Table S1. *Miscanthus* lines screened in vitro for specific characteristics.

| Entry | Accession # | Source(s) | Callus | Callus quality* | Regeneration ** | Transformation efficiency (%)*** | Lines used to test editing | Abreviations used in the manuscript |
|---|---|---|---|---|---|---|---|---|
| Miscanthus sinensis '10UI-008-2011-1-Row Replicated - CHA-115-7' | 10UI-008-2011-1-Row Replicated - CHA-115-7 | University of Illinois | Yes | Very good | Very good | 3.7 (Biolistics) | **Yes** | **UI1** |
| Miscanthus sinensis 'Purpurascens, Flame Grass' | Msi-P2 | Sow_Blessed_Seeds | Yes | Very good | Very good | 4 (Agrobacterium) | No | P2 |
| Miscanthus sinensis 'PMS-014' | PMS-014 | Wuhan Botanical Garden | Yes | Very good | Very good | 3 (Agrobacterium) | No | PMS-014 |
| Miscanthus sinensis 'Outdsidepride' | Msi-O1 | Outsidepride Seeds, LLC | Yes | Good | Very good | 0 (Agrobacterium) | No | O1 |
| Miscanthus sinensis 'Outdsidepride New Hybrid' | Msi-ONH1 | Outsidepride Seeds, LLC | Yes | Good | Very good | 0 (Agrobacterium) | No | ONH1 |
| Miscanthus sinensis 'Silberfeder (Silver Feather)' | UI10-00085 | Bluemel | Yes | Good | Good | Not tested | No | |
| Miscanthus sinensis 'Undine' | Msi-UN1 | HudsonAlpha | Yes | Good | Fair | 0 (Agro and biolistics) | No | UN |
| Miscanthus sinensis 'US47-0011' | CANE9233 | USDA-NPGS | Yes | Good | Not tested | Not tested | No | US47 |
| Miscanthus sinensis 'PMS-009' | PMS-009 | WBG | Yes | Fair | Good | 0 (Biolistics) | No | PMS-009 |
| Miscanthus sinensis 'Malepartus' | UI12-00002 | Walla Walla | Yes | Fair | Fair | Not tested | No | |
| Miscanthus sinensis 'Onna-1b' | Onna-1b | University of Illinois | Yes | Fair | Fair | Not tested | No | |
| Miscanthus sinensis 'Onna-1a' | Onna-1a | University of Illinois | Yes | Fair | Fair | Not tested | No | |
| Miscanthus sinensis 'Grosse Fontaine' | UI10-00053 | Bluemel | Yes | Fair | Fair | 0 (Agro and biolistics) | No | |
| Miscanthus sinensis 'KS1' | Msi-KS1 | Polish Academy of Sciences | Yes | Fair | Fair | 0 (Agro and biolistics) | No | KS1 |
| Miscanthus sinensis 'NC-2010-001 Nat Pop 2011-3-2-8' | UI14-00001 | University of Illinois | Yes | Fair | Poor | Not tested | No | |
| Miscanthus sinensis 'Purpurascens, Flame Grass' | Msi-P1 | Sow_Blessed_Seeds | Yes | Good | Good | 5.7 (Agrobacterium) | **Yes** | **P1** |
| Miscanthus sinensis 'Silberturm (Silver Tower)' | UI10-00088 | Bluemel | Yes | Poor | Poor | Not tested | No | |
| Miscanthus sinensis 'Gracillimus' | PI295764 | USDA-NPGS | Yes | Poor | Poor | Not tested | No | |
| Miscanthus sinensis var. condensatus 'Cosmopolitan' | UI10-00015 | ECG | Yes | Poor | Poor | Not tested | No | |
| Miscanthus sinensis 'Kaskade' (Digging Dog) | UI11-00014 | Juvik | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'Grosse Fontaine 4x' | UI11-00008 | Juvik | No | N/A | N/A | N/A | N/A | GF |
| Miscanthus sinensis 'November Sunset' | UI10-00075 | Bluemel | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'Variegatus' | UI10-00097 | ECG | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'Blondo' | UI10-00030 | Walla Walla | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis ssp. condensatus 'US64-0007-01' | PI294605 | USDA-NPGS | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'US64-0004-02' | PI294602 | USDA-NPGS | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'Strictus' | UI10-00092 | Bluemel | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'PMS-038' | PMS-038 | WBG | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'Hokkaido Univ-selection-4' | JPN-2011-024 | HU | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'Miyazaki' | UI11-00001.5 | Japan | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'PF20014.001' | UI11-00029 | NEF | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis 'Emerald Shadow' x FP1-2 | 13UI-013R-36 | University of Illinois | Yes | Fair | Fair | Not tested | No | |
| Miscanthus x giganteus 3x '12UI-001-007' | 12UI-001-007 | UI | Yes | Fair | Fair | Not tested | No | |
| Miscanthus x giganteus 3x | 10UI-032-001 | University of Illinois | Yes | Fair | Fair | Not tested | No | |
| Miscanthus x giganteus 3x 'MSU-MFL1', 'Freedom' | MSU-MFL1 | Mississippi State University | Yes | Fair | Fair | 1-2 (Agrobacterium) | **Yes** | |
| Miscanthus sinensis x Miscanthus sacchariflorus BC 'Andante' | UI10-00023 | Bluemel | Yes | Fair | Fair | Not tested | No | |
| Miscanthus floridulus 'US56-0022-03' | PI230189 | USDA-NPGS | Yes | Poor | Poor | Not tested | No | |
| Miscanthus floridulus 'PI295762' | PI295762 | USDA-NPGS | Yes | Poor | Poor | Not tested | No | |
| Miscanthus x giganteus 3x '64-1' | UI10-00123 | | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis x Miscanthus sacchariflorus 2x 'Mt. Washington' | UI10-00072 | Long's Garden | No | N/A | N/A | N/A | N/A | |
| Miscanthus 'M75-062' | PI423566 | USDA-NPGS | No | N/A | N/A | N/A | N/A | |
| Miscanthus 'US64-0016-03' | PI302423 | USDA-NPGS | No | N/A | N/A | N/A | N/A | |
| Miscanthus sinensis Kaskade x M. sacchariflorus 4x 'Tohuku' | 13UI-008-R-001 | University of Illinois | No | N/A | N/A | N/A | N/A | |
| Miscanthus x giganteus 4x | 13UI-013R-66 | University of Illinois | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 4x 'Tohoku-2010-020' | JPN-2010-008 | HU | Yes | Very good | Very good | 9 (Biolistics) | **Yes** | **S1** |
| Miscanthus sacchariflorus 'RU2012-081' | RU2012-081 | USDA-VIR**** | Yes | Very good | Very good | Not tested | No | S4 |
| Miscanthus sacchariflorus 'RU2012-110' | RU2012-110 | USDA-VIR | Yes | Good | Good | 5.7 (Agrobacterium) | **Yes** | **S13** |
| Miscanthus sacchariflorus 4x 'EMI-5' | UI11-00027 | USDA-Canal Point | Yes | Good | Good | Not tested | No | S2 |
| Miscanthus sacchariflorus 'Robustus-Bluemel' | UI10-00009 | Bluemel | Yes | Good | Fair | Not tested | No | RB |
| Miscanthus sacchariflorus 'RU2012-062' | RU2012-062 | USDA-VIR | Yes | Fair | Fair | Not tested | No | |
| Miscanthus sacchariflorus 'RU2012-179' | RU2012-179 | USDA-VIR | Yes | Fair | Fair | Not tested | No | S3 |
| Miscanthus sacchariflorus 'RU2012-182' | RU2012-182 | USDA-VIR | Yes | Fair | Fair | Not tested | No | |
| Miscanthus sacchariflorus 'RU2012-185' | RU2012-185 | USDA-VIR | Yes | Fair | Fair | Not tested | No | S7 |
| Miscanthus sacchariflorus ssp. lutarioriparius 'PF30022' | UI11-00031 | NEF | Yes | Poor | Poor | Not tested | No | |
| Miscanthus sacchariflorus 4x 'Bluemel' | UI10-00117 | Bluemel | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 4x 'Gotemba Gold' | UI11-00005 | Glasshouse Works | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 4x 'JM11-019' | JM11-019 | HU | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 4x 'Hokkaido Univ-selection-3' | JPN-2011-023 | HU | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-016' | RU2012-016 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 4x 'RU2012-023' | RU2012-023 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-025' | RU2012-025 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-027' | RU2012-027 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-036' | RU2012-036 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-038' | RU2012-038 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-044' | RU2012-044 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-049' | RU2012-049 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 4x 'RU2012-055' | RU2012-055 | USDA-VIR | No | N/A | N/A | N/A | N/A | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Miscanthus sacchariflorus 'RU2012-059' | RU2012-059 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-064' | RU2012-064 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-068' | RU2012-068 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-070' | RU2012-070 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-082' | RU2012-082 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-087' | RU2012-087 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-096' | RU2012-096 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-097' | RU2012-097 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-101' | RU2012-101 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-126' | RU2012-126 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-136' | RU2012-136 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-144' | RU2012-144 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-145' | RU2012-145 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-151' | RU2012-151 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-153' | RU2012-153 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-165' | RU2012-165 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-197' | RU2012-197 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-203' | RU2012-203 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-206' | RU2012-206 | USDA-VIR | No | N/A | N/A | N/A | N/A | |
| Miscanthus sacchariflorus 'RU2012-209' | RU2012-209 | USDA-VIR | No | N/A | N/A | N/A | N/A | |

