

Supplemental Data. Myers et al. (2011). Plant Cell 10.1105/tpc.111.087205

ZmMGD1 1 -----MPAPTAEPALPAAFLCVPSPLLSTPLPGAALSASPAPSSHHASFLPRPR
 OsMGD1 1 -----MPAPTASSLAAAADPALPAAFLSLPSLLPASPP---LPAAPAPSSNAFCVPRGPA
 AtMGD1 1 -----MQNPSTVTQESAAPVDFDFPRLRGLTSRNRSPCSNSDGYALSSSNALYFNGFRTL
 PpMGD1 1 MDCSVELAGLGESSVVRFSKPVVNASLSSSFSAAGNVSSRRRCWDGIRANGVRDVTQGVQGGVPLRQKRSR
 PpMGD2 1 -----
 AtMGD3 1 -----MMKVVV
 AtMGD2 1 -----MAT
 ZmMGD2 1 -----MAAS
 OsMGD3 1 -----MAASSSSSSS
 OsMGD2 1 -----MVISVAT
 ZmMGD3 1 -----MVISV
 PtMGD 1 -----

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ZmMGD1 50 GGPRLSVAISVPGPTFSVASRLHQMWGEFAEFVRLHGNQIAPLGFASMGGLGFGGGGGDNVGGGGGGGGG
 OsMGD1 54 RAVAVSVSAYGAGSTAAASRLHRMWAEESEFVRLHGNQIAPLGFASLGLVGGGGGGSGEGAGGGGGG
 AtMGD1 57 SRRMGKTLASLSFNTKSSAGSSLRRFISDFNSFIRFHCDKVPESFASVGG----VGLSSDENGIRENG
 PpMGD1 71 QEIGVFAAAKTVGDLQSTSKGLQNSFARHFNDLIRRHCCERVPLG---WASISQQPNGKLEGGDDGKGIEL
 PpMGD2 1 -----
 AtMGD3 6 SPRTSRSDSIEKVFRRVYSNFNISTVEDEVIHRQRSSDYEKESRLRKRGLE-----
 AtMGD2 4 TVMALAEKVLERVYGTSKSAVSVTSGDGEKTRHRTHHHIHR--IKSYDDID-----
 ZmMGD2 5 MASPRGRSRETVLETVAAYHQQRRAKCKLRKSLTYAGGELSSAGRARGDG--PASTSVSSSLCGPDDDE
 OsMGD3 11 MASPRGRSRETVLETVAAYHQQRMRKRKSLSYAG-ELSSAGRARGEAGSSASTTSLCGPDEDE
 OsMGD2 6 PRRSIRDVAVLGGVLAGGRQLYQPLRCAFYDGAAGGGLTAALSEDGAEGGVP-----
 ZmMGD3 8 PAARRSTSIRDAVLLGGGRQLYQPLRCAFYDGLAAALSESEEPSD-SDAAR-----
 PtMGD 1 -----

