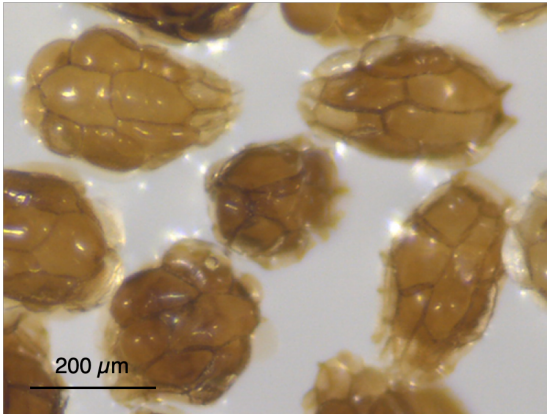
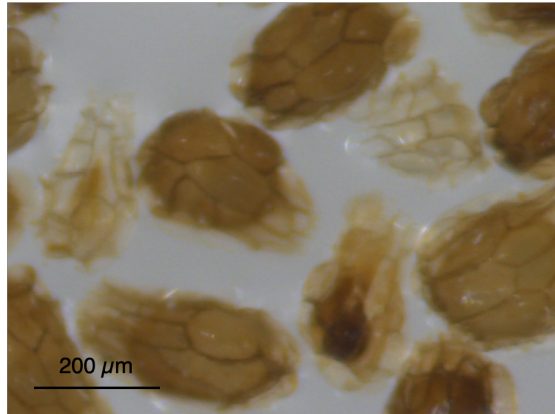


**Supplemental Fig. S1.** MALDI–MS/MS spectra of raffinose (A), a planteose isomer, and purified planteose (B). 2,5-Dihydroxybenzoic acid solution was mixed with 0.01 mg, 0.1 mg, or 1 mg mL<sup>-1</sup> of each compound solution and spotted onto ITO glass slides. After crystallization, the spots were analyzed by the iMScope TRIO with  $m/z$  527.16 [M + Na]<sup>+</sup> as a precursor ion.

**A****B**

**Supplemental Fig. S2.** *In vivo* visualization of  $\alpha$ -galactosidase activity in germinating seeds of *O. minor* with 5-bromo-4-chloro-3-indolyl- $\alpha$ -d-galactopyranoside (X- $\alpha$ -Gal). The seeds before *rac*-GR24 treatment after conditioning (0 DAT) (A) or 2 DAT (B) were treated with X- $\alpha$ -Gal solution ( $40 \mu\text{g mL}^{-1}$ ), indicating no pigmentation was observed before radicle emergence.

**A**

```

OmAGAL1      1 -----
AtAGAL1      1 MSRRAMVIKMPILMILISSMVMTMVESSRSVNNGHDDSEILRRHLLTNGLGVTFFPMGWNS

OmAGAL1      1 -----QGYLVPKNDTFPSSG
AtAGAL1      61 WNHFSCNIDEKMIKETADALVTTGLSKLGYNYVNIDDCWAEISRDSKGS LVPKKS TFPSSG

OmAGAL1      15 IKALADYVHSKGLKLG IYSDAGYFTCSK KMPGSLGHEEQDAETFA SWGIDYLYKDYDNCNTD
AtAGAL1      121 IKAVADYVHSKGLKLG IYSDAGYFTCSK TMPGSLGYEEDAKTFAEW GIDYLYKDYDNCNSD

OmAGAL1      75 GSRPTVRY PVMTRALMK TGRPIFFSLCEW GDMHPALWGFNVGNSWR TTNDIADNWD SMIS
AtAGAL1      181 GSKPTVRY PVMTRALMK SGRPIFHSLCEW GDMHPALWGS PVGNSWR TTNDIKDTWLS MIS

OmAGAL1      135 RADQNEVWAEHARPGGWNDPDMLEVGNGG MTKDEYI IHFISIWAISKAPLLIGCDVRNTTK
AtAGAL1      241 IADMNEVWAEHARPGGWNDPDMLEVGNGG MTKDEYI VHFISIWAISKAPLLIGCDIRNMTK

OmAGAL1      195 YVMDI IANT EVI AVNQDKLGVQAKKVRMEGDLEVWAGPLSNYRVAVVLLNRGPGRNAITA
AtAGAL1      301 ETMEI VANKEVIA INQDPHGVQAKKVRMEGDLEVWAGPLSGYRVALLLN RGPSTRSITA

OmAGAL1      255 YWDDIGI PPS SSVVARELWEHKTLKPRFVGNMTATVD SHACKMYILKPV S
AtAGAL1      361 LWEDIEI PANS IVEARDLWEHQTLKQKRFVGNLTATVD SHACKLYVLPKVA

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**B**