Contact for inquiries about this germplasm collection - esacks@illinois.edu

Ratings and corresponding colors relate to percentage of explants able to generate embryogennic calli, or percentage of embryogenic calli capable of shoot regeneration

Poor (orange): 1-20% responded

Fair (yellow): 21-40% responded

Good (blue): 41-60% responded

Very good (green): 61-80% responded

Excellent (none rated in this category): 81-100% responded

*Callus quality referred to percentage of calli that were embryogenic

**Regeneration referred to percentage of calli capable of shoot regeneration; 8-12 embryogenic calli (2-3 mm diameter) were transferred to regeneration media (RM; Table 1) for shoot regeneration, and assessed for ability to generate shoots after 4 weeks

***Efficiency based on reporter gene expression

****USDA-VIR Collection Expedition 2012

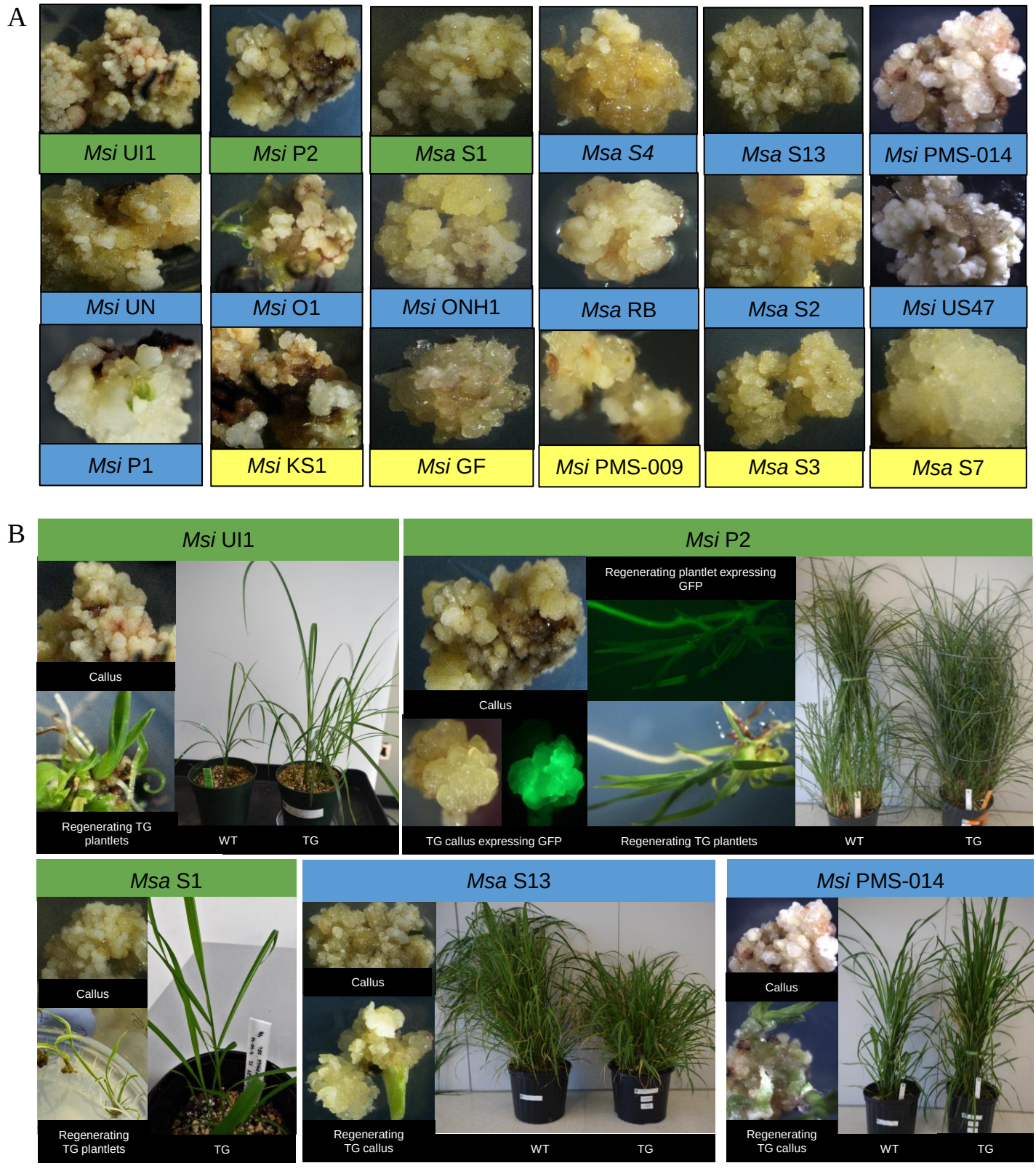
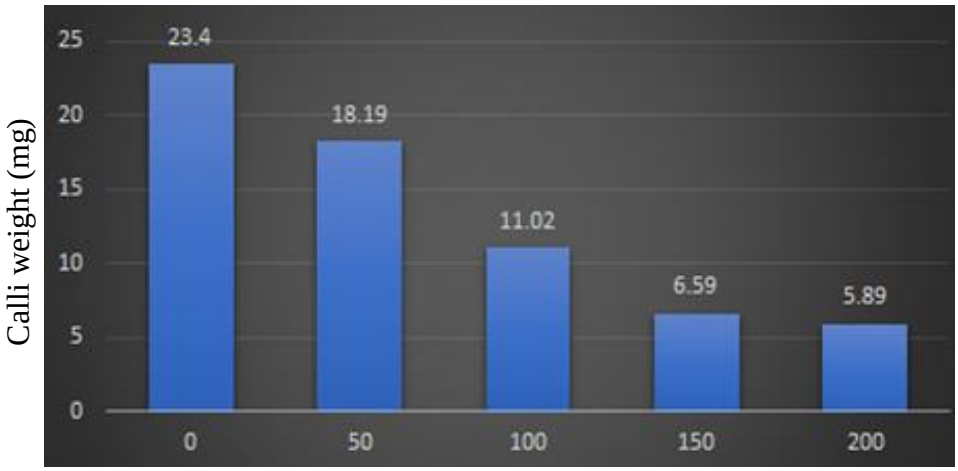



Fig. S1. Examples of *Miscanthus* calli, regenerants and transformants generated in initial genotype screenings. (A) Calli generated from immature inflorescences; 18 genotypes shown. (B) Genotypes were assessed for ability to regenerate shoots, and some were also assessed for ability to be transformed via biolistics or *A. tumefaciens*, screening for GFP expression. Footer/header colors referred to classifications based on percentage of calli that were embryogenic or percentage of calli capable of shoot regeneration: green=very good response (61-80%), blue=good response (41-60%), yellow=fair response (21-40%). TG=transgenic tissues/plants, WT=wild-type.

A




B

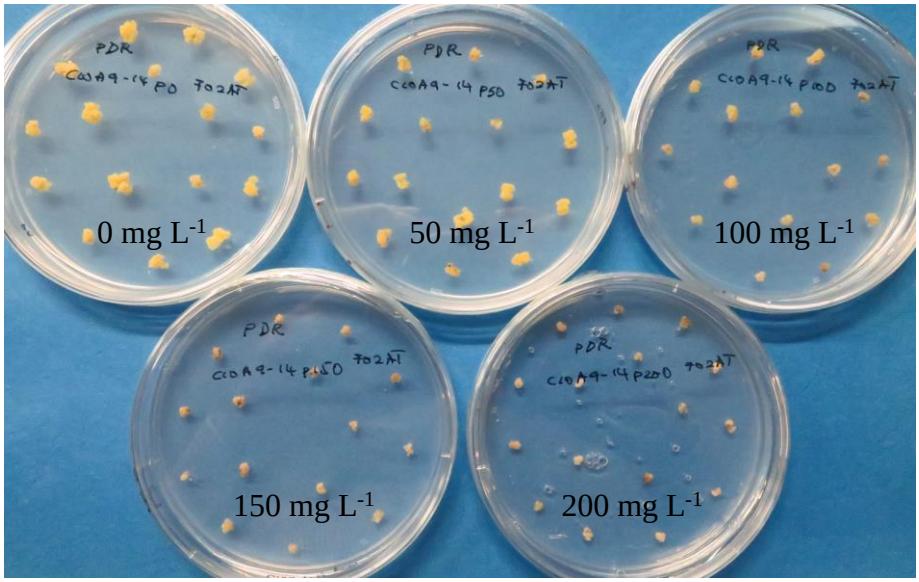



Fig. S2. Dose-response curve for *Miscanthus* using paromomycin.
Per treatment, 15 pieces of *Msi* UI1 calli ~2-3 mm in diameter were individually weighed then plated on CMM-1 + paromomycin at the following concentrations: 0, 50, 75, 100, 125, 150, 175, and 200 mg $L^{-1}$. After 3 wk incubation in the dark, the EC were individually weighed and visually evaluated for growth inhibition. (A) Average weight gain per callus on media containing different paromomycin concentrations. (B) Visual appearance of calli after 3 wk incubation on media containing paromomycin.

