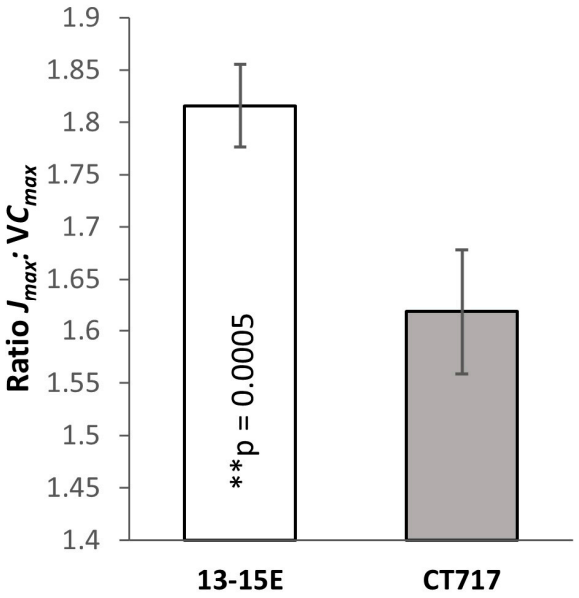
b.


115
110
105
100
95
90
85
Jmax (µmol m-2 s-1)
* p = 0.0235
13-15E
CT717

c.


64
62
60
58
56
54
52
VC_{max} (µmol m^{-2} s^{-1})
p = 0.227
13-15E
CT717

d.


1.9
1.85
1.8
1.75
1.7
1.65
1.6
1.55
1.5
1.45
1.4
Ratio J_{max}: VC_{max}
**p = 0.0005
13-15E
CT717

e.

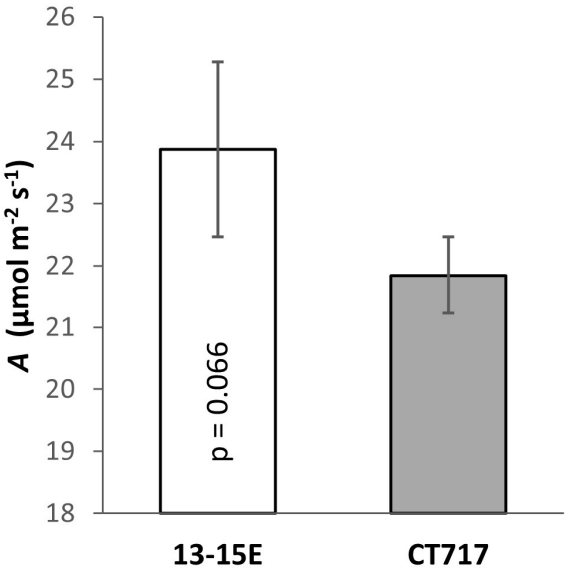


Figure S7. Photosynthetic activity measurement from plants of another C2 repeat experiment. a. A-Ci curve of 14 weeks old plants. Number of ramets: 3 for each event. Error bars indicate SEM. **b-e.** Determination of photosynthetic parameters J_{max} (**b**), Vc_{max} (**c**), J_{max}:Vc_{max} ratio (**d**), and A_{max} (**e**) in plants of 9-11 weeks old. Number of ramets: Event 13-15E, 7; Events 7 and 5C, 5 each; Transgenic escape 16-20 and non transgenic control CT717, 7 each. All *p*-values were calculated using t-test and represent the difference compared to the non-transgenic CT717 control plants.

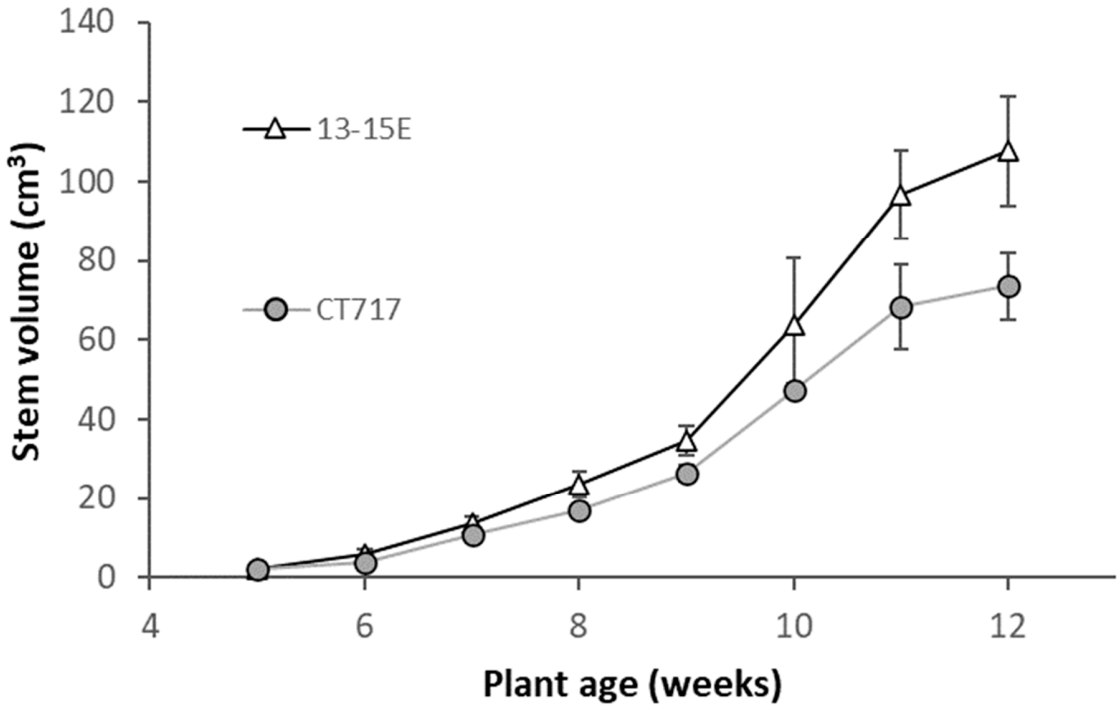


Figure S8. Stem growth rate comparison on C2 experimental plants. Weekly stem volume growth curves from week 4 to 12 for events 13-15E and CT717. Number of ramets 5 for each event.