

Supplementary material: Mid-season Leaf Glutamine Predicts End-Season Maize Grain Yield and Nitrogen Content in Response to Nitrogen Fertilization under Field Conditions

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Table S1. Pearson correlation between maize (alternate variety Pioneer P9329) *GlnLux* glutamine and end-season measurements at an alternate field location in 2014^z.

Growth stage	V3	V6	V12	V14
N application rate	0.24	0.28	0.55*	0.74**
Grain yield ^y	0.15	0.15	0.64**	0.66**
PSNT ^x	0.41	0.60*	0.79**	0.80**

^zMeasurements were performed across four replications.

^yGrain yield at 15.5% moisture content.

^xPSNT, pre-sidedress soil nitrate test (sampled on June 20, 2014).

One, and two asterisks represent significant Pearson correlation at $P < 0.05$ and 0.01 respectively.

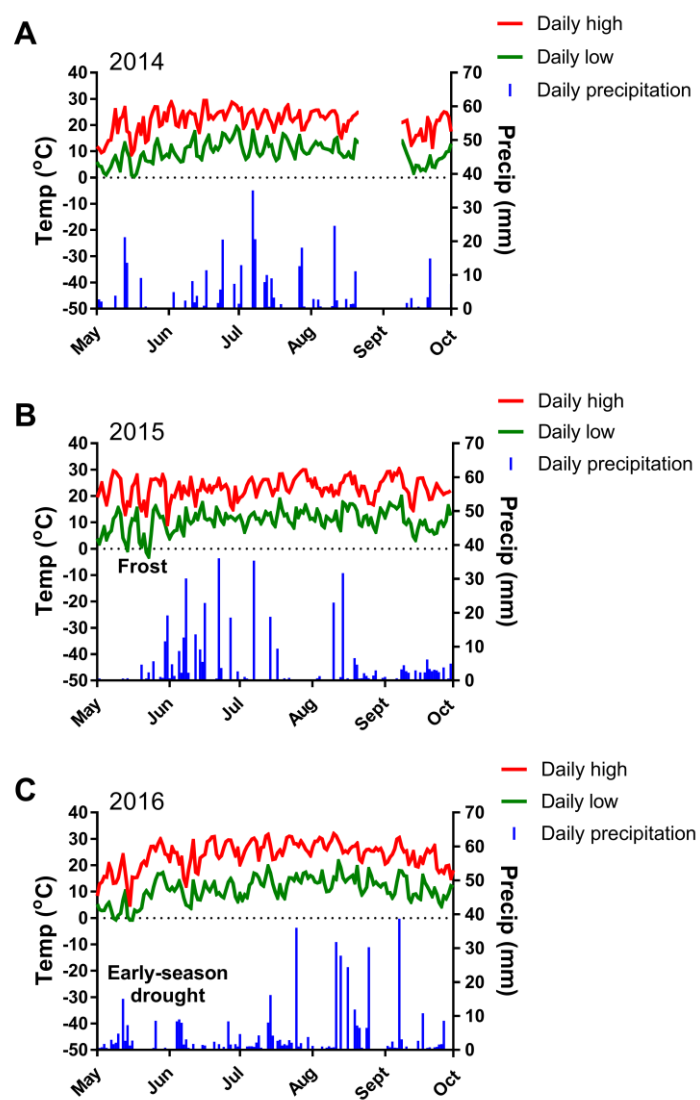


Figure S1. Daily precipitation (mm), high, and low temperatures (°C) during the growing season at the experimental site in (A) 2014, (B) 2015 and (C) 2016. Notable weather events are labeled. In 2014 there was data missing from August 22 to September 9 [55].