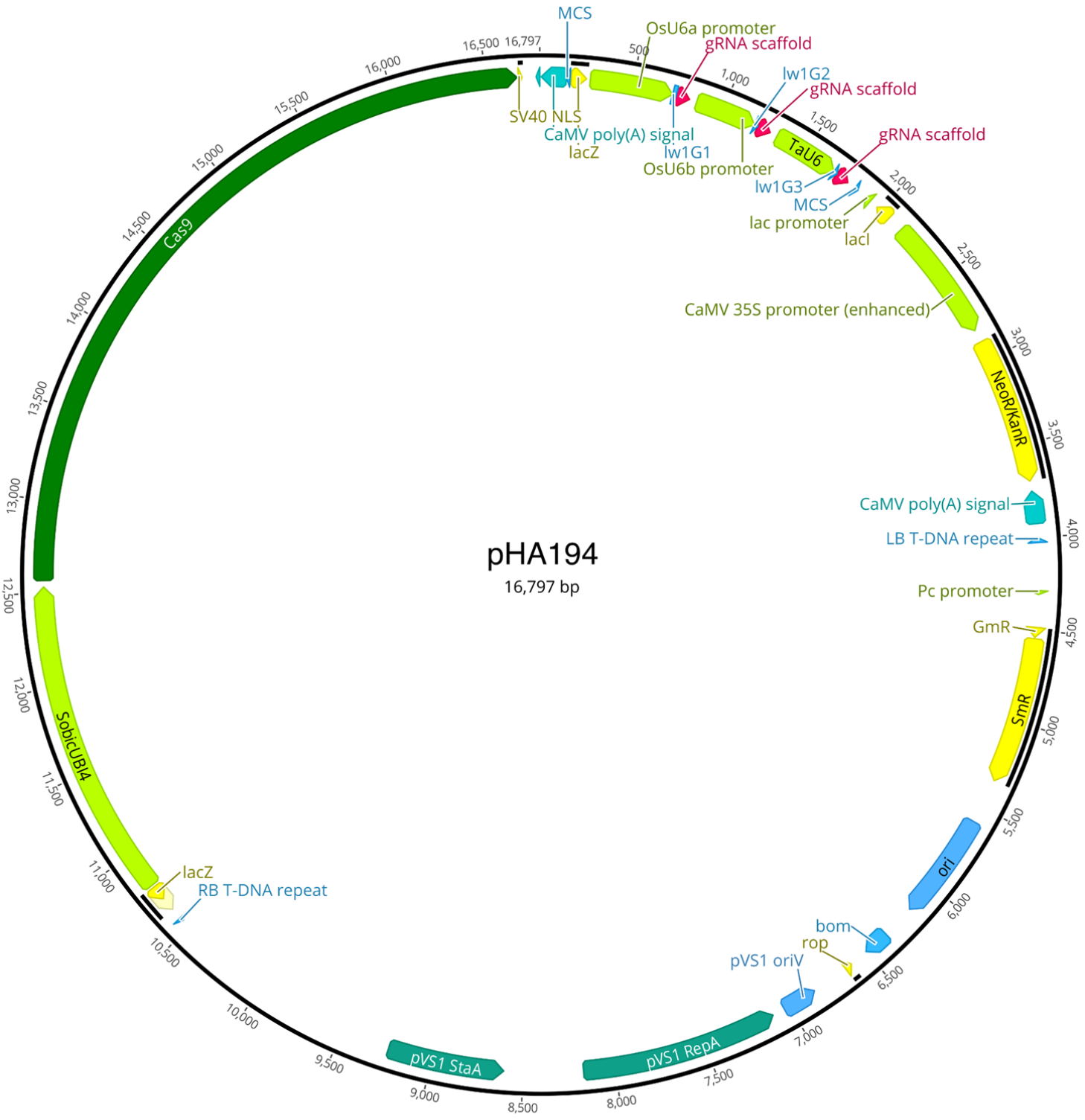



Fig. S3. Map of plasmid pHA194.
SobicUBI4, sorghum ubiquitin promoter; Cas9, sorghum codon-optimized Cas9; SV40 NLS, nuclear localization signal; CaMV poly(A) signal, terminator and poly(A) signal; OsU6a, OsU6b, & TaU6, class/type III RNA polymerase III promoters; gRNA scaffold, tracrRNA backbone sequence; NeoR/KanR, kanamycin/paromomycin resistance marker; SmR, spectinomycin resistance marker. Refer to Additional file 4.