```

OmAGAL3      1 -----
AtAGAL3      1 MVIMKKMKDSVLFVVG LFLSLSVLVSQS IAGRVKAPLLQSNTGG LVFSKSFNSIYDTSMY

OmAGAL3      1 -----FFACD INE EVIKETADALVSTGLAK LGYTYVNI DD CWSE
AtAGAL3      61 GRLQLNNG LARTPQMGWNSWN FFACN INE TVIKETADALVSSGLAD LGYI HVNI DD CWSN

OmAGAL3      40 IIVRDSK GQLA F DAKS F P SGIK FLADY M HSKD LKLG IYSDAGAF TCQV R P G S L F H E S D D A E
AtAGAL3      121 LLRDSE GQL V P H P E T F P S G I K L L A D Y V H S K G L K L G I Y S D A G V F T C E V H P G S L F H E V D D A D

OmAGAL3      100 I F A S W G V D Y L K Y D N C F N L G I A P E K R Y P P M R D A L N A T G R M I F Y S L C E W G V H D P A L W A G K V G
AtAGAL3      181 I F A S W G V D Y L K Y D N C F N L G I K P I E R Y P P M R D A L N A T G R S I F Y S L C E W G V D D P A L W A K E V G

OmAGAL3      160 H S W R T T D D I N D S W A S M T M I A D I N D K W A A Y A G P G G W N D P D M L E V G N G G M T Y Q E Y R A H F S I W
AtAGAL3      241 N S W R T T D D I N D T W A S M T T I A D L N N K W A A Y A G P G G W N D P D M L E I G N G G M T Y E E Y R G H F S I W

OmAGAL3      220 A L M K A P L I I G C D V R N I T S E T L K L L T N E E V I A V N Q D P L G V Q G R K V Y S Y G P L D C Y Q V W A G P L
AtAGAL3      301 A L M K A P L I I G C D V R N M T A E T L E I L S N K E I I A V N Q D P L G V Q G R K T Q A N G E N D C Q Q V W S G P L

OmAGAL3      280 S G R R L A V V L W N R C L E A A T I T A K W A V L G L E S S I S V S I K D L W K H E Y V S K E T V A S F S A R V D G H
AtAGAL3      361 S G D R M V V A L W N R C S E P A T I T A S W D M I G L E S T I S V S V R D L W Q H K D V T E N T S G S F E A Q V D A H

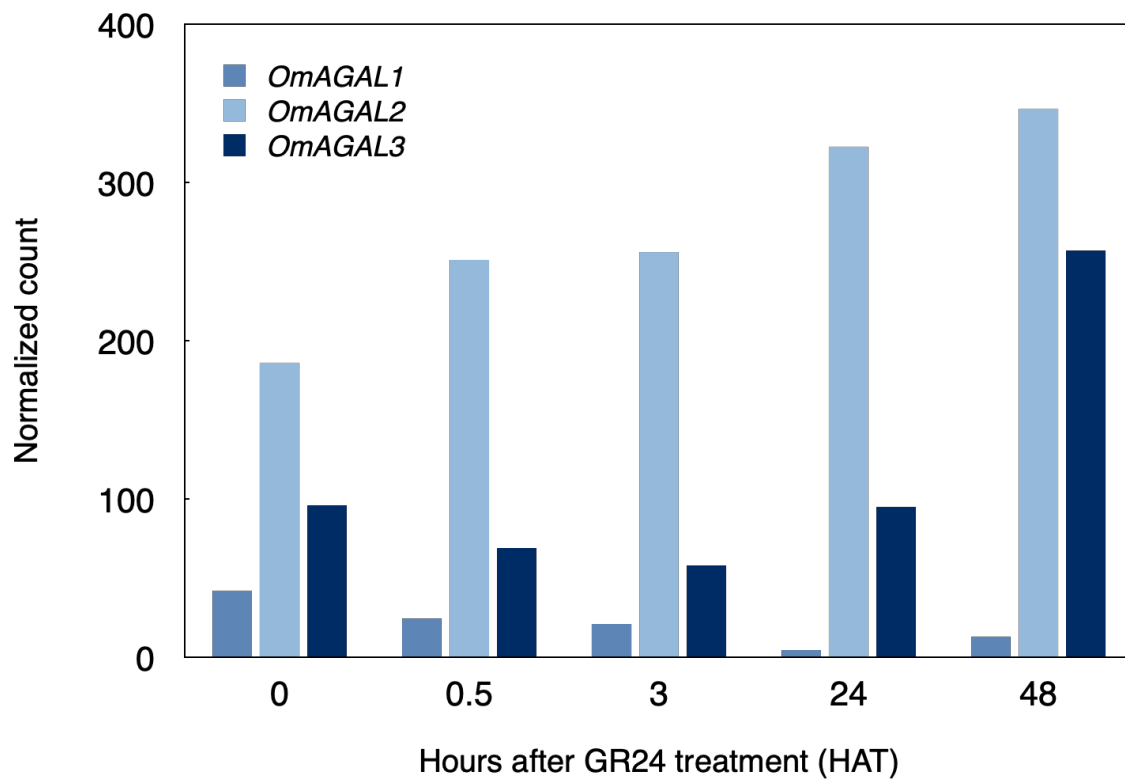
OmAGAL3      340 A C E M Y I F T Q L T E S R S S S
AtAGAL3      421 D C H M Y V L T P Q T V S H S D V

