INTRODUCTION

Plant-parasitic nematodes are soilborne pathogens of crops that cause tremendous yield losses (Jones et al., 2013). Sedentary plant-parasitic nematodes invade plant roots and establish feeding sites in the root tissue, which are unique organs in plant roots, characterized by a higher metabolic activity and different cell wall composition compared to normal root cells (Kyndt et al., 2013; Zhang et al., 2013).
et al., 2017). The sedentary plant-parasitic nematodes withdraw nutrients from these feeding sites for their growth and reproduction (Grundler & Hofmann, 2011).

Source-to-sink transport of sucrose, which is the major product of photosynthesis in plants (Zhu et al., 2013), includes three major steps: phloem loading, long-distance transport in phloem, and phloem unloading. This transport is mediated by both plasmodesmata (PD) and sugar transporters, such as the long-known sucrose transporters (SUTs) as well as the more recently found Sugars Will Eventually be Exported Transporters (SWEETs) (Chen et al., 2010; Jeena et al., 2019; Lemoine et al., 2013), among which OsSWEET11 to -15 are reported to be responsible for sucrose efflux (Chen et al., 2012; Eom et al., 2015; Zhou et al., 2015). Pathogens hijack plant sugar transport to acquire plant-derived sugars, facilitating their infection and colonization on plants (Verdier et al., 2012; Yamada & Osakabe, 2018). However, there is limited knowledge on how plant-parasitic nematodes modulate plant sugar location to enhance their infection success. Until now, two different mechanisms of sucrose supply to nematode-caused feeding sites had been reported in Arabidopsis thaliana. In Heterodera schachtii-caused syncytia in A. thaliana, the sucrose supply is mediated by AtSUC4 at early stages and by PD at later stages (Hofmann et al., 2007). The mechanism of sucrose transport into syncytia induced by H. schachtii in A. thaliana and by Globodera tabacum and H. schachtii in tobacco seem to be similar because fewer PD exist in the walls of G. tabacum- and H. schachtii-caused syncytia in tobacco toward neighbouring cells at early stages and lots at later stages (Hofmann et al., 2010). Unlike H. schachtii-caused syncytia, Meloidogyne incognita-caused giant cells (GCs) in A. thaliana are symplastically isolated from their neighbouring cells, and sucrose transport to the GCs is mediated by AtSUC4 (Hofmann, Kolev, et al., 2009). However, the opposite observation is found in tomato, where a symplastic transport pathway exists between M. incognita-caused GCs and the tomato root phloem (Dorhout et al., 1993). In addition, the GCs of M. incognita in tobacco are symplastically connected to their neighbouring cells at later stages because few PD exist in walls toward neighbouring cells at early stages, but more PD at later stages (Hofmann et al., 2010). These data elucidate that connections between nematodes’ feeding sites and their neighbouring cells and sucrose supply from phloem to their feeding sites are different, and we still have only a rudimentary understanding of the transport processes.

Meloidogyne graminicola (Mg) is an obligate sedentary plant-parasitic nematode of rice with a noticeable difference in the life cycle compared to other root-knot nematodes, where Mg lays eggs inside the rice root tissue, which makes it well adapted to irrigated conditions (Kyndt et al., 2014). How sucrose is transported from phloem to Mg-caused GCs in rice is unknown. Therefore, we analysed the expression of the OsSWEET11 to -15 and OsSUTs in Mg-infected compared to control rice plants. The role of PD was also analysed. PD can be involved in sucrose transport in plants, and PD permeability is negatively modulated by callose deposition along PD (Lemoine et al., 2013; Sevilem et al., 2015). OsSGL2 and OsGNS5 have been reported to encode a callose synthase and degradation enzyme, respectively (Dong et al., 2005; Hao et al., 2008; Yamaguchi et al., 2006). Here, OsSGL2- and OsGNS5-overexpressing rice lines were developed to investigate the role of PD in sucrose supply to Mg-caused GCs through manipulating callose deposition. Therefore, this work aimed to assess the roles of OsSWEET11 to -15 and OsSUTs as well as PD-mediated sucrose transport in the rice–Mg interaction.

2 | RESULTS

2.1 | Expression of OsSWEET11 to -15 and OsSUT genes was not induced in Mg-caused galls