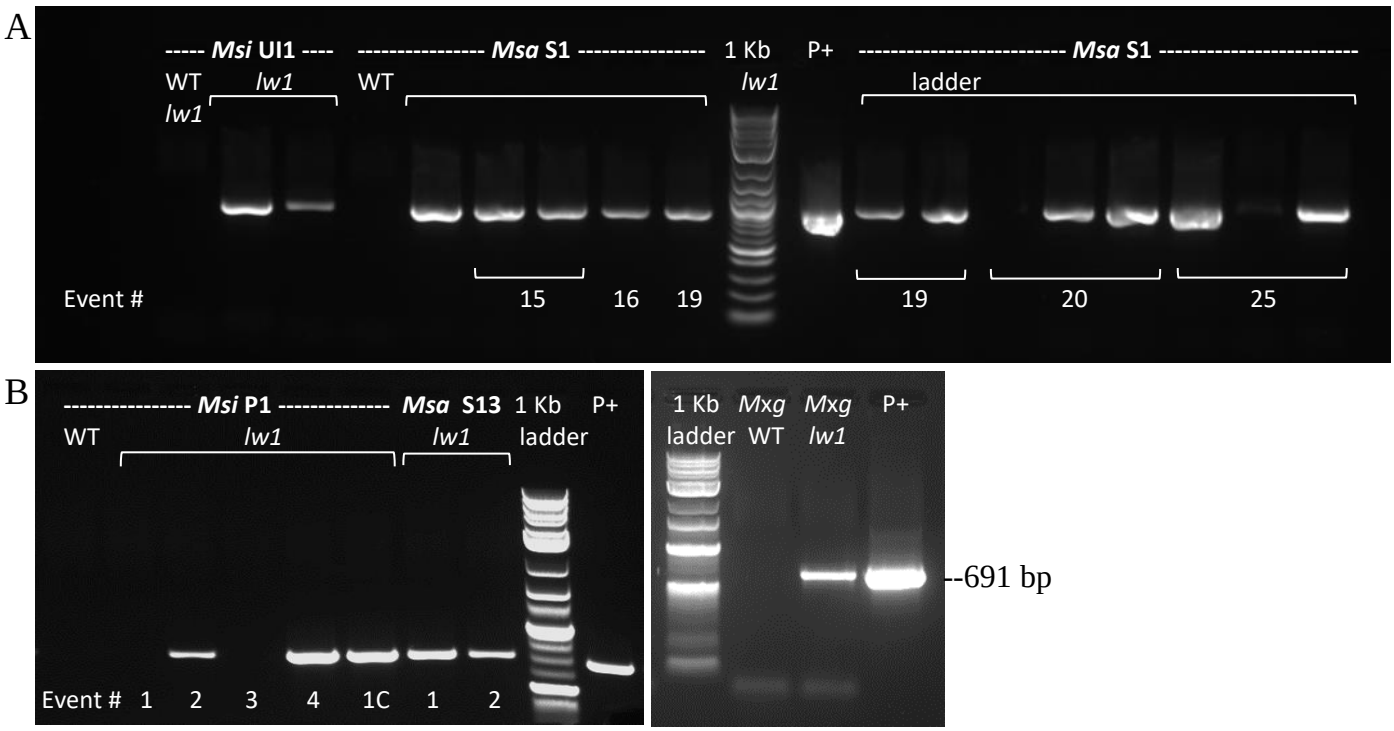



Fig. S4. PCR screening of putative *lw1* edited *Miscanthus* plants, amplifying *nptII* in all five genotypes. Leaf samples were harvested from putative transgenic plants and isogenic wild-type plants. Extracted DNA were amplified via PCR using *nptII* primers, then analyzed via agarose gel electrophoresis. (A) Gel showing *nptII* amplification in *Msi* UI1 and *Msa* S1 plant samples. (B) Gels showing *nptII* amplification in *Msi* PI, *Msa* S13 and *Mxg* Freedom plant samples. Abbreviations: WT=wild-type, *lw1*=putative gene-edited plants, P+=positive control (pHA194). Event # indicates transgenic plant(s) arose from that individual (numbered) callus piece. Plants arising from different calli were independent transformants.