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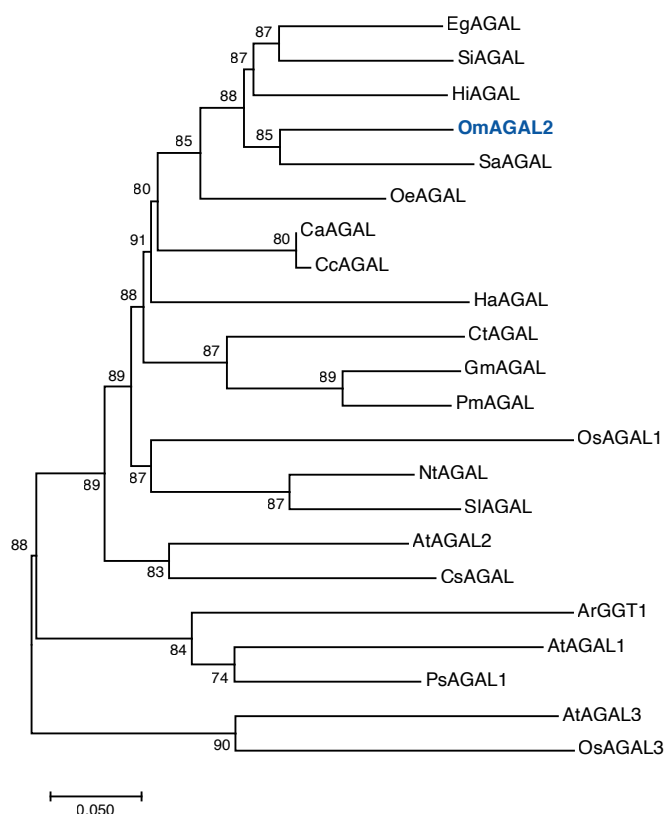
**Supplemental Fig. S3.** Alignments of a partial sequence of OmAGAL1 (LC636199) with Arabidopsis  $\alpha$ -galactosidase AtAGAL1 (At5g08380) (A) and a partial sequence of OmAGAL3 (LC636201) with Arabidopsis  $\alpha$ -galactosidase AtAGAL3 (At3g56310) (B) using COVALT ([https://www.ncbi.nlm.nih.gov/tools/cobalt/re\\_cobalt.cgi](https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi)) and BoxShade (version 3.21, [https://embnet.vital-it.ch/software/BOX\\_form.html](https://embnet.vital-it.ch/software/BOX_form.html)). Blue shading indicates identical amino acids, and gray shading indicates similar residues.

