

Supplementary Table IV. C₄ genes from maize (Chang et al., 2012; Li et al., 2010) were used to identify syntenic orthologs in *Setaria* using the grass synteny database (Schnable et al., 2012). The majority of genes recruited into the C₄ pathway in maize and *Setaria* were syntenic. For *CA*, *RBCS*, *GDC*, and *SBP* no syntenic regions were found in the database, however phylogenetic inference has identified orthologous *CA* genes in *Setaria* and maize (Christin et al., 2013). The number of genes in each gene family from *Setaria* and maize are listed.

Gene	Maize ID	Syntenic ortholog	C4 gene ID	Matched	Role	Genes per family - <i>Setaria</i>	Genes per family - Maize
<i>CA</i>	GRMZM2G121878	Si003882m.g	Si003882m.g	Y	Core pathway	5	5
<i>NADP-ME</i>	GRMZM2G085019	Si000645m.g	Si000645m.g	Y	Core pathway	9	8
<i>PEPC</i>	GRMZM2G083841	Si005789m.g	Si005789m.g	Y	Core pathway	6	6
<i>NADP-MDH</i>	GRMZM2G129513	Si013632m.g	Si013632m.g	Y	Core pathway	4	4
<i>AK</i>	GRMZM2G178192	Si014186m.g	Si014186m.g	Y	Core pathway	11	17
<i>ASP-AT</i>	GRMZM5G836910	Si017156m.g	Si017156m.g	Y	Core pathway	4	3
<i>PPDK</i>	GRMZM2G306345	Si021174m.g	Si021174m.g	Y	Core pathway	2	2
<i>RBCACT</i>	GRMZM2G162200	Si026402m.g	Si026402m.g	Y	Core pathway	3	3
<i>PPDK-RP</i>	GRMZM2G131286	Si032116m.g	Si032116m.g	Y	Core pathway	1	2
<i>PCK</i>	GRMZM2G001696	Si034404m.g	Si034404m.g	Y	Core pathway	1	2
<i>MEP3b</i>	GRMZM2G305851	Si024315m.g	Si000451m.g	N	Transport	10	9
<i>PPT</i>	GRMZM2G174107	Si013874m.g	Si013874m.g	Y	Transport	8	10
<i>MEP3a</i>	GRMZM2G138258	Si005376m.g	Si024315m.g	N	Transport	10	9
<i>OMT1</i>	GRMZM2G383088	Si024403m.g	Si024403m.g	Y	Transport	4	3
<i>DIT1</i>	GRMZM2G040933	Si029415m.g	Si029415m.g	Y	Transport	4	3
<i>DCT2</i>	GRMZM2G086258	Si013503m.g	Si035016m.g	N	Transport	4	3
<i>TKL</i>	GRMZM2G033208	Si005927m.g	Si005927m.g	Y	Calvin cycle	5	2
<i>RPI</i>	GRMZM5G874903	Si038812m.g	Si030780m.g	N	Calvin cycle	4	5
<i>FBA</i>	GRMZM2G046284	Si010312m.g	Si010312m.g	Y	Calvin cycle	7	7
<i>PRK</i>	GRMZM2G162529	Si017390m.g	Si017390m.g	Y	Calvin cycle	7	10
<i>GAP-DH</i>	GRMZM5G845611	Si035707m.g	Si035707m.g	Y	Calvin cycle	6	8
<i>PGK</i>	GRMZM2G083016	Si021917m.g	Si021917m.g	Y	Calvin cycle	4	5
<i>TPI</i>	GRMZM5G852968	Si030636m.g	Si030636m.g	Y	Calvin cycle	3	5
<i>RPE</i>	GRMZM2G026807	Si036994m.g	Si036994m.g	Y	Calvin cycle	2	3
<i>FBP</i>	GRMZM2G306732	Si035941m.g	Si035941m.g	Y	Calvin cycle	5	5
<i>SHMT</i>	GRMZM2G135283	Si035240m.g	Si035240m.g	Y	Photorespiration	5	7
<i>GCH</i>	GRMZM2G399183	Si011200m.g	Si011200m.g	Y	Photorespiration	4	4
<i>GOX</i>	GRMZM2G129246	Si029923m.g	Si040072m.g	N	Photorespiration	6	4