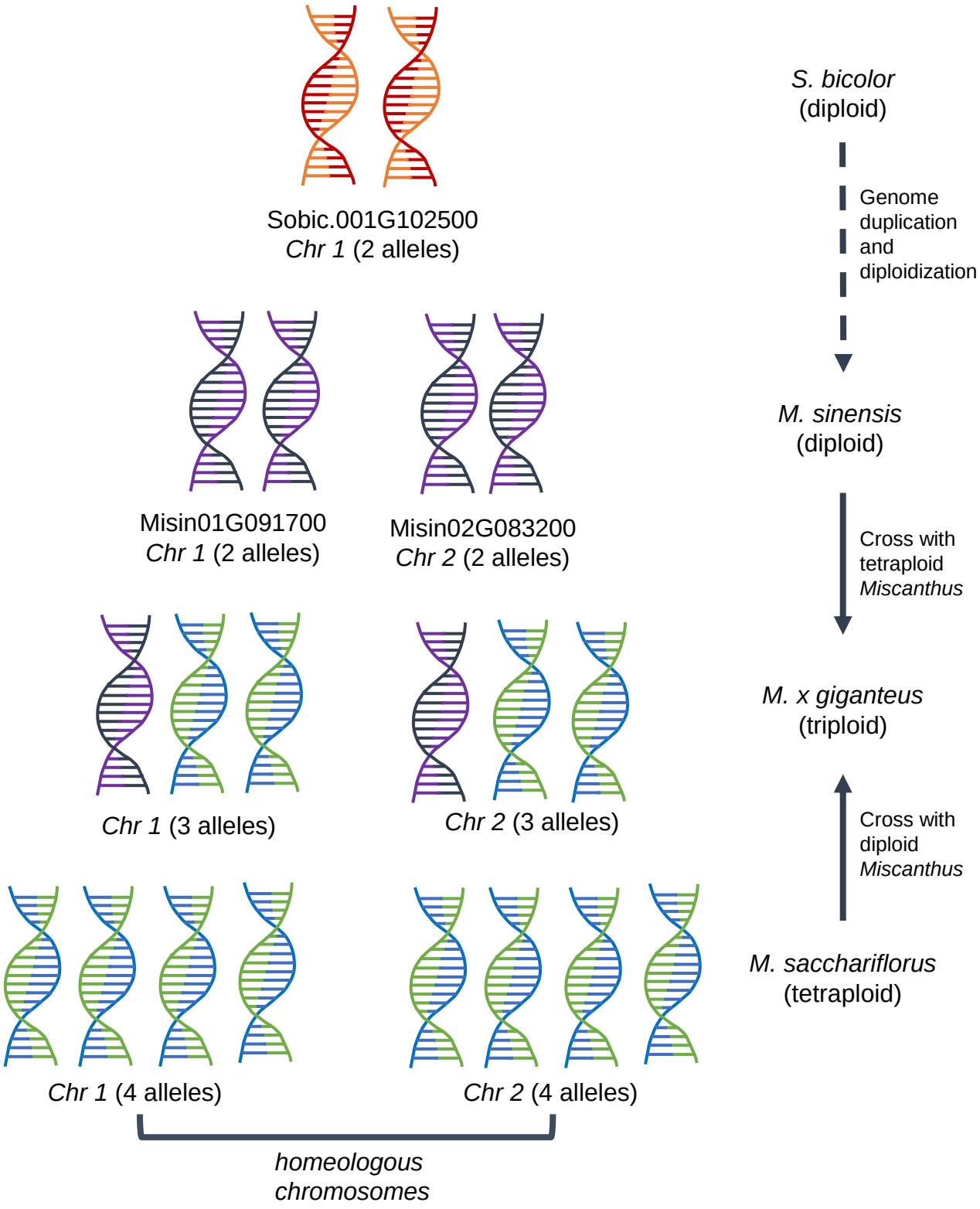



Fig. S5. A graphical representation of gene/allele copy number, specifically *lemon white1* (*lw1*), in paleo-allopolyploid *Miscanthus*.
The number of *lw1* alleles that need to be targeted to get complete loss of function mutants in *Miscanthus* ranges from four to eight. The *lw1* ortholog in sorghum resides on chromosome (Chr) 1. In *Miscanthus* the *lw1* orthologs reside on homeologous Chr 1 and Chr 2. In diploid *Miscanthus* genotypes (all *M. sinensis* and some *M. sacchariflorus*), there are two alleles per chromosome, resulting in four nearly identical copies of *lw1*. Similarly, tetraploid *M. sacchariflorus* genotypes have eight copies of *lw1*. The triploid hybrid *M.* x *giganteus*, a cross between a tetraploid *M. sacchariflorus* and diploid *M. sinensis*, has six copies of *lw1*.

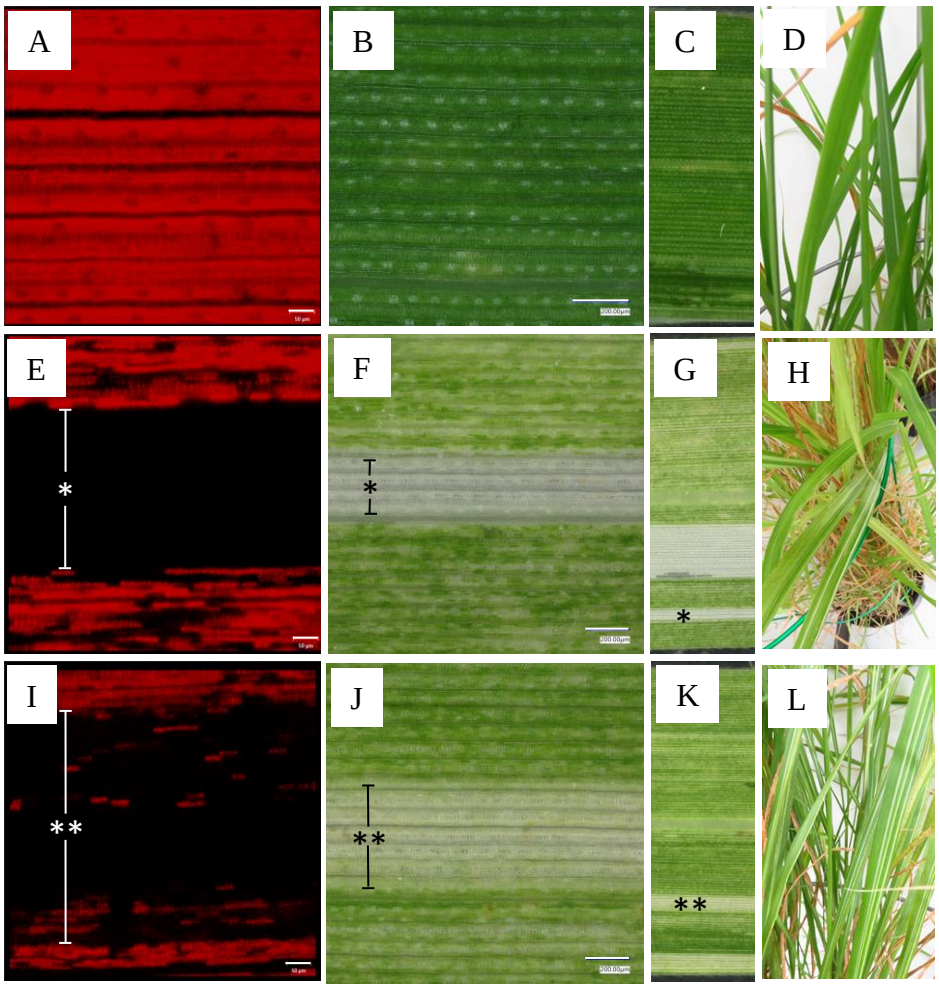
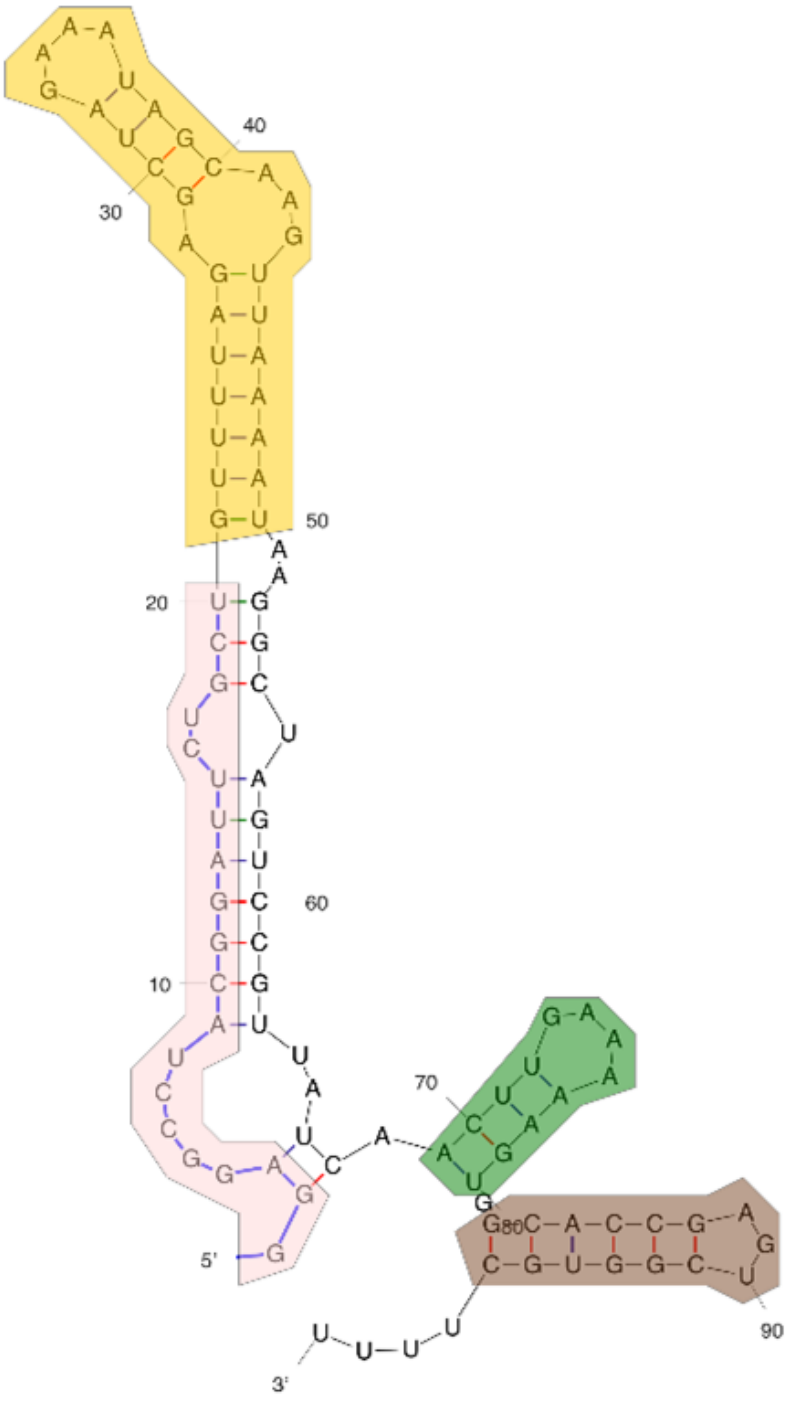



