

Constitutive expression of *eIF5A3* increases biomass yield in an elite alfalfa cultivar

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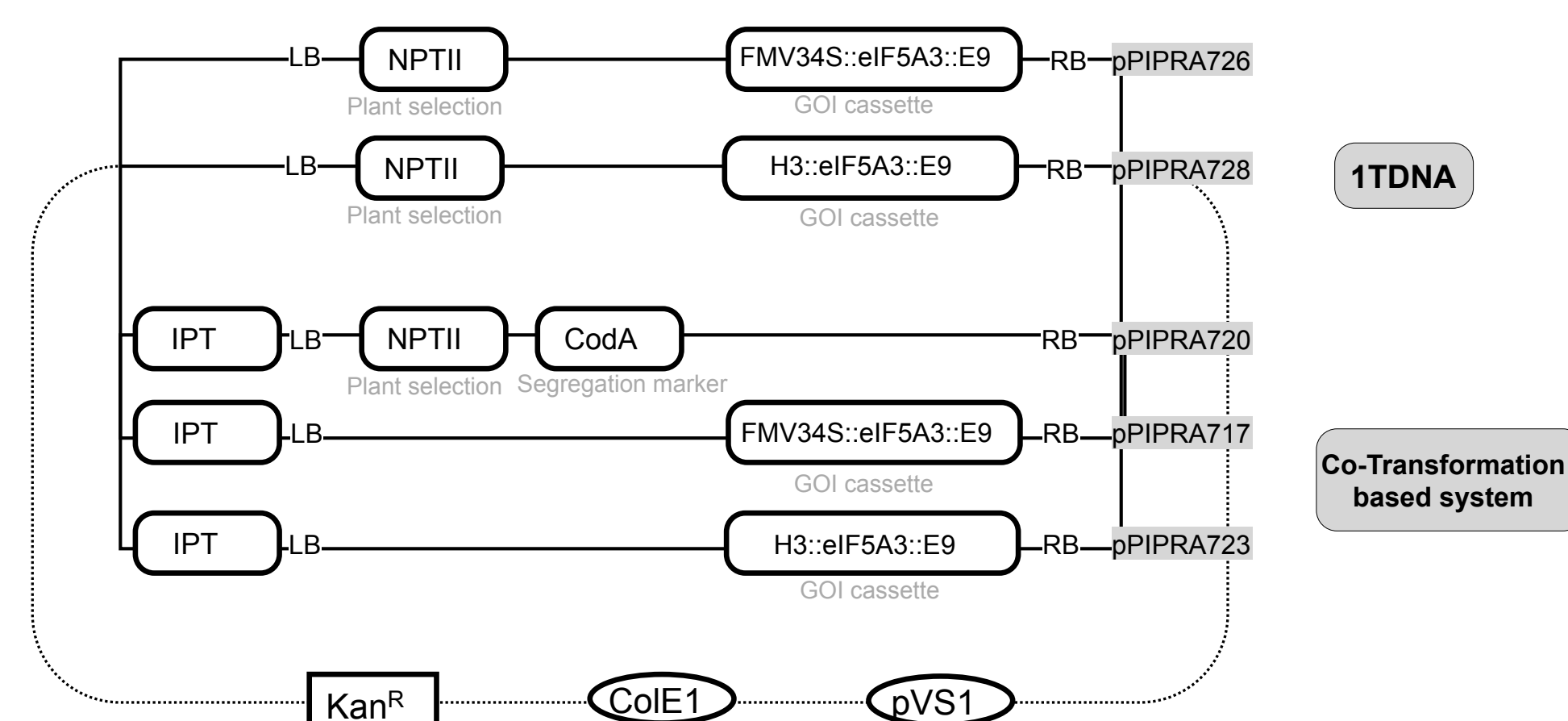
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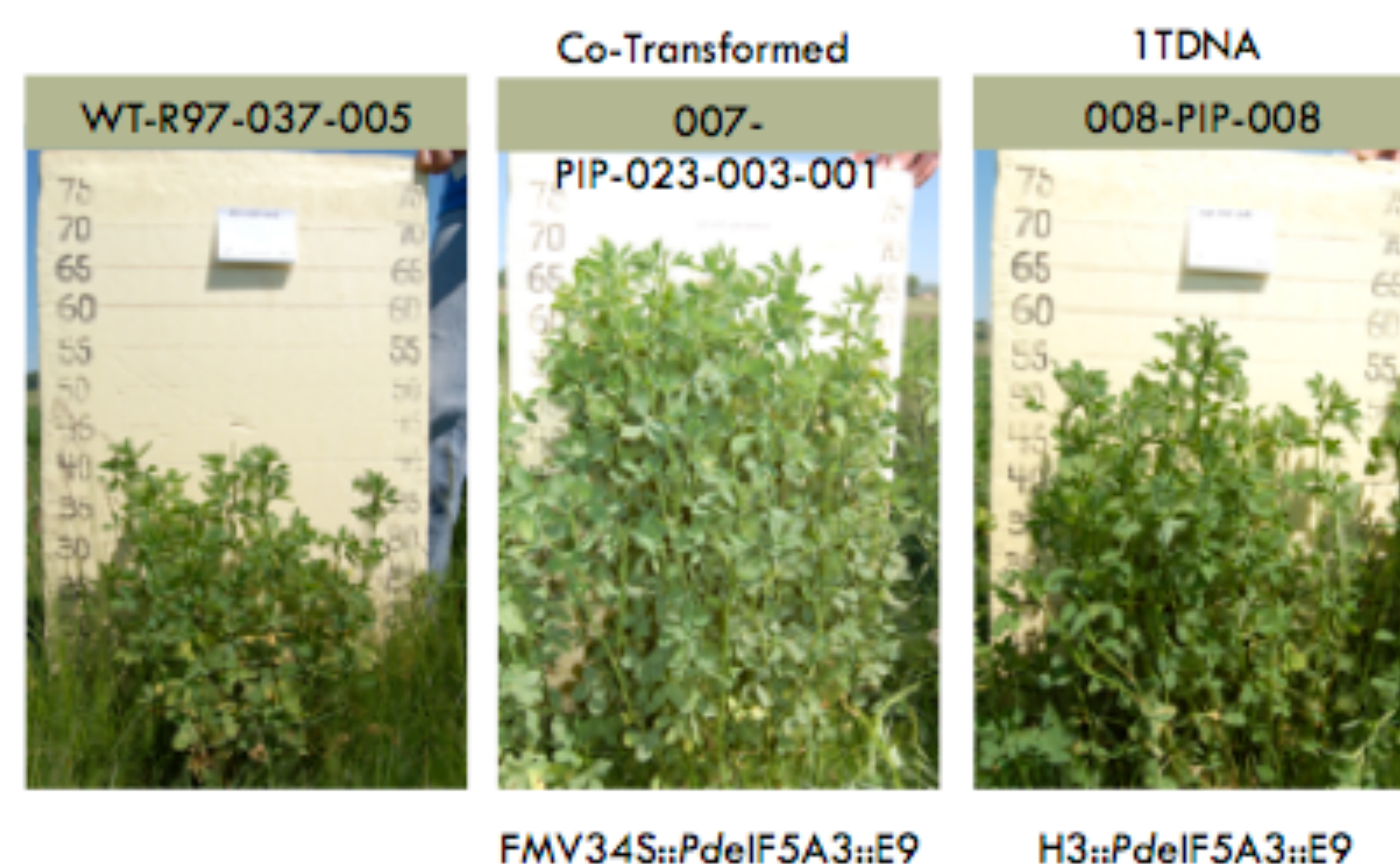
INTRODUCTION

