Molecular interactions among regulatory factors influencing shoot maturation in maize

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Shoot maturation in maize is characterized by three major phase changes, the transition from juvenile to adult vegetative development, inflorescence initiation, and the specification of floral organ identity. Molecular genetic studies in maize and Arabidopsis have identified important factors for the general process of phase change and shoot maturation, including genes that function in small RNA biogenesis and metabolism, microRNAs, transcription factors, growth regulators (GA and ABA) and sensitivity to photoperiod. Detailed analysis of molecular interactions within the vegetative phase change pathway has clarified the functional relationships among some of these regulatory factors. Analysis of mutations and natural allelic variation affecting vegetative phase change indicates that the observed antagonistic effects of miR156 and miR172 are mediated by independent pathways that converge to regulate Glossy15. Experiments involving exogenous GA application and GA-deficient mutants demonstrate that GA both represses miR156 and promotes miR172 expression, but acts most strongly on miR172. We also show that prolonged Glossy15 expression could lead to delayed shoot maturation, suggesting novel strategies to increase maize biomass.

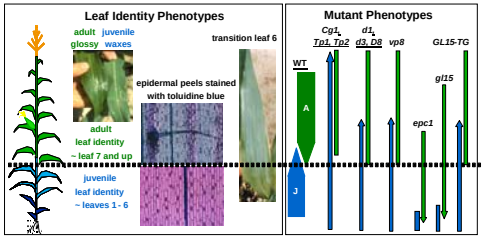

Fig. 1: **Maize leaf identity traits and known regulatory factors.** Phenotypes distinguishing juvenile and adult leaf identity include leaf waxes, macrohairs and bulliform cells (purple cell files in adult leaves), and differences in cell wall biochemistry visualized by staining with toluidine blue. Transition leaves exhibit juvenile then adult phenotypes in a proximal-distal pattern. The dashed line marks the boundaries that typically define the transitions from juvenile to adult leaf identity in wild-type (WT). The relative expression of juvenile (blue) and adult (green) leaf identities in WT and mutant genotypes is indicated by the position and height of the blue or green colored bars.

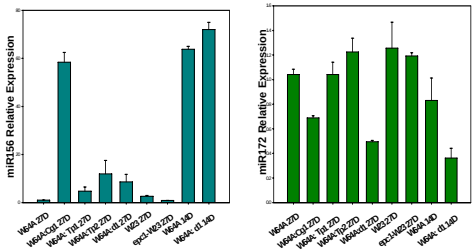

Fig. 2. Vegetative phase change mutants (Corngrass1-Cg1, Teopod 1-Tp1, Teopod 2-Tp2, dwarf1- d1 and Early Phase Change 1- epc1) have altered expression of miR156 and miR172. Shoot apical meristem samples were taken from Moose Lab 2008 summer research field except samples from 14 days, which were from plants in green house. miRNA quantitation is through real-time PCR by ABI taqman assays and calculated by ΔΔCt method. (a) Delayed phase change mutants could maintain a high level of miR156 in later developmental stages (27D), a time when wild-type plants have enter adult stage and marked by minimum miR156 expression (14 Days Vs 27 Days). (b). At 27 days, miR172 has increased to a level that represents the adult leaf stage, except in Cg1 and d1 mutants. d1 mutants have half miR172 as isogenic W64A, which could not be changed as plants develop.

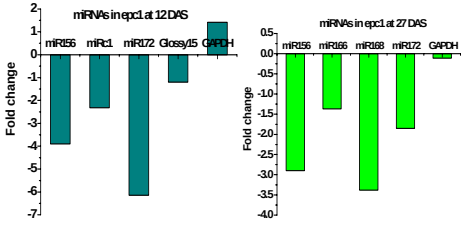

Fig. 3. epc1 mutant has lower levels of all tested miRNAs. A. epc1 mutant has lower miRNA levels at 12 days. miRNA quantitation was done by ABI taqman miRNA assays with all corresponding miRNAs in W23 as reference, + means increase in miRNA level in epc1 relative to W23 and – means decrease in miRNA level relative to W23. Comparison among different miRNAs is not drawn to actual scale. B. Epc1 mutant has lower miRNA levels at 27 days.

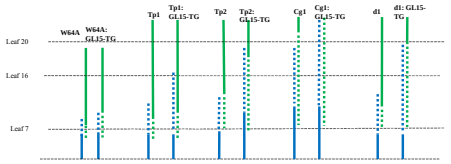

Fig. 3. Glossy15 transgene further dalys vegetative phase change in maize vegetative phase change mutant Tp1, Tp2, Cg1 and d1, indicating Glossy15 acting downstream of these factors. Blue lines marks the juvenile wax and green lines marks the adult macrohair; solid lines represent total coverage and dash lines represent incomplete coverage.