Fig. S6. Confocal and bright field images of *M. sacchariflorus* S1 TG-25 leaves: wild-type vs. *lw1* edited.

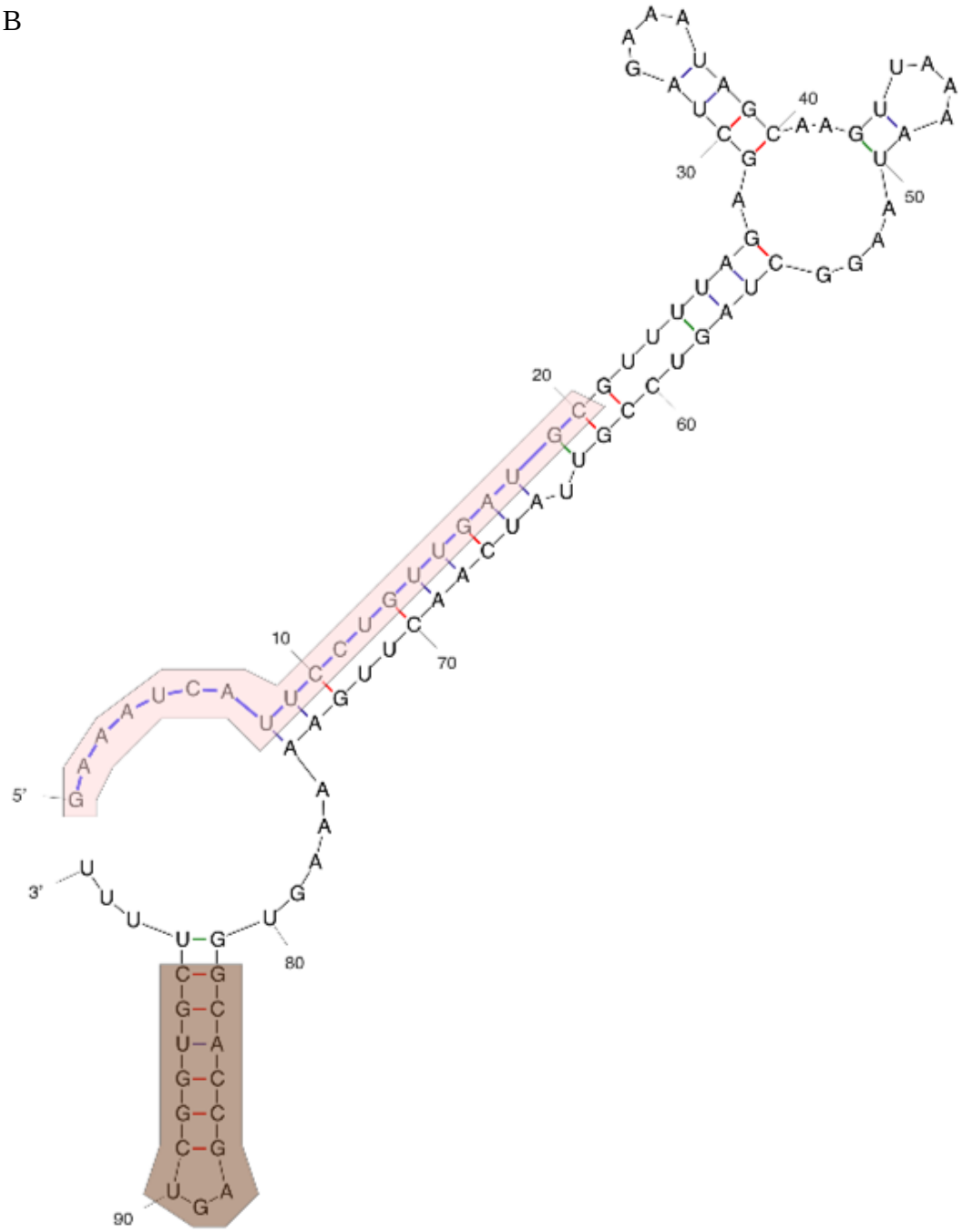
Wild-type (A-C), *lw1* edited TG-25h (E-G) and TG-25c (I-K) leaf samples; one leaf sample was taken from each corresponding intact plant (D, H, L) for visualization and imaging under different magnifications. Asterisks identify same yellow/white stripe in each set of images; vertical lines in E-F and I-J span the widths of yellow/white stripes at the different magnifications. Confocal images (A, E, I), using a Zeiss LSM 510 confocal mounted on a Zeiss Axiovert 200 M inverted microscope, show autofluorescence (red) of chloroplasts. Bright field images, captured using a Keyance VHX7000 digital microscope, show magnified (B, F, J) and entire leaf width (C, G, K) of samples. Size bars: A, E, I = 50 µm, B, F, J = 200 µm.