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ZmMGD1 120 DVDGVG--EVEEAAARAEPKQVILILMSDTGGGHRASAEAIKAAFTQEFGDDYQVVFITDLWTDHTP-WPF
 OsMGD1 124 GGGEVDGLVEEGVARAEAPKQVILILMSDTGGGHRASAEAIKAAFIQEFGDDYQVVFITDLWTDHTP-WPF
 AtMGD1 122 TGGVLGEGEPLNGVEADRPKQVILILMSDTGGGHRASAEAIRAAFNQEFGDEYQVFIITDLWTDHTP-WPF
 PpMGD1 138 KGEEVGNEEAQPSCQSERKHKTVLILMSDTGGGHRASAEAIKSTFELEYGDEYKVFVIDLWKEHTP-WPF
 PpMGD2 1 -MEEVGDETTQSVGQDKRQKNVILILMSDTGGGHRASAEAIKATFELEYGDKYKVVVVDLWKEHTP-WPF
 AtMGD3 57 ---DKEEVMEQMGAEIRIKTVLILMSDTGGGHRASAEAIRDAFKIEFGDDYRIIILKDVWKEYTG-WPL
 AtMGD2 53 ---EDSSLELIQIGAERTKQVILILMSDTGGGHRASAEAIRDAFKIEFGDKYRVIVKDVWKEYTG-WPL
 ZmMGD2 73 PFWEEEEETVELVQLGANRAKNVILILMSDTGGGHRASAEAIKDAFRIEFGDEYRVFVKDLCKDEAG-WPL
 OsMGD3 80 PFWEEEEETVELVQLGANRAKNVILILMSDTGGGHRASAEAIKDAFRIEFGDDYRVFVKDLCKDEAG-WPL
 OsMGD2 60 ---LPCGRKTAANKVILILMSDTGGGHRASAEALRDAFRLEFGDAYQVVFVVDLWKEYYGG-WPL
 ZmMGD3 57 ---VPACCKRQAARNVILILMSDTGGGHRASAEALRDAFRIEFGDAYKVFVVDLWKEYYGG-WPL
 PtMGD 1 -----VQILMSDTGGGHRASAEALRDAFRITLHPGRIQCDIVDIYTYEYGFWPY

ZmMGD1 187 NQLPRYSYFLVKHGPLWKMTYYGTAPRVVHQPFAATSTFIAREVAKGLMK-----YQPDV IISVHPLM
 OsMGD1 193 NQLPRYSYFLVKHGPLWKMTYYGTAPRVVHQPFAATSTFIAREVAKGLMK-----YQPDV IISVHPLM
 AtMGD1 191 NQLPRSYNFLVKHGTLWKMTYYGTSPRIVHQSNAFAATSTFIAREIAQGLMK-----YQPD I IISVHPLM
 PpMGD1 207 NOVPRITYSFLVKHENLWRFTFHSTAPKLVHQSMAAATAPFVAREVAKGLAK-----YQPDV IISVHPLM
 PpMGD2 69 NOVPRLYSFFVKHEILWKFYFYSTAPKLVHQSSEMRAATSLFISREVANGLAK-----YQPDV IISVHPLM
 AtMGD3 122 NDMERYQKFMVKHVGLWSVAFHGTSPKWIHKSYLSALAAAYAKEIEAGLME-----YKPD I IISVHPLM
 AtMGD2 118 NDMERSYKFMVKHVQLWKVAFHSTSPKWIHSCYLAALAAAYAKEVEAGLME-----YKPE I IISVHPLM
 ZmMGD2 142 NNMESSYKFMVKHVQLWKVAFHSTSPRVVHGFYLAALASFYAKKVEAGLKK-----YKPD I IISVHPLM
 OsMGD3 149 NNMESSYKFMVKHVQLWKVAFHSTSPRVVHGFYLAALASFYAKKVEAGLKK-----YKPD I IISVHPLM
 OsMGD2 119 NDMERSYKFMIRHVRLWKVAFHGTSPRVVHGMVLAALAYFYANVAVAGIMR-----YNPDI IISVHPLM
 ZmMGD3 116 NDMERSYKFMIRHARLWKVAFHGTSPRVVHGVYLAALAYLYANVAVAGITK-----YEPDI IISVHPLM
 PtMGD 49 DSYIELYKFAAKYPITWDLFYVHFATDFTGIWLNRLMLELFCFEPFKTCLSRPSGNSGKKADMVVSVHPLT