Expression of the OsSWEET clade III members, OsSWEET11 to OsSWEET15, and the five OsSUT family members, OsSUT1 to OsSUT5, in Mg-caused galls at 3, 7, and 14 days postinoculation (dpi) were analysed by quantitative reverse transcription PCR (RT-qPCR). Results showed that the transcript levels of the five OsSWEETs in Mg-caused galls were extremely low or could even not be detected at all tested time points (data not shown). The RNA level of OsSUT1 was significantly reduced at 3 and 14 dpi compared to noninfected roots, but it was higher than the control at 14 dpi (Figure 1a). OsSUT2 was down-regulated at the three time points (Figure 1b), whereas a slight decrease was detected in the expression level of OsSUT4 at 3 dpi (Figure 1c). Down-regulation of OsSUT5 was observed at 7 and 14 dpi (Figure 1d). In addition, the expression level of both OsSUT3 and OsSUT5 at all the time points was extremely low, and that of OsSUT3 was too low to be detected (data not shown). Moreover, the promoter activities of OsSUT1 to OsSUT5 during Mg parasitism were studied using pOsSUT1 to -5::GUS rice lines. The pOsSUT1 to -5::GUS rice lines were validated by β-glucuronidase (GUS) staining of leaves, PCR amplification, and RT-qPCR analysis of GUS (Figure S1). No GUS signals were visible in roots and Mg-caused galls, whereas noticeable GUS signals were found in positive controls (Figure 1e), implying that the promoters of OsSUT1 to -5 were not active enough in roots and Mg-caused galls to yield a detectable GUS signal.

2.2 | Mg-caused GCs are symplastically connected to the phloem of rice roots

On phloem loading with carboxyfluorescein diacetate (CFDA), fluorescent signals of CFDA were observed in both Mg-caused galls and nematodes isolated from these galls at both J2 and adult stages, whereas no fluorescent signals were detected in the control (Figure 2). These data imply that symplastic connections exist between Mg-caused GCs and the phloem of rice roots.

2.3 | Callose deposition inside galls negatively affects Mg parasitism on rice

Callose deposition inside Mg-caused galls was analysed in rice plants overexpressing OsGNS5 and OsSGL2. OsGNS5 and OsSGL2 had
78- and 115-fold higher expression levels in OsGN5- and OsGSL2-overexpressing lines, respectively, than in nontransformed control rice (Figure 3a). No changes in root growth were observed in 15-day-old seedlings of the overexpressing lines compared with control rice (Figure 3b,c). Overexpressing the callose-degradation OsGN5 led to less callose deposition inside Mg-caused galls compared with control.
rice, and callose deposition was significantly increased in rice overexpressing the callose synthase OsGSL2 (Figure 3d).

There was no difference in the number of Mg-caused galls between the overexpressing and control lines (Figure 4a, b). However, in OsGNS5-overexpressing rice plants more juveniles and adult females were found compared with the control rice (Figure 4c), while their numbers were significantly reduced in OsGSL2-overexpressing rice plants (Figure 4d). Moreover, the size of the GCs was found to be significantly reduced in OsGSL2-overexpressing plants compared with that in control rice (Figure 4e, f and Video S1). No difference in the giant cell size was observed between OsGNS5-overexpressing plants and the control ($p < .05$; Figure 4e, f).

### 2.4 OsGSL2-overexpressing rice reduced sucrose content in Mg-caused galls

The effect of callose deposition on the sucrose supply to the galls was assessed by gas chromatography-mass spectrometry in OsGNS5- and OsGSL2-overexpressing rice compared to the control. The results indicated that the sucrose content was significantly higher in noninoculated roots of both OsGNS5-overexpressing rice (659 μg/g fresh weight [FW]) and OsGSL2-overexpressing rice roots (179 μg/g FW) compared with that of the control rice roots (32 μg/g FW; Figure 5). There was no significant difference in sucrose content of Mg-caused galls between control and OsGNS5-overexpressing rice (Figure 5a). On the contrary, sucrose content in Mg-caused galls was noticeably reduced from 608 μg/g FW in wild-type rice to 441 μg/g FW in OsGSL2-overexpressing plants (Figure 5b).

### 3 DISCUSSION

Here, we showed that none of the OsSWEET11 to -15 and OsSUT genes were induced in Mg-caused galls, and PD played a key role in sucrose supply from rice root phloem to Mg-caused GCs.

Sugar compartmentation has been shown to be an important strategy in plant-pathogen interactions (Yamada & Osakabe, 2018).
Plant pathogens can hijack host sugar transporters to regulate host sugar distribution and facilitate their parasitism. For instance, it has been found that *Puccinia striiformis* infection in wheat led to abscisic acid biosynthesis, which induced the sugar transporter gene *TaSTP6* (Huai et al., 2019). During the *Ustilago maydis*–*maize* interaction, *U. maydis* causes the formation of a tumour in which *ZmSUT1* and several *ZmSWEETs* are up-regulated to enhance sugar accumulation (Sosso et al., 2019).