|         |     |                                                                           |
|---------|-----|---------------------------------------------------------------------------|
| OmAGAL2 | 1   | ----MAGNKNGNLMLLHNNIIRVVVVI FVGFNGSLACARLVR-----DGDSP--L                  |
| AtAGAL1 | 1   | ----MSRRAMVIKMPILMILISSMVM-----TMVESSRSVN-----NCHDDSEIL                   |
| AtAGAL2 | 1   | -----MVLLSFSLR-----FIAFTLTITLTQIA-----DGFQS----                           |
| AtAGAL3 | 1   | MVIMKMKKDSVLFLLVVGFLFSLVLSQSIAGRVKAPLLOSNNTGGLVFSSKSFNSIYDTSMY            |
|         |     | Signal peptide ↓                                                          |
| OmAGAL2 | 46  | <u>RQKLEENGLGPTPOMGWSSWNHFQCNIDESLIRETADAMISTGLASLGYQYINLDDCWAE</u>       |
| AtAGAL1 | 42  | <u>RRHLLTNGLGVTTPPMGWNSWNHFSNIDEKMIKETADALVTTGLSKLGYNYVNIDDCWAE</u>       |
| AtAGAL2 | 29  | <u>-RMLMNNGLALSPOMGWNSWNHFQCNINETLIKQTADAMVSSGLSALGYKYINIDDCWGE</u>       |
| AtAGAL3 | 61  | <u>GRLQLNNGLARTPOMGWNSWNHFFACNINETVIKETADALVSSGLADLGYIHVNIDDCWSN</u>      |
|         |     | AGAL motif                                                                |
| OmAGAL2 | 106 | <u>LNRDSQGNFVAKASTFPSPGIKALADYVHAKGLKGLGIYS DAGTQTCSNOMPGLGYEEQDA</u>     |
| AtAGAL1 | 102 | <u>ISRDSKGS LVPK KSTFPSPGIKAVADYVH SKGLKGLGIYS DAGYFTCSKTPMPSLGYEEHDA</u> |
| AtAGAL2 | 88  | <u>LKRDSQGS LVAKASTFPSPGIKALS DYVH SKGLKGLGIYS DAGTLTCSOTMPSLGH EEDA</u>  |
| AtAGAL3 | 121 | <u>LLRDSEGLVPHPETFPSPGIKLLADYVH SKGLKGLGIYS DAGVFTC-EVHPGSLFHEVDDA</u>    |
|         |     | ↓                                                                         |
| OmAGAL2 | 166 | <u>KTFASWGIDYLYKDYDNCNNNNISSKERYPAMAKALONSGRSIFFSLCEWGEDPATWAKTI</u>      |
| AtAGAL1 | 162 | <u>KTFAEWGIDYLYKDYDNCNSDGSKPTVRYPMTRALMKSGRPIFHSLCEWGDMPALW GSPV</u>      |
| AtAGAL2 | 148 | <u>KTFASWGIDYLYKDYDNCENTGTSRPRERYPKMSKALNSGRSIFFSLCEWGEDPATWAGDI</u>      |
| AtAGAL3 | 180 | <u>DIFASWGV DYLKDYDNC FN LGIKPIERYPPMRDALNATGRSIFVSLCEWGVDDPATWAK EV</u>  |
|         |     | ↓                                                                         |
| OmAGAL2 | 226 | <u>GNSWRTTGDISDNWD SMTSRADLNDKWASYAG PGGWNDPDMLEVNGGMTAE EYRSHFSI</u>     |
| AtAGAL1 | 222 | <u>GNSWRTTNDIKDTWLSMIS IADMN EYAEHAR PGGWNDPDMLEVNGGMTKDEYIVHFSI</u>      |
| AtAGAL2 | 208 | <u>GNSWRTTGDIQDNWKSMTLIADQNDRWASYARPGSWNDPDMLEVNGGMTKEEYMSHFSI</u>        |
| AtAGAL3 | 240 | <u>GNSWRTTDDINDTWASMTTIADLNNKWAAYAG PGGWNDPDMLEITNGGMTVEEYRSHFSI</u>      |
|         |     | ↓                                                                         |
| OmAGAL2 | 286 | <u>WALVKAPLLIGCDIRSV DNETLATLSNREVI AVNODKLGVOGKKIKKDGD---LEVWGGP</u>     |
| AtAGAL1 | 282 | <u>WAI SKAPLLIGCDIRNMTKETMEIVANKEVIAINODPHGVQAKKVRMEGD---LEVWAGP</u>      |
| AtAGAL2 | 268 | <u>WALAKAPLLIGCDLRSM D KVTFFELSNKEVIAVNODKLGIQGKKVKKEGD---LEVWAGP</u>     |
| AtAGAL3 | 300 | <u>WALMKAPLLIGCDVRNMTAETLEILSNKEIIAVNODPLGVQGRKIQANGENDCQQVWSGP</u>       |
|         |     | ↓                                                                         |
| OmAGAL2 | 343 | <u>LSGNRVAVVLWNRGSTETKITATWS DVGFDSTTVVDARDLWAHST-QTSINCHI SANVAP</u>     |
| AtAGAL1 | 339 | <u>LSGYRVALLLN RGPSRTSIT ALWEDIEIPANSIVEARDLWEHQTLKQKFVGNLTATVDS</u>      |
| AtAGAL2 | 325 | <u>LSKKRVAVILWNRGSASANITARWAEIGLNSSDIVNARDLWEHST-YSCVKKQLSALVEBP</u>      |
| AtAGAL3 | 360 | <u>LSGDRMVVALWNRCS EPATITASWDMIGLESTISVSVRDLWQH KDV TENTS CSFEAQVDA</u>   |
|         |     | ↓                                                                         |
| OmAGAL2 | 402 | <u>HDCKMYVLSPK-----</u>                                                   |
| AtAGAL1 | 399 | <u>HACKLYVLKPVA-----</u>                                                  |
| AtAGAL2 | 384 | <u>HACKMYTLTRRKA-----</u>                                                 |
| AtAGAL3 | 420 | <u>HDCHMYVLTPTQTVSHSDV</u>                                                |