ZmMGD1 251 QHVPLRILRSKG-----LLDKIPFATVITDLSSTCHPTWFHKLVTTRCYCPSTEVEKRALKAGLQPSQI
 OsMGD1 257 QHVPLRILRSKG-----LLDKIPFTTIVITDLSSTCHPTWFHKLVTTRCYCPSAEVSKRALKAGLQPSQI
 AtMGD1 255 QHVPLRVLRSKG-----LLKKIVFTTIVITDLSSTCHPTWFHKLVTTRCYCPSTEVAKRAQKAGLETQSI
 PpMGD1 271 QHIPLRVLRLRG-----LLDKIPFTTIVITDLSSTCHPTWFHKLVTACFCPTKEVADRALKAGLRQSQI
 PpMGD2 133 QHVPLRVLRLRG-----LLDKIPFTTITDLSSTCHPTWFHQLVTACFCPTKEVAKRALEAGLRQSQI
 AtMGD3 186 QHIPLWVWKWQG-----LHKKVI FVTVITDLSSTCHPTWFHFGVSRCYCPSKEVAKRALVDGLDSSQI
 AtMGD2 182 QHIPLWVWKWQE-----LQKRVLVFTVITDLSSTCHPTWFHFGVNRVRCYCPSEVAKRALFDGLDSSQV
 ZmMGD2 206 QHIPLWVWKWQG-----LQNRVVFVTVITDLSSTCHPTWFHADVNRVRCYCPSEVAKRALDDLQPSQI
 OsMGD3 213 QHIPLWVWKWQG-----LQNRVVFVTVITDLSSTCHPTWFHADVNRVRCYCPSEVAKRALDDLQPSQI
 OsMGD2 183 QHIPLWVWKWQS-----LHPKVPFVTVITDLSSTCHPTWFHFGVTRCYCPSAEVAKRALRLGLDPSQI
 ZmMGD3 180 QHIPLWVWKWQSS--LRQHPKAVVPFVTVITDLSSTCHPTWFHFGVTRCYCPSAEVASRALRLGLDPSQV
 PtMGD 119 QDIPLRILAEI DSN GAT RERTGRKTFCTVITDLSSTCHPTWFHFGVTRCYCPSAEVAVASRALRLGLDPSQI

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ZmMGD1 313 K V Y G L P V R P S F V K P V ----- R P K D E L R R E L G M D E D L P A V L L M G G G E G M G P I E A T A K A L G D S L Y D E N L
 OsMGD1 319 K V Y G L P V R P S F V K P I ----- R P E D E L R R E L G M D E Y L P A V L L M G G G E G M G P I E A T A R A L G D A L Y D E V L
 AtMGD1 317 K V Y G L P V R P S F V K P V ----- R P K V E L R R E L G M D E N L P A V L L M G G G E G M G P I E A T A R A L A D A L Y D K N L
 PpMGD1 333 R V H G L P I R P S F A T F T ----- R P K D E L R K E L D M D E S L P A V L L V G G G E G M G P V E Q T A R A L G Q S L Y D A N T
 PpMGD2 195 R V H G L P I R P S F A T F S ----- R S K D E L L K E L E M D K D L P A V L L V G G G E G M G P V E Q T A R A L G E S L Y D A N T
 AtMGD3 248 R V F G L P V R P S F P R T I ----- L N K N E L R K E L E I D L N L P A V L L M G G G E G M G P V Q K T A L A L G D S L Y N S K E
 AtMGD2 244 R V F G L P V R P S F A R A V ----- L V K D D L R K E L E M D Q D L R A V L L M G G G E G M G P V K E T A K A L E E F L Y D K E N
 ZmMGD2 268 R V F G L P I R P S F C R A V ----- L V K D D L R R E L E L D P E L P A V L L M G G G E G M G P V K K T A R A L G E S L F D K E L
 OsMGD3 275 R V F G L P I R P S F C R A V ----- L V K D D L R K E L E L D P E L P A V L L M G G G E G M G P V K K T A K A L G E S L F D K E L
 OsMGD2 245 R V Y G L P I R P S F C R A V ----- L D K D E L R K E L D M D P D L P A V L L M G G G E G M G P V E E T A R A L S D E L Y D R R R
 ZmMGD3 248 R V F G L P I R P S F C R A V ----- L D K D E V R K E L G L D P Q L P A V L L M G G G E G I G P V E E T A R A L G E E L Y D R R R
 PtMGD 189 V Q Y G L P I R Q G E F W A N S E S A H V A P E K V R K S L R R Q L G L D E N L P T V L I V G G G D G M G G I V E I S K S L G V A L G --- T