Plant-parasitic nematodes, such as *Meloidogyne* spp. and *Heterodera* spp., infect host roots and establish feeding sites within the root tissues (Kyndt et al., 2013). It has been suggested that plant sugars are transported into the feeding sites and are stored as starch for nematodes’ carbohydrate needs (Hofmann et al., 2008; Vilele et al., 2019). Two different mechanisms on sucrose supply to feeding sites of sedentary plant-parasitic nematodes have been uncovered in *Arabidopsis*. Sucrose influx in *H. schachtii*-caused syncytia is mediated by AtSUCs (AtSUC2 and AtSUC4) at early and PD at later infection stages, but only mediated by the two AtSUCs in *M. incognita*-caused GCS (Hofmann et al., 2007; Hofmann, Kolev, et al., 2009; Juergensen et al., 2003; Zhao et al., 2018). OsSWEET11 to -15 function in sucrose efflux in rice (Chen et al., 2012; Eom et al., 2015; Zhou et al., 2015), and there are five sucrose transporter genes (OsSUT1 to OsSUT5) in the rice genome that are responsible for sucrose influx (Aoki et al., 2003). In this study, gene expression patterns of OsSWEET11 to -15 and OsSUT1 to -5 in Mg-caused galls were analysed by using noninoculated rice roots as control. Using noninoculated rice roots as control is acceptable when assessing RNA levels of genes in *Meloidogyne* spp.-caused galls, such as that in Balestrini et al. (2019) and Yamaguchi et al. (2017). In addition, even uninfected roots excluding meristematic roots or root tips have also served as the control in these kinds of analyses (Banora et al., 2011; Holbein et al., 2019). Moreover, according to the results obtained by Kyndt, Denil, Haegeman, Trooskens, De Meyer et al. (2012), rice sucrose transporter genes are not differentially expressed between rice root tips and mature root tissues. Data from the gene expression pattern analysis showed that RNA levels of OsSWEET11 to -15 were relatively low in both noninoculated rice roots and Mg-caused galls at each time point tested, indicating that OsSWEET11 to -15 do not function in sucrose supply from rice phloem to Mg-caused GCS. Although a slight up-regulation of OsSWEET13 in Mg-infected roots was found at 7 dpi in our RT-qPCR (data not shown), and the up-regulation has also been reported by Petitot et al. (2017), the RNA abundances of OsSWEET13 in both Mg-caused galls and uninfected rice roots were extremely low, suggesting that OsSWEET13 does not function in sucrose supply from rice phloem to Mg-caused galls. Other previous papers did not find up-regulation of OsSWEET in Mg-infected roots and Mg-caused GCS (Ji et al., 2013; Kyndt, Denil, Haegeman, Trooskens, Bauters et al., 2012). Meanwhile,
OsSUT2 was down-regulated in rice roots during Mg parasitism, and OsSUT3 and OsSUT5 had very low RNA levels, which was also found by Takahashi et al. (2017) for aerial tissues. Only OsSUT1 and OsSUT4 have similarly high transcript levels in both noninoculated and infected rice roots at later parasitism stages. OsSUT1 has been reported to be expressed in the sieve element-companion cells of leaf blades and sheaths (Matsukura et al., 2000; Scofield et al., 2007; Sun & Ward, 2012). In addition, OsSUT1 is induced by phosphorium deficiency in rice roots, and overexpressing OsSUT1 in rice leads to an increase in phosphorus content in both root and shoot (Feng et al., 2018), which indicates that OsSUT1 is involved in phosphorus uptake in rice. OsSUT4 is expressed in the seed aleurone layer, scutellum, and embryonic vascular bundle during early seed germination (Chung et al., 2014). Expression of OsSUT1 and OsSUT4 in roots indicates that these genes might play a role in sucrose supply to Mg-caused GCs in rice, which needs further assessment. However, the investigated pOsSUTs::GUS lines showed no promoter activities of the five OsSUTs in Mg-caused galls at all tested time points. No observable GUS signal in Mg-caused galls from the pOsSUT1- and pOsSUT4::GUS lines might result from their low promoter activities. Our finding is different from that reported in the M. incognita–Arabidopsis interaction (Hofmann, Hess et al., 2009; Zhao et al., 2018), but supported by the data from Kyndt, Denil, Haegeman, Trooskens, Bauters et al. (2012) and Petitot et al. (2017) that no OsSUT was induced in Mg-infected rice roots. However, the gene expression patterns of OsSUT1 to -5 in Mg-caused galls are partly supported by Ji et al. (2013) in that OsSUT2 is down-regulated in Mg-caused GCs at 7 dpi but up-regulated for OsSUT4. This difference could signify OsSUT4 induction specifically in the GCs that stayed undetected in the gall and root samples. It might also be due to biological variation and/or to differences in the applied algorithms for assessing expression levels. There is a
high sugar metabolism in GCs (Kyndt et al., 2013), and PD-mediated sucrose influx alone might be inadequate to meet the need for a large quantity of sugars. It has been widely reported that expression of some plant sugar transporter genes, such as SISUTs and SISWEETs, goes up in root tissues on nematode infection (Hofmann, Hess et al., 2009; Maboreke et al., 2016; Szakasits et al., 2009; Zhao et al., 2018), indicating their potential roles in sugar accumulation of nematode feeding sites. Although no up-regulation or observable promoter activities of OsSWEET11 to -15 and OsSUT1 to -5 in Mq-caused galls were observed here, it is a reasonable to assume that Mq could orchestrate rice sugar transport by hijacking rice sugar transporters somehow to enhance sucrose supply to Mq-caused GCs. Further analysis of OsSUT1 and OsSUT4 is warranted to figure this out.