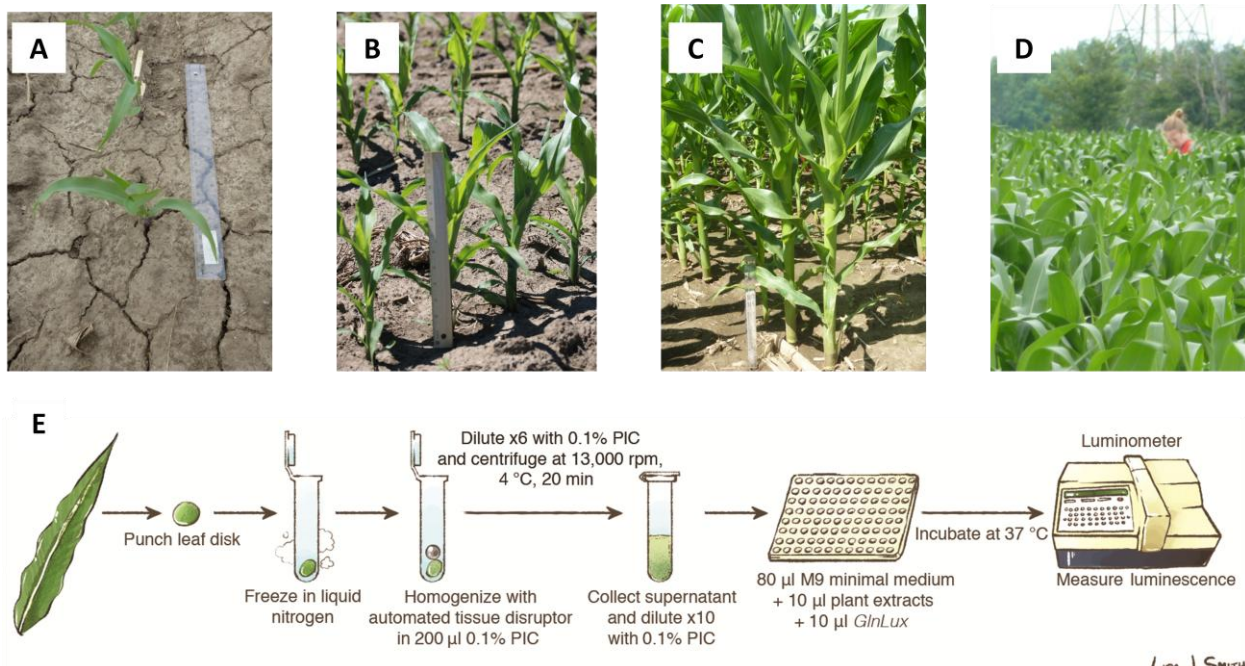


Figure S2. Methodology of field sampling and the *GlnLux* glutamine assay. Maize leaves were sampled at the (A) V3, (B) V6, (C) V12, and (D) V14 vegetative growth stages. (E) Extracts of leaf punches were incubated with *GlnLux* biosensor cells in 96-well plates, and luminescence was quantified with a luminometer to measure relative levels of Gln.

