Protein arginine methyltransferase expression affects ectomycorrhizal symbiosis and the regulation of hormone signaling pathways

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Abstract

The genomes of all eukaryotic organisms, from small unicellular yeasts to humans, include members of the protein arginine methyltransferase (PRMT) family. These enzymes affect gene transcription, cellular signaling and function through the post-translational methylation of arginine residues. Mis-regulation of PRMTs results in serious developmental defects, disease or death, illustrating the importance of these enzymes to cellular processes. Plant genomes encode almost the full complement of PRMTs found in other higher organisms, plus an additional PRMT found uniquely in plants: PRMT10. Here we investigate the role of these highly conserved PRMTs in a process that is unique to perennial plants – the development of symbiosis with ectomycorrhizal fungi. We show that PRMT expression and arginine methylation is altered in the roots of the model tree *Eucalyptus grandis* by the presence of its ectomycorrhizal fungal symbiont *Pisolithus albus*. Further, using transgenic modifications, we demonstrate that *E. grandis*-encoded PRMT1 and PRMT10 have important, but opposing, effects in promoting this symbiosis. In particular, the plant specific EgPRMT10 has a potential role in the expression of plant hormone pathways during the colonization process and its over-expression reduces fungal colonization success.

Introduction

Ectomycorrhizal (ECM) fungi associate with the roots of most tree species in temperate and sub-tropical forests worldwide, improving plant access to nutrition and protecting them from biotic and abiotic stressors in exchange for photosynthates (Smith and Read 2008). The establishment of a successful mutualistic relationship between plant and fungus requires a carefully balanced exchange of signaling molecules and appropriate transcriptomic alteration on the part of both partners. Several recent studies have considered the transcriptional changes in host roots throughout the colonization process (Plett et al. 2015a; Plett et al. 2015b; Sebastiana et al. 2014; Tarkka et al. 2013). Typically, plant roots will launch a general defense response upon perception of the fungus, although less pronounced than with a pathogen attack. At later stages of the interaction this response will shift to include hormone signaling, and changes to cell wall architecture and nutrient shuttling to accommodate the establishing symbiotic partnership. Proper regulation of hormone signaling within plant tissues is crucial to the interaction (Daguerre et al. 2016; Martin et al. 2001), however, the underlying mechanisms and signaling pathways to produce these necessary transcriptional changes are not well understood.

One mechanism by which both transcription and protein function can be altered is via protein post-translational modifications (PTMs), which can include phosphorylation, acetylation, glycosylation, ubiquitination or methylation. PTMs of histones, transcription factors or DNA binding proteins can directly alter transcription of specific genes or gene clusters (DesJarlais and Tummino, 2016) or PTMs can change the function of a given protein through changes to its structure, stability or localization. Post-translational modifications such as phosphorylation are known to affect the outcome of some beneficial plant-microbe interactions (Antolin-Llovera et al. 2012). One emerging PTM of interest, protein methylation at arginine residues, is catalyzed by protein arginine methyltransferases (PRMTs) using S-adenosyl-L-methionine (SAM) as a methyl donor (Bedford and Clarke, 2009; Ahmad and Cao, 2012; Blanc and Richard, 2017; Peng and Wong, 2017). PRMTs are well conserved in all eukaryotic organisms and have been implicated in altered transcription, RNA processing, transport and translation, signal transduction, DNA repair, chromatin structure, cell cycle regulation and cellular differentiation (Bedford and Clarke, 2009;

Blanc and Richard, 2017; Raposo and Piller, 2018). Common targets of PRMTs are histones (Liu et al. 2010), although many non-histone methylated proteins have also been identified as PRMT substrates (Lee and Stallcup, 2009; Wei et al. 2014).