CFDA has been widely used to assess PD permeability in rice and other plants (Botha et al., 2008; Julius et al., 2018; Wang et al., 2013). Here, leaf phloem loading with CFDA was performed at parasitic J2 and adult stages because *Meloidogyne* spp. can take up nutrients from GCs only at these two developmental stages (Moens et al., 2009). CFDA signals inside the feeding Mq isolated from rice galls demonstrated functional symplastic connections between Mq-caused GCs and the root phloem, which is similar to GCs caused by *M. incognita* in tomato (Dorhout et al., 1993). However, the opposite was found for *M. incognita*-caused GCs in *A. thaliana*, where the GCs are symplastically isolated from their neighbouring cells (Hofmann, Kolev et al., 2009). The difference might be due to the diversity of biological features of the feeding sites induced by different nematodes. For example, it has been reported that the cell wall composition of the feeding sites can differ depending on the specific nematode–plant interaction (Zhang et al., 2017).

PD permeability is negatively affected by callose deposition along PD (Tilsner et al., 2016). OsGSL2, a callose synthesis gene, is expressed in anthers, leaves, and roots and was suggested to play a pivotal role in callose synthesis during rice microsporogenesis (Yamaguchi et al., 2006). OsGNS5 encodes a callose degradation enzyme and was found to be up-regulated in rice leaf sheaths by *Nilaparvata lugens* and suggested to facilitate *N. lugens*–rice feeding by reducing callose deposition on the sieve plates of the leaf sheaths (Hao et al., 2008). To assess the role of PD in sucrose supply to Mq-caused GCs in rice, OsGSL2- and OsGNS5-overexpressing rice lines were analysed. Infection of those plants revealed that increased callose deposition attenuated Mq parasitism on rice while decreased callose had the opposite effect. Moreover, although increased callose deposition significantly increased the sucrose content of noninoculated roots compared with noninoculated control rice, the sucrose content in galls was reduced compared with control rice. These results reveal that increased callose deposition inside Mq-caused galls could decrease Mq parasitism by the reduction of PD-mediated sucrose supply from root phloem to Mq-caused GCs. In addition, relatively fewer adult females were observed in OsGSL2-overexpressing rice plants compared to rice control. It has been reported that sufficient nutrients in feeding sites are necessary for the development of juveniles into females (Grundler & Hofmann, 2011). The lower development into adult females might be due to the limited nutrition in the GCs of OsGSL2-overexpressing rice plants because smaller GCs and less sucrose in Mq-caused galls were found in OsGSL2-overexpressing rice plants compared to rice control. Although the OsGNS5-overexpressing line led to observable reduction of callose deposition in Mq-caused galls, there were no significant differences in GC size and sucrose content in the galls between the transgenic line and wild-type rice. This result could be explained by the already low callose deposition in Mq-caused galls in control rice. Despite no difference in GC size and sucrose content, more females developed in OsGNS5-overexpressing rice compared to the control. This could be due to the inhibition of callose-dependent rice resistance to Mq (Ellinger & Voigt, 2014; Schneider et al., 2016). Notably, both OsGSL2- and OsGNS5-overexpressing rice lines led to a significant increase of sucrose content of their noninoculated root tissues compared with control rice. To some extent this has also been evidenced by previous works. A rice grain setting defect 1 mutant that results in the
increase of PD permeability increased sucrose content in both flag leaves and grains (Gui et al., 2014). In contrast, callose deposition in PD under heat stress also increased sucrose content in leaf and sheath-stem (Zhang et al., 2018).

It is concluded that sucrose in root phloem is transported into Mg-caused GCs through PD, and OsSWEET1 to OsSWEET15 as well as OsSUT2, OsSUT3, and OsSUT5 do not function in this process, whereas the roles of OsSUT1 and OsSUT4 need further investigation. The present research underlines the importance of sugar supply to nematode feeding sites and the diversity among feeding sites of different nematodes.

4 | EXPERIMENTAL PROCEDURES

4.1 | Plant and nematode culture

The nematode Mg was originally isolated from rice in Haikou, Hainan Province, China and was cultured on rice (Oryza sativa ‘Nipponbare’) in controlled environmental conditions (28 °C under a 12 hr/12 hr light/dark regime). For all the following experiments, unless otherwise stated, rice plants were grown at 28 °C with 16 hr/8 hr light/dark cycles.

4.2 | Nematode infection test

Mg infection on OsGSL2- and OsGNS5-overexpressing rice lines was compared to nontransformed controls as follows. First, Mg eggs were isolated from 3-month-old infected roots by hand, followed by hatching at 28 °C for 2 days to collect the second-stage juveniles (J2s). Rice seedlings were grown in polyvinylchloride tubes that were filled with sand absorbent polymer (Reversat et al., 1999); when 14 days old they were then inoculated with 300 J2s per plant. Infected root samples were collected at 7 dpi. Juveniles inside roots were observed by staining as described (Bybd et al., 1983), and the numbers of juveniles inside roots and galls per plant were recorded. Ten seedlings were analysed for each treatment, and all the experiments were repeated three times.