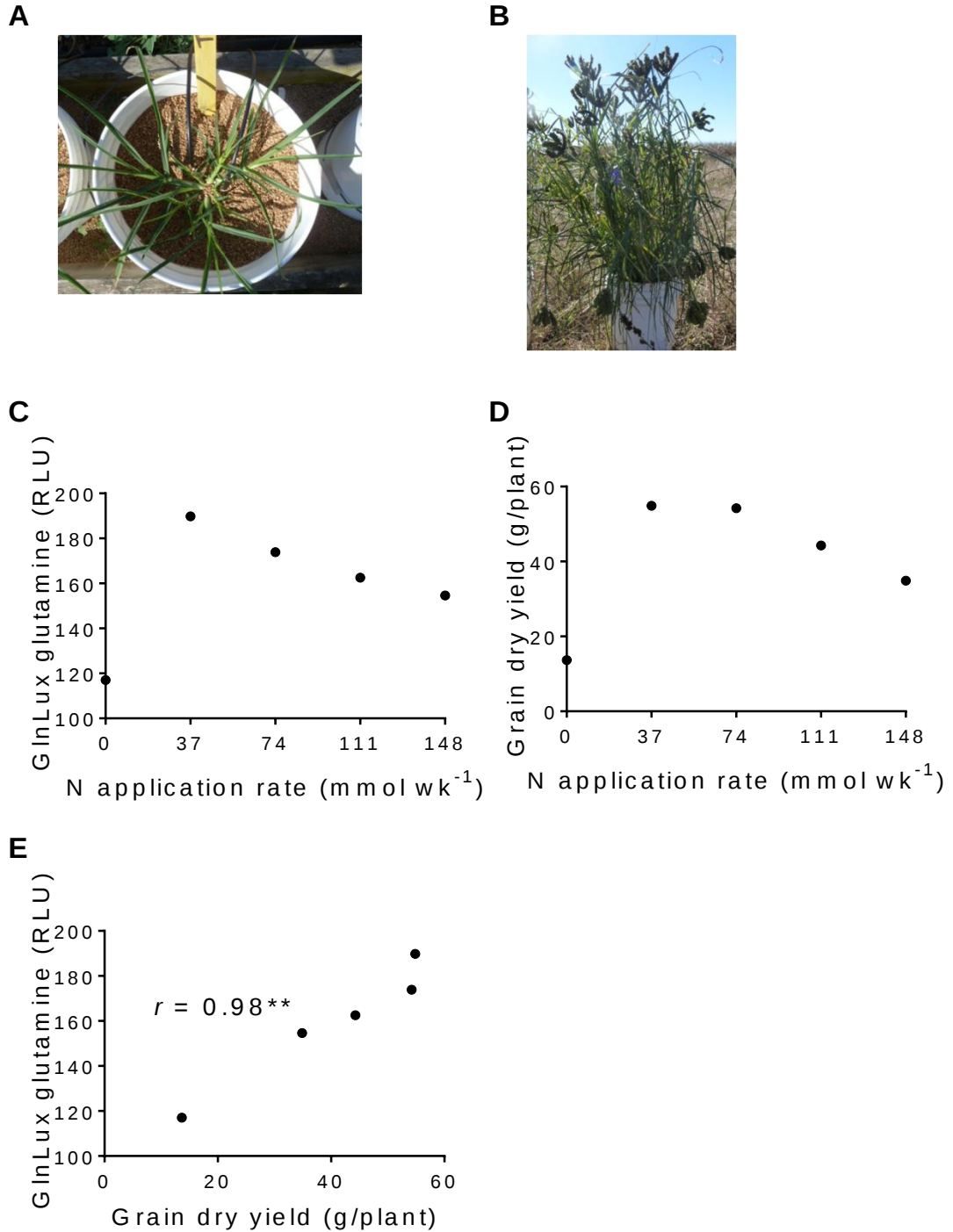


Figure S3. Correlation of early-season *GlnLux* glutamine and end-season grain yield in finger millet (*Eleusine coracana* Gaertn.). Plants were grown in 2013 in an outdoor pot experiment, and provided with weekly N application rates of 0, 37, 74, 111, or 148 mmol total N, dissolved into 1 L ddH₂O. (A) Young leaf tissue was sampled 39 days after germination, and (C) *GlnLux* glutamine was measured from leaf disks. (B) Plants were harvested at maturity 142 days after germination, and (D) grain dry yield was measured. (E) Pearson correlation of grain dry yield was performed against the early-season *GlnLux* biosensor output. Data points represent the mean of five replicate plants in each N treatment. RLU, relative light units intercepted by the luminometer in a one second interval. Two asterisks represent significant Pearson correlation at $P < 0.01$.