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ZmMGD1 375 G E P T G Q I I V I C G R N K K L V N R L Q S I N W K -- V P V Q V K G F V T K M E E C M G A C D C I I T K A G P G T I A E A M I R G L P I
 OsMGD1 381 G E P T G Q I I V I C G R N K K L T S R L Q S I N W K -- V P V Q V K G F V T K M E E C M G A C D C I I T K A G P G T I A E A M I R G L P I
 AtMGD1 379 G E A V G Q V L I I C G R N K K L Q S K L S S L D W K -- I P V Q V K G F I T K M E E C M G A C D C I I T K A G P G T I A E A M I R G L P I
 PpMGD1 395 G K A V G Q L V V V C G R N K R L V K K L E A M N W N -- I P V K I N G F V T N M S E W M A A S D C I I T K A G P G T I A E A M I R G L P M
 PpMGD2 257 G K A L G Q L V V V C G R N K G L V K K L Q K I Q W N -- I P V K I N G F V T N M S E W M A A C D C I I T K A G P G T I A E A L I R G L P M
 AtMGD3 310 S N P I G Q L I V I C G R N K V L A S I L A S H E W K -- I P V K V R G F E T Q M E K W M G A C D C I I T K A G P G T I A E A L I C G L P I
 AtMGD2 306 R K P I G Q M V V I C G R N K K L A S A L E A D W K -- I P V K V R G F E T Q M E K W M G A C D C I I T K A G P G T I A E S L I R S L P I
 ZmMGD2 330 G K P I G Q L I V I C G R N K T L S S L Q A V E W K -- I P V K V R G F E T Q M E K W M G A C D C I I T K A G P G T I A E A L I R G L P I
 OsMGD3 337 G K P I G Q L I V I C G R N K T L S S L Q A L E W K -- I P I K V R G F E T Q M E K W M G A C D C I I T K A G P G T I A E A L I R G L P I
 OsMGD2 307 R R P V G Q I V V I C G R N Q V L R S T L Q S S R W N -- V P V K I R G F E K Q M E K W M G A C D C I I T K A G P G T I A E A L I R G L P I
 ZmMGD3 310 R R R V G Q V V V V C G R N R A L R S T L Q S L R W K -- V P V K I R G F E T Q M E K W M A A C D C I I T K A G P G T I A E A L I R G L P I
 PtMGD 256 A S T T T Q M V V V C G N N Q E A K A S L E K E S W G T T V R V N V Q G F V E N M D E W M K A S D A L V T K A G P G T I A E A S I C G L P C