4.3 | Development of transgenic rice lines

For construction of promoter-GUS lines, promoter fragments of OsSUT1 to -5 were chosen as reported (Chung et al., 2014; Eom et al., 2011; Li et al., 2020; Scofield et al., 2007), and they were amplified by PCR from genomic DNA of rice cv. Nipponbare using KOD-Plus (TOYOBIO). Sequence-specific primers were used as described in Table S1, and a BamHI restriction site was added in each primer. The promoter fragments of OsSUT1 to -5 amplified were 2,306, 2,194, 2,257, 2,415, and 2,239 bp, respectively. The promoter fragments of OsSUT1 to -5 were then cloned in the BamHI restriction site in front of the β-glucuronidase (GUS) reporter gene of the binary vector DX2181 (Du et al., 2011). This vector carries a hygromycin resistance gene for selection of transformed rice calli. All clones were confirmed by DNA sequencing. To overexpress OsGSL2 (GenBank accession no. AP003223) and OsGNS5 (GenBank accession no. U72251) in rice, the coding regions of OsGSL2 and OsGNS5 (1,449 and 1,032 bp, respectively) were amplified from rice cv. Nipponbare cDNA using gene-specific primers to which a KpnI restriction site was added (Table S1). The products were gel purified, cloned in the KpnI restriction site downstream of the ubiquitin promoter in the binary vector pU1301 (Hu et al., 2020), and confirmed by DNA sequencing. The hygromycin resistance gene carried by pU1301 was used for the selection of transformed rice calli. The binary vectors constructed above were introduced into Agrobacterium tumefaciens AH105 using electroporation, which was then used for transformation of rice cv. Nipponbare (Toki et al., 2006). Regenerated transgenic rice plants were grown in the greenhouse. GUS-positive transgenic plants of pOsSUT1 to -5::GUS were identified by GUS staining of leaves and propagated to obtain their homozygous progeny. All the rice lines used for GUS staining in this study were confirmed to be transgenic for GUS by PCR amplification with GUS-specific primers on their genomic DNA (Table S1). RT-qPCR was used to analyse the GUS RNA level in the roots (see below). Rice plants overexpressing OsGSL2 or OsGNS5 were selected by germinating seeds on agarose plates containing hygromycin (50 mg/L; Roche) and propagated to obtain their homozygous progeny, of which the relative transcript abundance of OsGSL2 and OsGNS5 compared with nontransformed rice was characterized through RT-qPCR as described below. The rice transgenic lines for use in this study were selected as follows: pOsSUT1 to -5::GUS homozygous rice lines with GUS RNA levels in roots comparable to that of corresponding OsSUTs in roots of wild-type rice and OsGSL2- and OsGNS5-overexpressing homozygous rice lines with high relative transcript abundance of the target gene in roots.

4.4 | Histochemical GUS assay

Histochemical GUS assay was performed according to Barthels et al. (1997). GUS staining was observed with a fluorescence stereomicroscope (Leica). For testing the expression of OsSUT1 to -5 in GCs at different developmental stages, promoter-GUS lines of pOsSUT1 to -5 were inoculated with Mg, and galls were sampled at 3, 7, and 14 dpi. The 35S promoter::GUS rice line was used as a positive control for the GUS assay. More than four plants from each group were tested.

4.5 | Gene expression analyses by RT-qPCR

For gene expression analysis of OsSWEET11 to -15 and OsSUT1 to -5 in rice root tissues during Mg parasitism on rice, seedlings were inoculated by Mg as described above. Control rice roots and galls caused by Mg were sampled at 3, 7, and 14 dpi. Noninoculated rice
14-day-old rice seedlings were sampled. Roots of nontransformed rice were used as the calibration sample for each time point, and the gene expression levels of these samples were defined as 1. For transcript abundance assessments of OsGSL2 and OsGNS5 in their corresponding overexpressing rice lines, root tissues of 14-day-old rice seedlings were sampled. Roots from nontransformed rice plants were used as the calibration samples and the gene expression levels of these samples were defined as 1. In addition, for the analysis of genes, the mRNAs corresponding overexpressing rice lines, root tissues of 14-day-old rice seedlings were sampled. Roots of nontransformed rice were used as controls and transcript levels of the corresponding OsSUTs in those samples were defined as 1.