As a source of high quality forage for ruminants, alfalfa (*Medicago sativa* L.) makes major, yet often unrecognized, contributions to global food production¹. Not only a key source of protein used by dairy farmers throughout the world, alfalfa is also an important component of sustainable agricultural ecosystems due to its ability to “fix” nitrogen, improve soil tilth, serve as an insectary for beneficial insects and prevent soil erosion¹. Increasing the biomass yield of alfalfa forage would not only increase the sustainability of producing dairy- and livestock-related food products for human consumption, by e.g. improving the water-use efficiency of this key agricultural crop¹, but could also make alfalfa an even more desirable candidate for biofuel production². Substantial increases in biomass production were recently achieved in the model plant *Arabidopsis thaliana* by constitutively expressing a gene encoding the eukaryotic translation initiation factor *eIF5A3*. In *Arabidopsis*, overexpression of poplar *eIF5A3* (*PdeIF5A3*) enhanced seed yield by 50-300% and it was proposed that this yield enhancement was due to the ability of the transformed plants to overcome growth inhibition in response to sub-lethal episodes of water or nutrient stress³. Using an efficient transformation method, we produced alfalfa lines that constitutively express *PdeIF5A3*, maintain high forage quality and, over two years of field trials, produced yields averaging 20-45% higher than the non-transgenic controls.