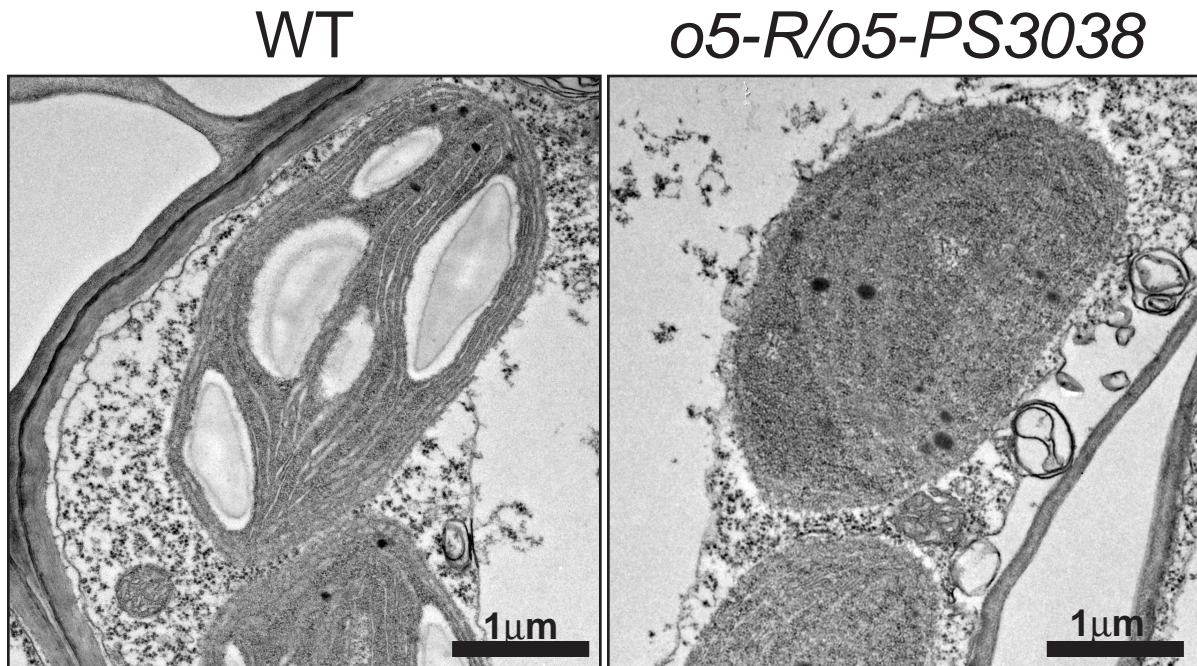
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ZmMGD1 443 I L N D Y I A G Q E A G N V P Y V V E N G C G K F S K S P K Q I A K I V A D W F G P R S D --- E F R V M S Q N A L K L A R P D A V F K I V
 OsMGD1 449 I L N G Y I A G Q E A G N V P Y V V D N G C G K F S K S P E Q I A K I V A D W F G P R S D --- E L K M M S Q N A L K L A R P D A V F K I V
 AtMGD1 447 I L N G Y I A G Q E A G N V P Y V V E N G C G K F S K S P K E I S K I V A D W F G P A S K --- E L E I M S Q N A L R L A K P E A V F K I V
 PpMGD1 463 I L F D F I A G Q E V G N V S F V V E N G A C T F C E E P K E I S R I I A D W F G F K A D --- Q L S K M A E Q C K K L A Q P D A V F K I V
 PpMGD2 325 V L F D F I A G Q E V G N V S F V V D N G A G A F C D K P K V I S K I I A D W F G A S S S --- K L K E M T E K C K M L G Q P D A V F K I V
 AtMGD3 378 I L N D Y I P G Q E K G N V P Y V V D N G A G V F T R S P K E T A K I V A D W F S N N K E --- E L K K M S E N A L K L S Q P E A V F D I V
 AtMGD2 374 I L N D Y I P G Q E K G N V P Y V V E N G A G V F T R S P K E T A R I V G E W F S T K T D --- E L E Q T S D N A R K L A Q P E A V F D I V
 ZmMGD2 398 I L N D F I P G Q E V G N V P Y V V D N G A G V F S K S P K E T A S L V A R W F G P D S E --- E L K K M S E K A L K L A Q P E A V F D I V
 OsMGD3 405 I L N D F I P G Q E V G N V P Y V V D N G A G V F S K S S R E T A K I V A R W F G P D S D --- E L K R M S E K A L K L A Q P E A V F D I V
 OsMGD2 375 I L N D F I P G Q E V G N V P Y V V D N G A G V F S K D P R E A A R Q V A R W F T T H T N --- E L R R Y S L N A L K L A Q P E A V F D I V
 ZmMGD3 378 I L N D F I P G Q E V G N V P Y V V D N G A G V F S K D P G E A A R Q V A R W F S S S S T D G D E L R R Y S R N A L R L A Q P E A V F H I V
 PtMGD 326 M L F S Y L P G Q E E C N I P F V E A C F G K Y S G D A S V I A N T V S S W L L S P E K --- L E A M R N A A L A A A R P O A T L N I A