Total RNA of each sample was isolated using TRIzol reagent according to the manufacturer's instructions (Invitrogen). The mRNAs were then reverse-transcribed using oligo(dT)18 primers (Invitrogen) and SuperScript III Reverse Transcriptase (Invitrogen). RT-qPCR was performed on an Applied Biosystems 7500/7500 Fast Real-Time PCR System (Life Technologies) with 20-μl PCR mix containing 2 μl cDNA, 200 nM of the appropriate oligonucleotide primers, 10 μl of 2 × SYBR Premix Ex Taq, and 0.4 μl of 50 × ROX Reference Dye II (TaKaRa). The mix was preheated at 95 °C for 15 s followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s. Melting curve analysis was performed from incubation at 65 °C followed by 0.5 °C incremental ramp up to 95 °C. Melting curve lines were used to validate product specificity. Two rice genes, Os1f4α (GenBank accession no. AK073620) and OsUBQ5 (GenBank accession no. AK061988), were used as internal references (Nahar et al., 2011). The primers used for RT-qPCR are listed in Table S1. Standard curve lines were established with four appropriate serial dilutions of first-strand cDNAs. Relative transcript abundance was calculated using the 2^ΔΔCT method (Livak & Schmittgen, 2001). At least six rice seedlings were pooled together for each sampling event. RT-qPCR was carried out in triplicate for each sample, and there were three independent biological replicates for each treatment.

4.6 | Quantification of callose deposition in Mg-caused galls

Callose depositions inside Mg-caused galls on the OsGNS5- and OsGSL2-overexpressing rice lines and the control Nipponbare were quantified at 7 dpi. Galls from each group were sampled and callose inside them was stained according to Millet et al. (2010) with some modifications, where samples were treated under vacuum for each step and galls were photographed under an SZX16 stereomicroscope (Olympus). Callose quantification was performed using ImageJ software (https://image.en.softonic.com/). At least 25 galls from at least six plants were quantified for each group, and all the experiments were repeated three times.

4.7 | Analysis of giant cell sizes

Mg-caused galls of OsGSL2- and OsGNS5-overexpression lines as well as control Nipponbare were collected at 7 dpi. GC measurement was then performed by using a TCS SP8 laser scanning confocal microscope (Leica) as described (Cabrera et al., 2018), where z series of GCs at adult female stage were stacked and the largest size area of each giant cell was recorded using ImageJ software. At least 11 GCs from at least five plants were recorded for each line. There were three independent biological replicates.

4.8 | Sucrose quantification by gas chromatography-mass spectrometry

For characterizing the effect of altered callose deposition on sucrose content of root tissues and Mg-caused galls, the transgenic rice lines overexpressing OsGSL2 and OsGNS5 as well as their control lines were inoculated with Mg and compared to the corresponding noninoculated rice plants. Galls from inoculated rice plants and roots from the control were washed and collected at 7 dpi. Soluble sugars were extracted in 1.5 ml of 75% methanol. After incubation for 15 min at 70 °C, 1.2 ml of each sample was taken and transferred into a new tube. Then, 100 μl of 50 μg/ml ribitol (Sigma-Aldrich) was added into each tube as internal standard and evaporated in vacuo to dryness. Finally, 50 μl of 15 mg/ml methoxyamine hydrochloride (Sigma-Aldrich) was added to each tube. The tubes were incubated for 2 hr at 65 °C, followed by adding 50 μl of N-methyl-N-trimethylsilyl trifluoroacetamide (Santacruze) to each tube, and then incubated for another 2 hr at the same condition. Samples prepared above were used for sucrose content analysis.

An Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer and a DB-5MS column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific) was employed for analysis of sucrose in the samples. Helium was used as carrier gas at a flow rate of 1 ml/min. The volume of injection was 1 μl and the split ratio was 10:1. The oven temperature was held at 70 °C for 4 min and then raised to 310 °C at 5 °C/min and held at that temperature for 10 min. All samples were analysed in both full-scan (mass range of 40–510 amu) and selective ion scan modes. The injector inlet and transfer line temperature were 290 and 280 °C, respectively. Sucrose was identified from the retention times and mass spectra using the corresponding reference materials. Quantification of sucrose was performed according to the standard curves of reference materials. Each sample consisted of about 15 mg of fresh root tissues or galls, where root tissues were pooled from at least six plants and galls from at least 15 plants. There were three independent sampling events and three independent biological replicates.

4.9 | Phloem loading with exogenous sucrose and CFDA

Phloem loading with CFDA was carried out as described in Wright and Oparka (1997) with some modifications. CFDA was loaded on leaves of infected seedlings at 3 and 14 dpi with Mg, where the
leaves were tweaked with tweezers and the applied absorbent cotton balls contained CFDA (0.65 mM; Sigma). Control plants were mock loaded with sterile distilled water. Seedlings were kept in darkness for 16 hr after CFDA was loaded. Finally, par-J2s and adult females were isolated from galls at 3 and 15 dpi, respectively, and the green fluorescence of CFDA in the nematodes and galls were tested with a fluorescence stereomicroscope (Leica) using UV light and an FITC filter. At least 15 galls from seven plants and nematodes isolated from the galls were observed for each experiment, and there were three independent biological replicates.