**Supplemental Fig. S4.** Multiple alignment of OmAGAL2 (LC636200) with three  $\alpha$ -galactosidases, AtAGAL1 (At5g08380), AtAGAL2 (At5g08370), and AtAGAL3 (At3g56310), of Arabidopsis using COVALT ([https://www.ncbi.nlm.nih.gov/tools/cobalt/re\\_cobalt.cgi](https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi)) and BoxShade (version 3.21, [https://embnet.vital-it.ch/software/BOX\\_form.html](https://embnet.vital-it.ch/software/BOX_form.html)). Blue shading indicates identical amino acids, and gray shading indicates similar residues. Putative signal peptide and  $\alpha$ -galactosidase (AGAL) motif sequences are underlined. A black arrow indicates a conserved aspartate for recognition of galactose in GH27 family enzymes (Imaizumi *et al.*, 2017). Blue arrows indicate conserved aspartate residues in active sites (Tapernoux-Lüthi *et al.*, 2004; Imaizumi *et al.*, 2017).



**Supplemental Fig. S5.** Expression of three  $\alpha$ -galactosidase homologous genes in germinating seeds of *Orobanche minor*. Transcriptome analysis using RNA-Seq was conducted for germinating seeds of *O. minor* after conditioning and 0.5–48 h after *rac*-GR24 treatment (Okazawa *et al.*, 2020). Three  $\alpha$ -galactosidase homologous genes, *OmAGAL1*, *OmAGAL2*, and *OmAGAL3* were identified in the transcriptome.



**Supplemental Fig. S6.** Phylogenetic tree of selected plant  $\alpha$ -galactosidases constructed using the neighbor-joining method with MEGAX (version 10.1.8). ArGGT1, *Ajuga reptans* galactan:galactan galactosyltransferase 1 (AAR02007.1); CaAGAL, *Coffea arabica*  $\alpha$ -galactosidase (Q42656.1); CcAGAL, *Coffea canephora*  $\alpha$ -galactosidase (CAI47560.1); CsAGAL, *Cucumis sativus*  $\alpha$ -galactosidase-like precursor (NP\_001267530.1); CtAGAL, *Cyamopsis tetragonoloba*  $\alpha$ -galactosidase (P14749.1); EgAGAL, *Erythranthe guttata*  $\alpha$ -galactosidase (XP\_012855605.1); GmAGAL, *Glycine max*  $\alpha$ -galactosidase precursor (NP\_001235022.1); HaAGAL, *Helianthus annuus*  $\alpha$ -galactosidase (BAC66445.1); HiAGAL, *Handroanthus impetiginosus*  $\alpha$ -galactosidase (PIN09377.1); NtAGAL, *Nicotiana tabacum*  $\alpha$ -galactosidase (AEB98600.1); OeAGAL, *Olea europaea* var. *sylvestris*  $\alpha$ -galactosidase-like (XP\_022885408.1); OmAGAL2, *Orobancha minor*  $\alpha$ -galactosidase 2 (LC636200); OsAGAL1, *Oryza sativa* Japonica Group  $\alpha$ -galactosidase isoform X1 (XP\_015613565.1); OsAGAL3, *Oryza sativa* Japonica Group  $\alpha$ -galactosidase 3 (XP\_015646774.1); PsAGAL, *Pisum sativum*  $\alpha$ -galactosidase (CAF34023.1); PvAGAL, *Phaseolus vulgaris*  $\alpha$ -galactosidase (AAA73964.1); SaAGAL, *Striga asiatica*  $\alpha$ -galactosidase (GER38410.1); SiAGAL, *Sesamum indicum*  $\alpha$ -galactosidase (XP\_011072096.1); and SIAGAL *Solanum lycopersicum*  $\alpha$ -galactosidase (AAF04591.1)