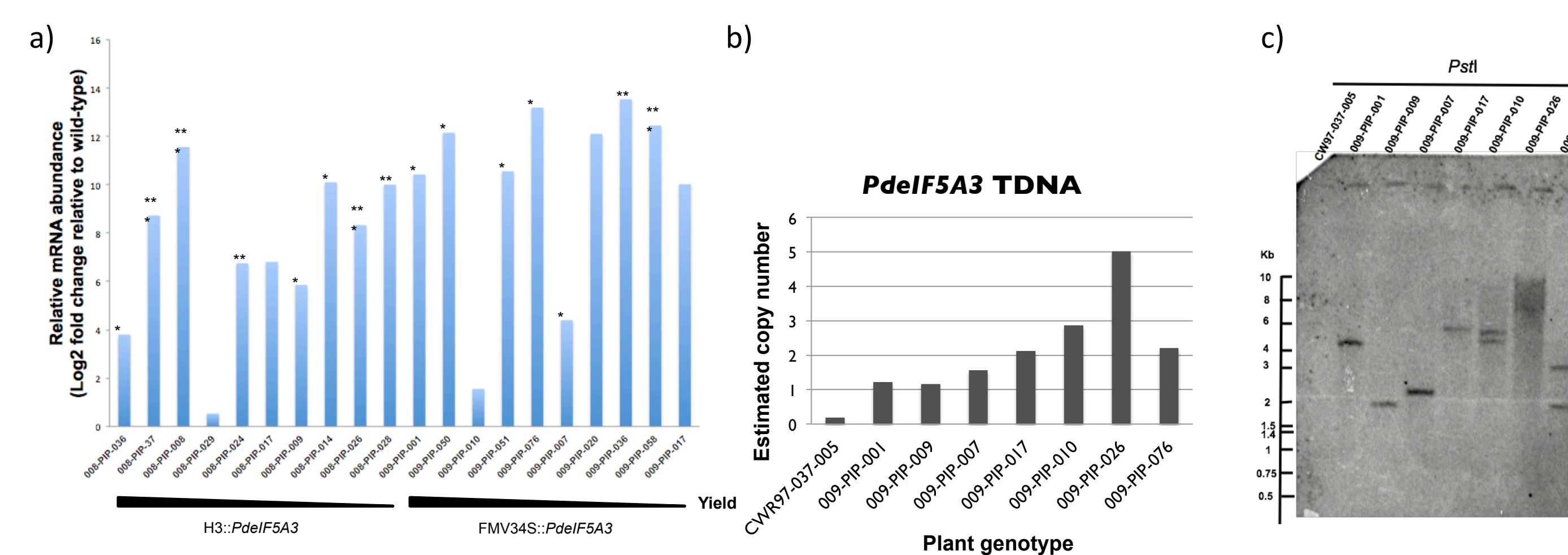
RESULTS



Chimeric gene constructs comprising the *PdeIF5A3* coding sequence expressed under the regulatory control of the *FMV34S* or alfalfa *H3* promoters were introduced into an elite alfalfa cultivar, using an optimized *Agrobacterium*-mediated transformation method.



