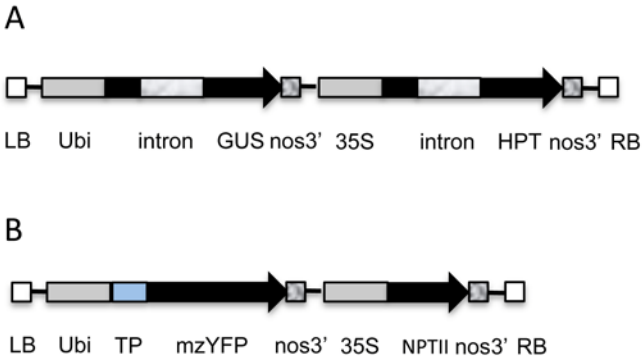
SUPPLEMENTAL FIGURES

Supplemental Figure 1. Mature *S. viridis* (left) and *S. italica* (right) at flowering.

Plants were grown in growth chambers under 16 hr L: 8 hr D cycles (31ºC L, 22ºC D, 50% relative humidity, light fluence=550 μmol/m^2/sec). The plant on the left was 19 days after planting (dap) and on the right was 38 dap when the photograph was taken. The scale bar on right is approximately 120 cm.



Supplemental Figure 2. Schematic of expression constructs.

Constructs used for:

(A) stable expression (see Vogel et al., 2006 for details of vector).

(B) transient expression (see Sattarzadeh et al., 2010 for details of vector).

SUPPLEMENTAL METHODS

Setaria viridis Stable Transformation

Callus Induction

Seed coats were removed from mature *S. viridis* seeds (Accession A10) and disinfested in a solution of 10% bleach and 0.1% Tween 20 (Fisher Scientific, Pittsburgh, PA) for 3 min followed by 3 rinses in sterile deionized water. Seeds were blotted dry on sterile paper towels and cultured on a callus induction medium (CIM) that contained Murashige and Skoog salts (MS) (Murashige and Skoog, 1962) (Caisson Laboratories, Sugar City, ID), 0.5 mg/l nicotinic acid (Sigma-Aldrich, St. Louis, MO), 0.5 mg/l pyridoxine HCL (Sigma), 0.1 mg/l thiamine HCl (Sigma), 1 mg/l d-biotin (PhytoTechnology Laboratories, Shawnee Mission, KS), 30 g/l sucrose (grade II; PhytoTechnology Laboratories), 100 mg/l myoinositol (Sigma), 0.6 mg/l $CuSO_4$ (Sigma), 0.5 mg/l kinetin (added after autoclaving) (PhytoTechnology Laboratories), 2 mg/l 2,4-dichlorophenoxyacetic acid (added after autoclaving) (PhytoTechnology Laboratories), and 4 g/l Phytagel (Sigma). The pH of the medium was adjusted to 5.8. Approximately 25 ml of medium was poured into each 100 x 20 mm Petri plate. Twenty-five seeds were cultured per plate. The cultures were maintained in the dark at 25ºC $\pm$ 2ºC. After 4 weeks, the calli were divided into 2 mm pieces and transferred to fresh CIM. Seven days prior to transformation, the calli were divided again and cultured onto fresh CIM. Plates were sealed with Nescofilm (Karlan Research Products, Cottonwood, AZ).

Transformation

Agrobacterium tumefaciens strain AGL1 (Lazo et al., 1991) was used for transformation that contained the pOL001 vector (Vogel et al., 2006) (Supplemental Figure 2A). The vector carries the b-glucuronidase gene driven by an intron-containing ubiquitin promoter and the hygromycin phosphotransferase gene driven by the cauliflower mosaic virus 35S promoter. Two days prior to transformation, the *Agrobacterium* was streaked from a glycerol stock onto medium that contained 5 g/l tryptone (Becton, Dickinson and Company, Sparks, MD), 2.5 g/l yeast extract (Becton, Dickinson and Company), 5 g/l NaCl (Fisher Scientific), 5 g/l mannitol (Fisher Scientific), 100 mg/l

$MgSO_4$ (Fisher Scientific), 250 mg/l K_2HPO_4 (Fisher Scientific), 1.2 g/l glutamic acid (Sigma), 15 g/l sucrose, 50 mg/l carbenicillin (PhytoTechnology Laboratories), 50 mg/l spectinomycin (PhytoTechnology Laboratories), and 15 g/l BactoAgar (Becton, Dickinson and Company). The pH of the medium was adjusted to 7.2. The culture was incubated for 48 hrs at 28C.

The day of transformation, the *Agrobacterium* was scraped off the plate and transferred to 2 ml of liquid CIM that did not contain $CuSO_4$. The OD_{600} was checked and the *Agrobacterium* solution was diluted to an OD_{600} of 0.6 with liquid CIM. Acetosyringone (PhytoTechnology Laboratories) from a stock solution prepared with DMSO and deionized water was added to the *Agrobacterium* solution to a final concentration of 200 mM. 10 ml of a 10% Synperonic PE/F68 (Sigma) solution was added per 1 ml inoculation media. Approximately 100 calli were transferred to a 50 ml sterile conical tube and covered with *Agrobacterium* solution. They were incubated for 5 min with gentle agitation to coat the calli.