### 4.10 Statistical analysis

All data were expressed as mean ± SE. The experimental data were analysed using SPSS v. 30.0 (SPSS Inc.), applying a Student’s t test for pairwise comparisons, or analysis of variance (ANOVA) and Tukey’s test for multiple comparisons of group means. Values with \( p < 0.05 \), \( p < 0.01 \), or \( p < 0.001 \) were considered statistically significant.

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### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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### REFERENCES

Aoki, N., Hirose, T., Scofield, G.N., Whitfeld, P.R. & Furbank, R.T. (2003) The sucrose transporter gene family in rice. *Plant and Cell Physiology*, 44, 223–232. https://doi.org/10.1093/pcp/pcg030

Balestrini, R., Rosso, L.C., Veronico, P., Melillo, M.T., De Luca, F., Fanelli, E. et al. (2019) Transcriptomic responses to water deficit and nematode infection in *Nicotiana.* *Frontiers in Microbiology*, 10, 1807. https://doi.org/10.3389/fmicb.2019.01807

Banor, M.Y., Rodic, N., Baldacci-Cresp, F., Smertenko, A., Bleve-Zacheo, T., Mellilo, M.T. et al. (2011) Feeding cells induced by phytoparasitic nematodes require γ-tubulin ring complex for microtubule reorganization. *PLoS Pathogens*, 7, e1002343. https://doi.org/10.1371/journal.ppat.1002343

Barthels, N., van der Lee, F.M., Klap, J., Goddijn, O.J., Karimi, M., Puzio, P. et al. (1997) Regulatory sequences of Arabidopsis drive reporter gene expression in nematode feeding structures. *The Plant Cell*, 9, 2119–2134. https://doi.org/10.2307/3870573

Botha, C.E.J., Aoki, N., Scofield, G.N., Liu, L., Furbank, R.T. & White, R.G. (2008) A xylem sap retrieval pathway in rice leaf blades: Evidence of a role for endocytosis? *Journal of Experimental Botany*, 59, 2945–2954. https://doi.org/10.1093/jxb/ern150

Bybd, D.W. Jr., Kirkpatrick, T. & Barker, K.R. (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology*, 15, 142–143.

Cabrera, J., Olmo, R., Ruiz-Ferrer, V., Abreu, I., Hermans, C., Martinez-Argudo, I. et al. (2018) A phenotyping method of giant cells from root-knot nematode feeding sites by confocal microscopy highlights a role for CHITINASE-LIKE 1 in Arabidopsis. *International Journal of Molecular Sciences*, 19, 429. https://doi.org/10.3390/ijms19020429

Chen, L.-Q., Hou, B.H., Lalonde, S., Takanaga, H., Hartung, M.L., Qu, X.-Q. et al. (2010) Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature*, 468, 527–532. https://doi.org/10.1038/nature09606

Chen, L.-Q., Qu, X.-Q., Hou, B.H., Sooso, D., Osorio, S., Fernie, A.R. et al. (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science*, 335, 207–211. https://doi.org/10.1126/science.1213351

Chung, P., Hsiao, H.H., Chen, H.J., Chang, C.W. & Wang, S.J. (2014) Influence of temperature on the expression of the rice sucrose transporter 4 gene, OsSUT4, in germinating embryos and maturing pollen. *Acta Physiologiae Plantarum*, 36, 217–229. https://doi.org/10.1007/s11738-014-1403-x

Dong, X.Y., Hong, Z.L., Sivaramakrishnan, M., Mahfouz, M. & Verma, D.P.S. (2005) Callose synthase (CalSS) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis. *The Plant Journal*, 42, 315–328. https://doi.org/10.1111/j.1365-313X.2005.02379.x

Dorhout, R., Gommers, F.J. & Kolloffel, C. (1993) Phloem transport of carboxyfluorescein through tomato roots infected with *Meloidogyne incognita*. *Physiological and Molecular Plant Pathology*, 43, 1–10. https://doi.org/10.1006/pmpp.1993.1035

Du, H., Liu, L., You, L., Yang, M., He, Y., Li, X. et al. (2011) Characterization of an inositol 1,3,4-trisphosphate 5/6-kinase gene that is essential for drought and salt stress responses in rice. *Plant Molecular Biology*, 77, 547–563. https://doi.org/10.1007/s11103-011-9830-9

Ellinger, D. & Voigt, C.A. (2014) Callose biosynthesis in Arabidopsis with a focus on pathogen response: What we have learned within the last decade. *Annals of Botany*, 114, 1349–1358. https://doi.org/10.1093/aob/mcu120

Eom, J.-S., Chen, L.-Q., Sooso, D., Julius, B.T., Lin, I.W., Qu, X.-Q. et al. (2015) SWEETs, transporters for intracellular and intercellular sugar translocation. *Current Opinion in Plant Biology*, 25, 53–62. https://doi.org/10.1016/j.pbi.2015.04.005