Examples of increased herbage in field-grown transgenic plants. Left panel: CW-R97-037-005 non-transformed control line; middle panel: CW-R97-037-005 co-transformed with one construct designed to constitutively express *PdeIF5A3* via the *FMV34S* promoter and another to express a selectable marker right panel: CW-R97-037-005 transformed with a single construct designed to constitutively express *PdeIF5A3* via a plant-derived promoter of a histone-encoding *H3* gene as well as a selectable marker gene.



a) Levels of *PdeIF5A3* mRNA in transgenic alfalfa lines. Changes (log₂-fold) in accumulation of *PdeIF5A3* mRNA were determined in three biological replicates using qPCR; Values were normalized against endogenous 18S rRNA. *, fold changes significant at $P \leq 0.05$; **, fold changes significant at $P \leq 0.005$; ***, fold changes significant at $P \leq 0.0005$ based on a two-tailed t test. The black triangles above the construct delineations reflect levels of increased yield in each transformed line, higher yielding lines toward the left and lower yielding lines toward the right for each triangle. b) Invader assay results for *PdeIF5A3* gene copy number in the non-transformed control alfalfa line (far left) and seven alfalfa lines independently transformed with the *FMV34S::PdeIF5A3* chimeric construct. c) Southern gel blot results with the control (CW97-037-005) and the same representative transgenic alfalfa plants as shown in a).