ZmMGD1 510 H D L H E L V R E K C F V P Q Y A C A T -----
 OsMGD1 516 H D L H E L V R Q K C F V P Q Y A C A S -----
 AtMGD1 514 H D M H E L V R K K N S L P Q L S C T A -----
 PpMGD1 530 H D L D D M V N N K H R Y L E H L N V R Y R G L I -----
 PpMGD2 392 H D L D D M I R N K H M Y Q D H L K I K Y R G L I -----
 AtMGD3 445 K D I H H I S Q Q Q O R I P L F N E F S Y -----
 AtMGD2 441 K D I D E L S E Q R G P L A S V S Y N L T S S F A S L V -----
 ZmMGD2 465 K D M H E L S Q E Q G V I S Q I S S S L T S S F F L P S P E S T P L Q L I -----
 OsMGD3 472 R D I H E L S R E Q G V I S Q I S S S L T S S F F I P S P E T T P I Q L M -----
 OsMGD2 442 K D I H K L Q Q - Q P A T V T R I P - Y S L T S S F S Y S I -----
 ZmMGD3 448 R D I H K Q L Q R Q P A A V T R I P S R S L T S S F P Y H M -----
 PtMGD 392 K D L -----

Supplemental Figure 1. Alignment of the Maize MGD1 Amino Acid Sequence with MGD Proteins from Other Species.

Sequences used to generate the phylogenic tree shown in Figure 2C of the published manuscript were subjected to multiple sequence alignment using CLUSTALW. Aligned residues were shaded using BOXSHADE (http://www.ch.embnet.org/software/BOX_form.html), with identical residues in black and conservative substitutions in grey. The aligned sequences are from maize (ZMMGD1, NP_001142118; Zm MGD3, NP_001170057; Zm MGD2, NP_001147778), rice (Os MGD1, Q69QJ7; Os MGD2, Q6UTZ2; Os MGD3, Q0DWQ), Arabidopsis (At MGD1, NP_194906; At MGD3, NP_565352; At MGD2, NP_568394), the moss *Physcomitrella patens* (Pp MGD1, XP_001758690; Pp MGD2, XP_001755870), and the diatom *Phaeodactylum tricornutum* (Pt MGD, XP_002181685). Substitutions present in mutant alleles, detected from mutant cDNA sequences, are noted. The pound symbol (#) indicates the position of the *Mu8* insertion in *o5-PS3038* and the delta (Δ) indicates the position of the deletion-containing intron in *o5-313328* relative to the amino acid sequence.



Supplemental Figure 2. Bundle sheath chloroplasts from wild type and *o5-Ref/o5-PS3038* mutant leaves. Wild type bundle sheath chloroplasts do not contain granal stacks but laminar thylakoid membranes are still evident. Several starch grains (white bodies) are visible. Although some laminar structure is evident in the mutant thylakoid membranes, the level of organization appears greatly reduced.

Supplemental Table 1. Summary of *o5* Mutations and Phenotypes

Allele Name	Mutation ^a	Kernel Phenotype	Seedling Phenotype	Reference
<i>o5-Ref</i>	G/T transversion, nt 1511; G430C	Slightly shrunken, opaque, slightly sugary, pale yellow	Virescent	1
<i>o5-PS3038</i>	<i>Mu8</i> insertion, exon 1	Pale yellow to white, slightly sugary, extremely shrunken, germination-defective	Not applicable, germination-deficient	2
<i>o5-5288</i>	G/A transition, nt 504; G94D A/G transition, nt 653; M144V	White, shrunken	Seedling lethal; white seedling turns brown and dies at 7-14 days post-emergence	3
<i>o5-313328</i>	79 bp deletion, intron 7	Variable: Pale yellow to white shrunken, slightly sugary	Virescent	3

^a The reference sequence for nucleotide numbering is Genbank accession no. BT042676.

References

1. **Robertson, D.S.** (1967) A new opaque gene located on chromosome 7. *Maize Genet. Newsl.* **41**, 94-95.
2. **Stinard, P.S., Schnable, P.S., and Robertson, D.S.** (1993) A unique white endosperm viviparous allele of *opaque5*. *Maize Genet. Newsl.* **67**, 9
3. This study