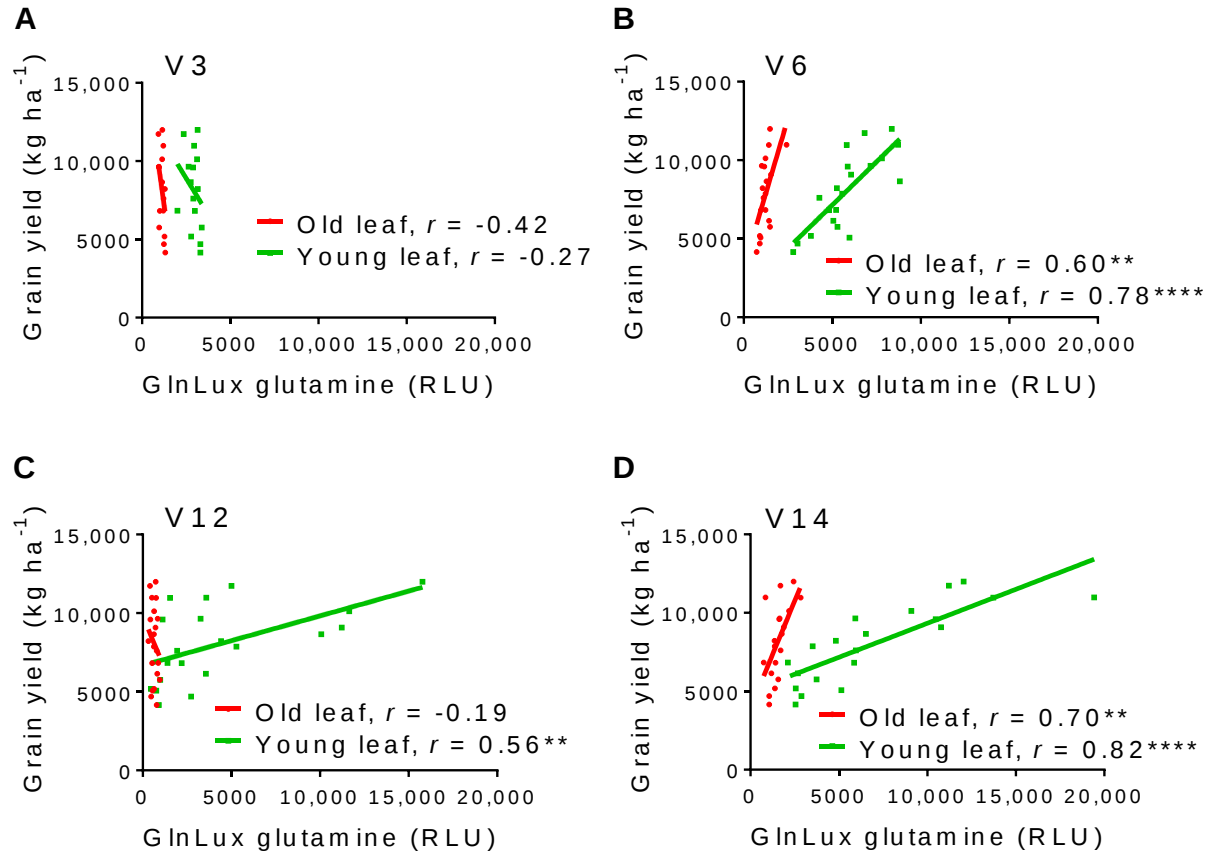


Figure S4. Maize leaf tissues sampled from young leaves within the same vegetative growth stage display increased *GlnLux* glutamine correlation with grain yield as compared to older leaves. The young leaf was the leaf located second from the top of the plant (not fully emerged). The old leaf was the lowest healthy leaf. Sampling was performed at the (A) V3, (B) V6, (C) V12, and (D) V14 growth stages in 2015. Pearson correlation was used to compare each sampled leaf to end-season grain yield. Asterisks represent significant correlations at $P < 0.05$, < 0.01 , < 0.001 , and < 0.0001 . RLU, relative light units intercepted by the luminometer in a one second interval.