PdeIF5A3 TDNA copy #	Hay Yield %	Hay Category	CP	ADF	NDF	TDN	RFV
2011							
H3::PdeIF5A3							
008-PIP-008	1	148	21.1	29.2	34.8	64.9	177.7
008-PIP-024	2	140	21.3	28.3	33.9	65.9	184.6
008-PIP-029	3	136	22.1	28.1	33.5	65.6	187.5
008-PIP-028	1	122	22.9	28.0	33.4	65.6	188.6
008-PIP-017	1	119	21.8	27.7	33.1	65.6	190.8
008-PIP-042	1	118	21.7	29.1	34.6	64.9	170.7
008-PIP-012	3	113	21.9	27.9	33.2	66.2	189.5
008-PIP-026	1	110	21.0	29.1	34.7	65.0	178.5
008-PIP-041	2	109	22.6	26.6	31.6	66.4	205.3
008-PIP-037	1	108	22.7	27.2	32.2	65.6	192.0
008-PIP-046	3	106	21.7	26.6	31.9	66.3	199.3
008-PIP-009	1	103	20.8	27.3	33.0	66.8	193.2
008-PIP-014	2	103	22.0	27.8	33.0	65.7	181.6
008-PIP-067	3	90	22.4	26.4	31.7	66.6	200.9
FMV34S::PdeIF5A3							
009-PIP-050	1	138	21.6	28.2	33.7	65.4	186.1
009-PIP-076	2	133	22.3	27.6	33.0	65.5	191.8
009-PIP-005	2	128	21.3	29.2	34.8	64.5	178.5
009-PIP-010	3	126	21.8	27.9	33.1	65.8	182.2
009-PIP-007	2	123	20.8	29.6	35.2	65.0	175.1
009-PIP-020	2	122	21.7	27.8	33.3	65.9	186.6
009-PIP-020	2	121	20.5	29.9	34.6	65.2	180.0
009-PIP-003	1	120	21.5	28.5	34.1	65.3	183.0
009-PIP-013	2	116	22.0	28.2	33.2	65.8	188.3
009-PIP-036	1	115	20.9	28.7	34.5	65.0	180.9
009-PIP-026	3	114	21.4	29.2	34.6	64.4	178.7
009-PIP-061	1	113	22.0	26.7	31.7	66.3	200.9
009-PIP-017	2	103	21.2	28.0	33.4	66.0	189.3
009-PIP-058	1	85	22.2	26.3	31.4	66.6	204.3
Control CW-R97-037-005	0	100	21.9	26.4	31.3	67.3	204.0
2012							
H3::PdeIF5A3							
008-PIP-036	1	137	18.3	31.4	37.6	63.5	160.0
008-PIP-037	1	130	18.5	31.5	37.6	63.7	160.0
008-PIP-008	1	124	18.9	30.6	36.6	64.2	165.8
008-PIP-029	3	121	18.4	31.1	37.1	64.0	162.7
008-PIP-024	2	119	18.2	31.5	37.7	63.9	159.2
008-PIP-042	1	118	18.5	31.5	37.6	63.7	159.4
008-PIP-046	3	114	19.0	29.9	35.7	64.4	171.8
008-PIP-013	2	111	18.3	30.3	36.2	64.4	167.7
008-PIP-009	1	108	17.7	31.0	37.3	63.8	162.3
008-PIP-014	2	104	18.2	30.4	36.4	64.2	167.1
008-PIP-028	1	111	18.4	31.2	37.3	63.6	161.2
008-PIP-027	3	98	19.1	29.3	35.2	64.9	175.5
008-PIP-028	1	95	18.4	30.9	37.0	64.1	163.6
008-PIP-012	3	86	18.5	30.0	36.0	64.7	169.4
008-PIP-041	2	87	18.4	29.8	35.9	64.8	171.2
FMV34S::PdeIF5A3							
009-PIP-001	1	143	18.6	31.3	37.3	63.3	161.1
009-PIP-050	1	132	18.4	30.7	36.8	63.8	164.8
009-PIP-010	3	130	18.5	30.0	36.0	64.4	170.0
009-PIP-051	2	126	18.3	30.7	36.8	64.1	165.1
009-PIP-076	2	114	19.0	30.2	36.1	64.5	168.4
009-PIP-007	2	110	18.4	31.1	37.4	63.6	162.2
009-PIP-020	2	109	18.9	29.4	35.4	65.0	174.4
009-PIP-036	3	106	18.8	30.1	36.0	64.4	170.1
009-PIP-058	1	108	18.5	30.1	36.2	64.8	169.2
009-PIP-026	1	89	18.5	30.9	37.0	64.0	161.7
009-PIP-005	2	92	19.0	28.9	34.8	65.1	177.8
009-PIP-013	2	82	18.7	30.1	36.1	64.3	169.5
009-PIP-017	2	81	18.1	30.5	36.6	64.3	165.8
009-PIP-003	1	89	18.2	30.7	36.8	64.4	164.9
Control CW-R97-037-005	0	100	18.7	29.6	35.5	65.0	173.0

Comparisons of transgene copy numbers, hay yields and forage quality in transgenic alfalfa constitutively expressing *PdeIF5A3* and control lines in field trials over years 2011 and 2012. Hay Yield % is as compared to the non-transformed control, CW-R97-037-005, which was set at 100% and measured as dry weight biomass. Categories of hay quality are from USDA quality guidelines adapted in 2002 (2006 USDA Livestock, Hay and Grain Market new, Moses Lake, WA). CP, crude protein; ADF, acid detergent fiber, NDF, neutral detergent fiber; TDN, total digestible nutrients; RFV, relative feed value.

CONCLUSIONS

Since the 1930s, alfalfa breeding programs have focused primarily on increasing biomass yield. And while conventional breeding in alfalfa has generated many high-yielding alfalfa varieties, the gains have been limited to about 0.2-0.3% per year and have been correlated with compromised forage quality. Our results suggest that this transgenic strategy of constitutively expressing *eIF5A3* in alfalfa can substantially increase biomass yield, up to 40% or more, with little loss of forage quality.

REFERENCES

- Putnam, D.H. et al. in California Alfalfa and Forage Association, 2001.
- Lamb, J.F. in Meeting Abstract 2011.
- Ma, F.S. et al. *Plant Cell Environ* **33**, 1682-1696 (2010).