The *Agrobacterium* solution was decanted and 50 calli were transferred to a 7 cm Whatman filter paper that was in a 100 x 15 mm Petri plate. This was repeated with a second plate. Excess solution was removed with a sterile pipet. The infected calli were incubated at 22C in the dark for 3 days.

Following a 3-day co-cultivation period, the calli were transferred to CIM containing 150 mg/l timentin (added after autoclaving) (GlaxoSmithKline, Research Triangle Park, NC) in 100 x 20 mm Petri plates and placed back in the dark. One week later, the calli were transferred to a selective CIM that contained 30 mg/l hygromycin and 150 mg/l timentin both added after autoclaving and placed back in the dark. After one week, the calli were transferred to a selective regeneration medium that contained MS salts, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine HCL, 0.1 mg/l thiamine HCl, 1 mg/l d-biotin, 20 g/l sucrose, 100 mg/l myoinositol, 2 mg/l kinetin (added after autoclaving), 150 mg/l timentin (added after autoclaving), 30 mg/l hygromycin (added after autoclaving), and 2 g/l Phytagel. The pH of the medium was adjusted to 5.8. Plates were sealed with

Nescofilm. The cultures were maintained at 25 ± 2C under lights at 70 µE m^{-2} s^{-1} with a 16 hr light/8 hr dark photoperiod.

Rooting

When the plants were well rooted, the medium was washed from the roots, and they were transferred to a soil-less potting mix (not commercially available) that contained the following: 538.6 g Unimix + III (Griffin Greenhouse and Nursery Supplies, Tewksberry, MA), 2.27 kg lime (Hummert International, Earth City, MO), 0.161 m^3 peat moss (Hummert International), 0.34 m3 vermiculite (Hummert International), 2.27 kg Osmocote 17-7-12 (Grower Supply Inc., Forest Hill, LA). The plants were covered with transparent plastic lids and placed in a growth chamber at 23ºC. After 4 days, the plastic lids were propped up slightly to allow a gradual acclimation and removed the next day. Plants flowered within three weeks of transfer to the greenhouse.

Setaria viridis **Transient Transformation by** ***Agrobacterium*****-infiltration**

S. viridis growth for transient transformation

S. viridis (accession A10) plants were grown using a soil mixture of MetroMix® 360 and 1/3 (v/v) sand in a growth chamber (Conviron, Pembina, ND). The light cycle is kept as 12 hours light and 12 hours of darkness. The relative humidity is set at 75%. Temperature was kept constant at 23^{o}C. The plants were watered as needed every the 3rd day and supplemented with 5 g/ liter of 20-10-20 fertilizer (Peters, Allentown, PA). Plants are typically grown for 12-16 days before agro-infiltration.

Agrobacterium-mediated infiltration of *S. viridis*

One day before the infiltration of *S. viridis*, *Agrobacterium* strain AGL1 (Lazo et al., 1991) carrying vector pPTN469 (Sattarzadeh et al., 2010) (Supplemental Fig.1b) was inoculated in fresh YEB culture (Beef extract 5 g; Yeast extract 1 g; Peptone 5 g; Sucrose 5 g; MgSO4.7H_2O 30.0 mg; Agar 2.0 g; Distilled water 1 liter) with 25 µg/ml of spectinomycin and 15 µg/ml of carbenicillin. Agrobacterium was grown overnight to the density of OD_{600}=0.4. On the day of inoculation, the *Agrobacterium* culture was spun

down at 5000 x g and washed twice with freshly-made infiltration buffer (MES 50 mM; $Na_3PO_4.12H_2O$ 2 mM; acetosyringone 5 mM; and D-glucose 0.25 g/ liter). The Agrobacterium pellet was re-suspended in infiltration buffer at a final density of OD_{600}=0.8. The *Agrobacterium* was infiltrated into the 4th true leaf of *S. viridis* using a 1ml syringe (Becton, Franklin Lakes, NJ). Expression of the transgene was visualized at 4 to 7 days post inoculation.

REFERENCES

Lazo, G., Stein, P., and Ludwig, R. (1991). A DNA transformation-competent Arabidopsis genomic library in Agrobacterium. Biotechnology **9,** 963-967.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant **15,** 473-497.

Sattarzadeh, A., Fuller, J., Moguel, S., Wostrikoff, K., Sato, S., Covshoff, S., Clemente, T., Hanson, M., and Stern, D.B. (2010). Transgenic maize lines with cell-type specific expression of fluorescent proteins in plastids. Plant Biotechnol J **8,** 112-125.

Vogel, J., Garvin, D., Leong, O., and Hayden, D. (2006). *Agrobacterium*-mediated transformation and inbred line development in the model grass *Brachypodium distachyon*. Plant Cell Tiss Org Cult **85,** 199-211.