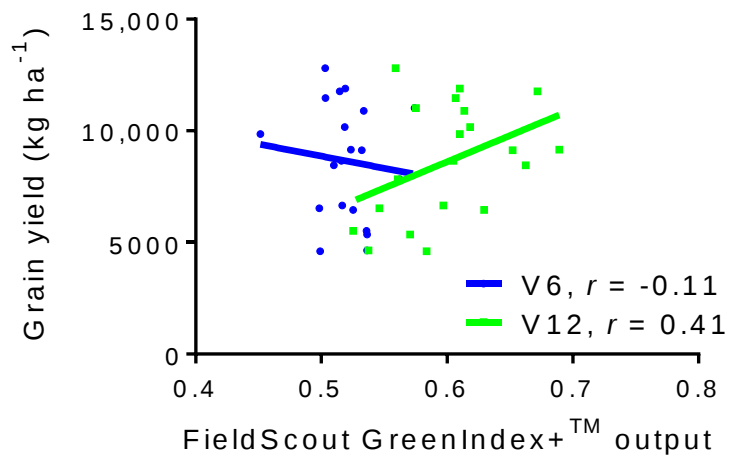


Figure S5. Pearson correlation of end-season maize grain yield (kg ha⁻¹) and FieldScout GreenIndex+™ (Smartphone application) output. Maize was provided with N application rates of 30, 58, 87, 145, and 218 kg ha⁻¹. Measurements were recorded at the V6 and V12 growth stages in 2014. End-season grain yield was adjusted to a moisture content of 15.5% prior to analysis.