A




dG = -32.30 [Initially -31.20] lw1G1

B




dG = -24.43 [Initially -27.30] lw1G2

C

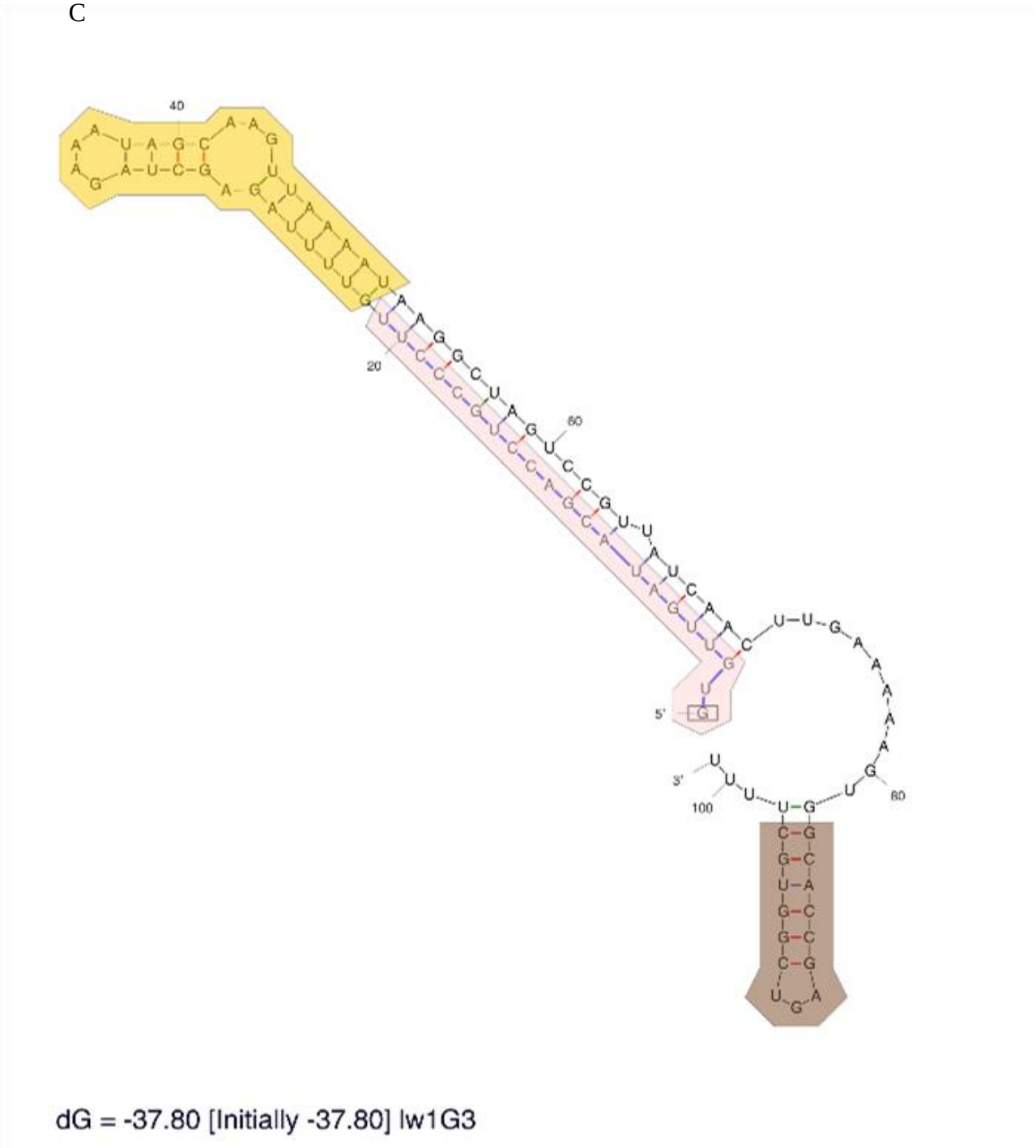



Fig. S7. Secondary structure prediction of three *lw1* single guide RNA (sgRNA) designs.
(A) lw1G1. (B) lw2G2. (C) lw1G3; nucleic acid base enclosed in box was added to the guide RNA to facilitate expression under U6 promoter. Guide RNA sequence is shaded in pink, repeat and anti-repeat stem loop is shaded in yellow, stem loop 2 is shaded in green, and stem-loop 3 is shaded in brown.