Eom, J.S., Cho, J.I., Reinders, A., Lee, S.W., Yoo, Y., Tuan, P.Q. et al. (2011) Impaired function of the tonoplast-localized sucrose transporter in rice, OsSUT2, limits the transport of vacuolar reserve sucrose and affects plant growth. *Plant Physiology*, 157, 109–119. https://doi.org/10.1104/pp.111.176982

Feng, B., Sun, Y., Ai, H., Liu, X., Yang, J., Liu, L. et al. (2018) Overexpression of sucrose transporter OsSUT1 affects rice morphology and physiology. *Chinese Journal of Rice Science*, 32, 549–556. http://www.ricesci.cn/EN/10.1681/j.1001-7216.2018.8015

Gruendler, F.M.W. & Hofmann, J. (2011) Water and nutrient transport in nematode feeding sites. In Jones, J., Gheyseyn, G. & Fenoll, C. (Eds.) *Genomics and Molecular Genetics of Plant-Nematode Interactions*. Springer, pp. 423–439.

Gui, J.S., Liu, C., Shen, J.H. & Li, L.G. (2014) *Grain setting defect 1*, encoding a remorin protein, affects the grain setting in rice through regulating plasmodesmatal conductance. *Plant Physiology*, 166, 1463–1478. https://doi.org/10.1104/pp.114.246769

Hao, P., Liu, C., Wang, Y., Chen, R., Tang, M., Du, B.O. et al. (2008) Herbivore-induced callose deposition on the sieve plates of rice: An important mechanism for host resistance. *Plant Physiology*, 146, 1810–1820. https://doi.org/10.1104/pp.107.111484

Hofmann, J., Hess, P.H., Szakasits, D., Blöchl, A., Wieczorek, K., Daxböck-Horvath, S. et al. (2009) Diversity and activity of sugar transporters in nematode-induced root syncytia. *Journal of Experimental Botany*, 60, 3085–3095. https://doi.org/10.1093/jxb/erp138
Vilele, R. M. I. F., Martini, V. C., Gonçalves, L. D., Kuster, V. C. & de Oliveira, W. R. (1997) Metabolic inhibitors induce symplasmic movement of solutes from the transport phloem of Arabidopsis root. The Plant Cell, 25, 2315–2329. https://doi.org/10.1105/tpc.113.110676

Wright, K. M. & Opara, K. J. (1997) Metabolic inhibitors induce symplasmic movement of solutes from the transport phloem of Arabidopsis root. Journal of Experimental Botany, 48, 1807-1814. https://doi.org/10.1093/jxb/48.10.1807

Yamada, K. & Osakabe, Y. (2018) Sugar compartmentation as an environmental stress adaptation strategy in plants. Seminars in Cell & Developmental Biology, 83, 106-114. https://doi.org/10.1016/j.semcdb.2017.12.015

Yamaguchi, T. Hayashi, T., Nakayama, K. & Koike, S. (2006) Expression analysis of genes for callose synthases and rho-type small GTP-binding proteins that are related to callose synthesis in rice anther. Bioscience, Biotechnology, and Biochemistry, 70, 639–645. https://doi.org/10.1271/bbb.70.639

Yamaguchi, Y. L., Suzuki, R., Cabrera, J., Nakagami, S., Sagara, T., Ejima, C. et al. (2017) Root-knot and cyst nematodes activate procambium-associated genes in Arabidopsis roots. Frontiers in Plant Science, 8, 1195. https://doi.org/10.3389/fpls.2017.01195

Zhang, C. X., Feng, B. H., Chen, T. T., Fu, W. M., Li, H. B., Li, G. Y. et al. (2018) Heat stress-reduced kernel weight in rice at anthesis is associated with impaired source–sink relationship and sugars allocation. Environmental and Experimental Botany, 155, 718–733. https://doi.org/10.1016/j.envexpbot.2018.08.021

Zhang, L., Lilley, C. J., Imren, M., Knox, J. P. & Urwin, P. E. (2017) The complex cell wall composition of syncytia induced by plant parasitic cyst nematodes reflects both function and host plant. Frontiers in Plant Science, 8, 1087. https://doi.org/10.3389/fpls.2017.01087

Zhao, D., You, Y., Fan, H. Y., Zhu, X. F., Wang, Y. Y., Duan, Y. X. et al. (2018) The role of sugar transporter genes during early infection by root-knot nematodes. International Journal of Molecular Sciences, 19, 302. https://doi.org/10.3390/ijms19010302

Zhou, J., Peng, Z., Long, J., Sosso, D., Liu, B. O., Eom, J.- S. et al. (2015) Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. The Plant Journal, 82, 632–643. https://doi.org/10.1111/tpj.12838

Zhu, X. G., Wang, Y., Ort, D. R. & Long, S. P. (2013) e-photosynthesis: A comprehensive dynamic mechanistic model of C3 photosynthesis: From light capture to sucrose synthesis. Plant, Cell and Environment, 36, 1711-1727. https://doi.org/10.1111/pce.12025

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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