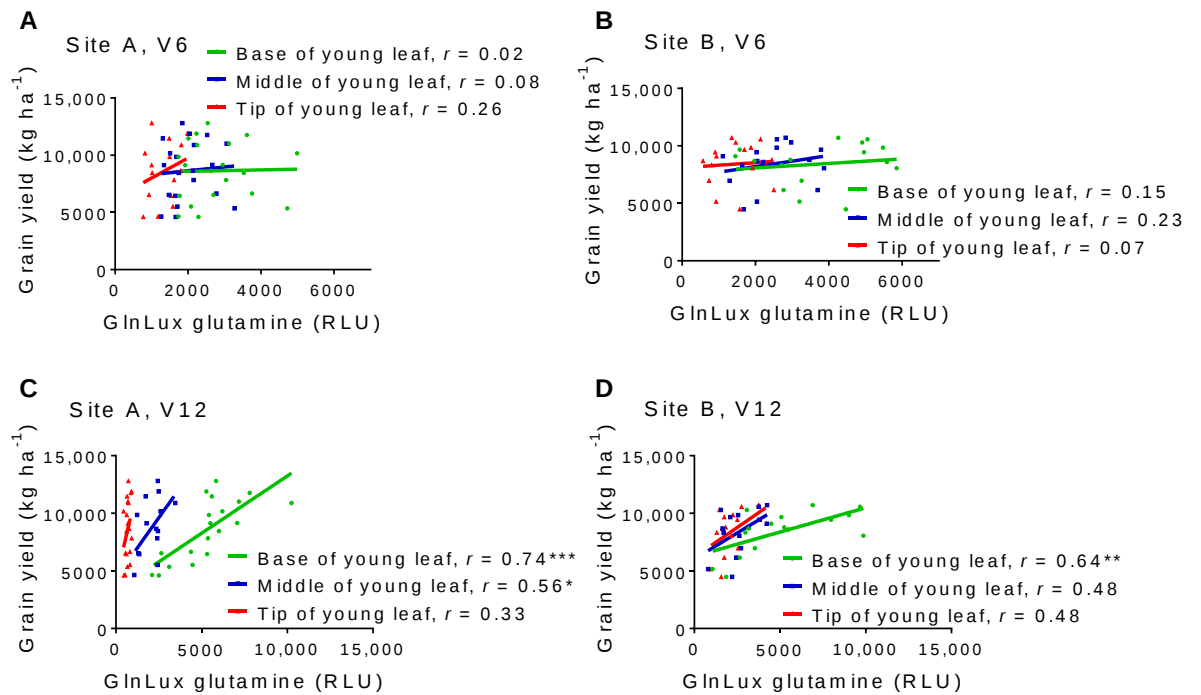


Figure S6. Maize leaf tissue sampled from different positions along the midrib of a young leaf (located second from the top of the plant, not fully emerged) displays increased *GlnLux* correlation with grain yield towards the base of the leaf compared to more distal locations. Plants were sampled at the (A-B) V6 and (C-D) V12 growth stages in 2014 in the (A, C) main experimental field (site A) and (B, D) an additional field site (site B). Pearson correlation was used to compare each sampling position to end-season grain yield. Asterisks represent significant correlations at $P < 0.05$, < 0.01 , and < 0.001 . RLU, relative light units intercepted by the luminometer in a one second interval.

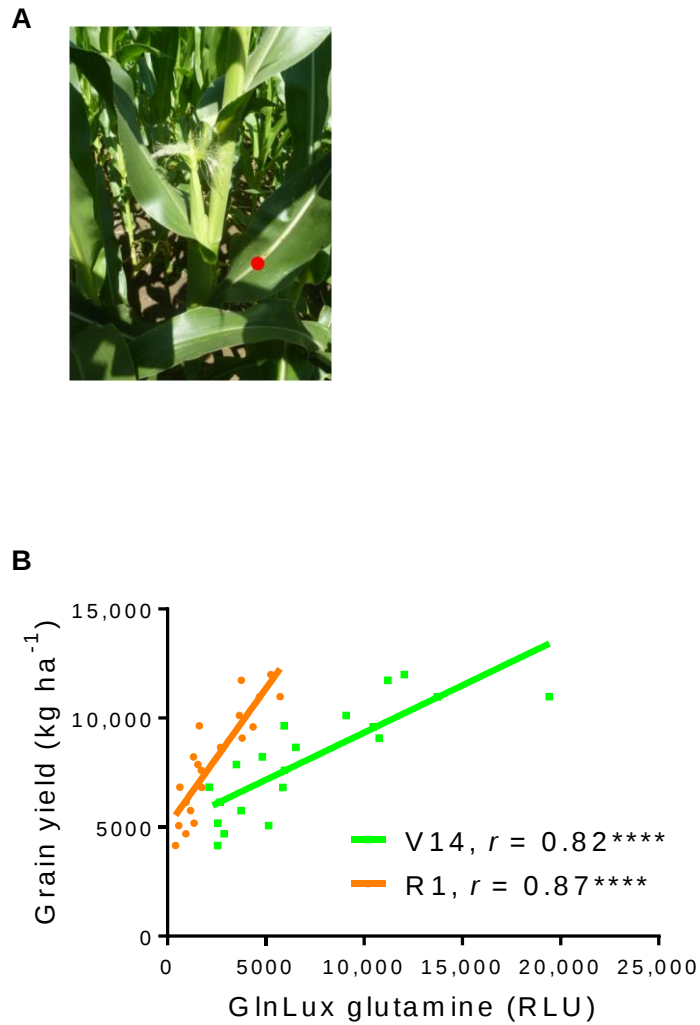


Figure S7. *GlnLux* glutamine correlation with grain yield shows a slight, but insignificant, increase when standardized by leaf number at silking (R1) since individual plants grew at slightly different rates. (A) The leaf directly opposite and below the developing ear was sampled at the same position along the midrib as the main experiment (red circle). (B) Pearson correlation of R1 *GlnLux* glutamine and end-season grain yield was compared to the correlation of V14 *GlnLux* glutamine and end-season grain yield. Although R1 displayed higher correlation, the increase was insignificant as determined by a z test approximation following Fisher transformation. RLU, relative light units intercepted by the luminometer in a one second interval.