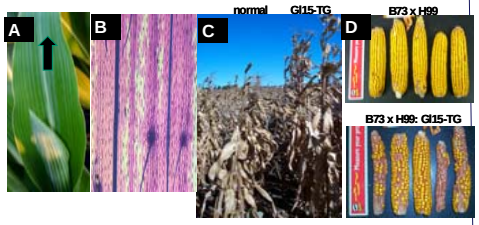

Fig. 5. Phenotypes of Glossy15-TG plants. A: Glossy15-TG plants have juvenile wax sectors in leaf tip (as the arrow head points); Glossy15-TG sectors usually go along with the veins in the upper leaves. B: Totuidine blue staining of Gl15-TG leaves, the purple staining areas along with the veins coexist with blue staining areas of adult trait. C: Gl15-TG plants are taller and bigger than their counterpart inbreds by delayed flowering. D. Gl15-TG plants have poor seeds fill in field.

Hybrid	Dry Weight (Mg/ha)	Stover Biomass (Mg/ha)	Total Biomass (Mg/ha)
B73 X H99	6.8	5.2	12
B73 X H99: GL15-TG	2.8	15	17.8
Fr1064 X H99	7.7	6.6	14.3
Fr1064 X H99 GL15-TG	2.9	15.9	18.8
IBM X H99	9.2	/	/
IBM X H99 GL15-TG	2.4	/	/

Table 1: Potential of GL15-TG plants to accumulate more biomass. Plants are grown in 2005 summer nursery without supplemental N, dry weight is calculated by the average dry weight in the nursery times 75000 plants /ha .

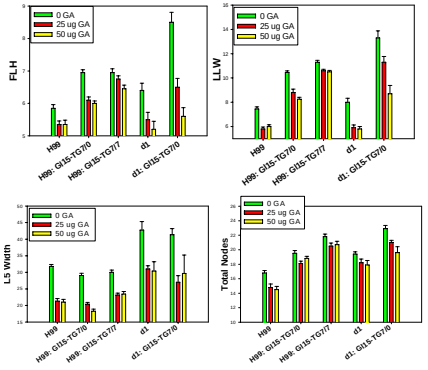

Fig. 6: GA application (25ug, 50ug) influences leaf identity in H99, H99 *GL15-TG7/0*, H99 *Gl15-TG7/7*, *d1* and *d1 GL15-TG7/0* in the following leaf identity phenotypes. (A) Fist Leaf Hair (FLH), (B) Last Leaf Waxy (LLW), (C) Leaf 5 Width (L5 width) and (D) Total Nodes. For leaf trait FLH and LLW and total nodes, delayed phase change that is brought by Glossy15-TG could be reversed by exogenous GA. GL15-TG doesn't have any effect for the leaf width brought by GA deficiency (H99 compared to d1), but exogenous GA can restore the leaf width to wild-type level. GA has little effect to reverse the GL15-TG's effect on total nodes.

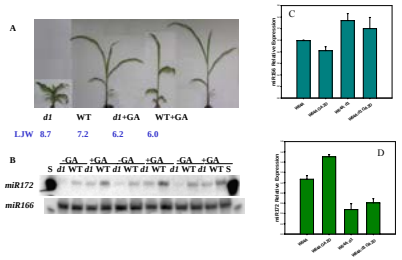

Fig. 7. Exogenous GA could increase the miR172 level in either wild-type or d1 plants. A: exogenous GA application could restore the dwarf phenotype by promoting internodes growth and reducing the leaf width. B. Northern blot of miR172 with or without GA treatment, note miR172 always has lower expression in d1 mutant. C. Quantitative real-time PCR of miR156 by taqman assay, this figure shows that GA could reduce miR156 level. D. qPCR shows that miR172 expression is promoted by GA treatment .

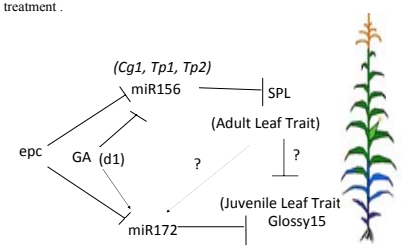

Fig. 8. Proposed vegetative phase change pathway. 1) epc1 influences phase change by reduced production of all miRNAs ; 2) GA promote vegetative phase change by increasing miR172 while decreasing miR156; 3) miR156 and miR172 have different pathways to influence by vegetative phase change, but converge to influence Glossy15 expression in plants.