Nine PRMT homologues have been identified in the *Arabidopsis thaliana* genome (Ahmad and Cao, 2012). PRMT homologues have also been identified in other annual plants such as rice (Ahmad et al. 2011) and soybean (Liew et al. 2013). While most of these PRMTs are conserved and present in other higher eukaryotic organisms, plants uniquely have evolved an additional type I PRMT - PRMT10 – which has proposed roles in histone modification and flowering time in *Arabidopsis* (Niu et al. 2007; Cheng et al. 2011). Type I PRMTs produce mono-methylated arginine as an intermediate and asymmetrically dimethylated arginines as the final product and, in *Arabidopsis*, have been implicated in transcription and RNA processing (Yan et al. 2007; Ahmad et al. 2011), ribosomal biogenesis (Hang et al. 2014) and flowering time (Niu et al. 2007; Niu et al. 2008; Liew et al. 2013). Type II PRMTs produce symmetrically dimethylated arginine as the final product and, in plants, have known roles in pre-mRNA splicing (Deng et al. 2010; Deng et al. 2016), flowering time (Deng et al. 2016; Wang et al. 2007), salt stress tolerance (Zhang et al. 2011), primary root length (Pei et al. 2007), root stem cell maintenance (Li et al. 2016) and circadian rhythms (Sanchez et al. 2010). While less is known about the role of PRMTs in perennial plants, previous research on *E. grandis* (Plett et al. 2017) has demonstrated an important role for type I PRMTs in the development of roots. *E. grandis* roots with transgenically down regulated type I PRMT genes had significantly shorter roots and fewer lateral roots formed (Plett et al. 2017). Repression of PRMT1 activity using chemical inhibitors resulted in a broad transcriptomic shift in the roots (Plett et al. 2017), highlighting the importance of PRMTs in cellular signaling and transcription regulation networks.

Here we consider the effect of protein methylation by two type I PRMTs, EgPRMT1 and 10, on the colonization outcomes and transcriptomic regulation of the interaction between *E. grandis* and *Pisolithus albus*, an ECM fungal symbiont. PRMT1 is considered to be the most active PRMT with as much as 85% of arginine methylation in mouse cells attributed to it (Ahmad and Cao, 2012). PRMT10 is of interest as it has uniquely evolved within plant genomes. While the role of PRMTs in mutualistic

plant-fungal associations has not been studied, there has been some work in pathogenic interactions. There are a number of examples during pathogenesis whereby aberrant histone methylation in the plant causes reduced immunity due to lower transcription of defense related genes (reviewed by Zhu et al. 2016). The majority of what is known concerning protein methylation, and its impact on the outcome of pathogenic interactions, however, focuses on lysine methylation rather than arginine methylation. One of the few studies considering asymmetric arginine dimethylation during pathogenesis focused on the activity of a type I PRMT from a fungal pathogen and found that it bolstered fungal virulence (Wang et al. 2012). Here we show that EgPRMT1 and EgPRMT10 have important, but opposing, effects in the promotion of mutualistic symbiosis between *E. grandis* and its ECM partner *P. albus*. Elevated expression of *EgPRMT1* in the roots of *E. grandis* results in a significant improvement in the number of lateral roots colonized by *P. albus*, while increased expression of *EgPRMT10* significantly decreases the colonization success of the fungus. Gene expression analysis demonstrates that EgPRMT10 has an effect on the expression of key hormone responsive genes in various hormone signaling pathways. These results suggest that PRMT10 may have evolved control over the plant specific hormone pathways and, concurrently, mycorrhizal symbiosis.

Results

PRMT genes are differentially regulated in roots undergoing colonization by ECM fungi

The roots of *E. grandis* seedlings were placed in contact with the ectomycorrhizal fungus *Pisolithus albus* strain SI-12 (Plett et al. 2015a) and expression of *E. grandis* PRMT genes was monitored over a two-week colonization time course. Within 24hrs of contact with the host plant, *P. albus* hyphae began growing around active root tips, increasing through 48hrs to 1 week of contact. Between 1-2 weeks of the colonization process, the hyphae condense around the root to form a thick mantle and establish the mycorrhizal root tip (Figure 1A-B). Expression of the seven PRMT genes encoded by *E. grandis* varied widely across this colonization timeline relative to axenic controls (Figure 1C). *EgPRMT1* is significantly repressed at nearly all of the time points, although this repression is quite small (expressed at about 65-75% of control levels). *EgPRMT10,* meanwhile, was universally over-expressed except for the 48hr time-point. At the 48hr time-point, *EgPRMT3* and *4* were also significantly over-expressed, while *EgPRMT5* expression was significantly reduced. *EgPRMT7* was also significantly up-regulated during the later time-points of the colonization process.

Colonization by *P. albus* alters protein methylation in *E. grandis*

Total protein was extracted from *E. grandis* control roots (grown without fungal contact), roots in indirect contact with *P. albus* for 48 hours (i.e. pre-symbiosis, an early symbiosis establishment time-point), and fully colonized roots (2 weeks post contact, a symbiosis maintenance time-point). The resulting proteins were analyzed by mass spectrometry to identify proteins with methylated arginine residues (Table 1). In some cases, proteins that were methylated in control roots, were not found to be methylated in roots exposed to *P. albus*, and *vice versa*. For example, at 48 hours in ATP synthase subunit beta proteins, R159 is methylated only in axenic controls, while in the elongation factor 1-alpha protein, R166 is non-methylated in axenic controls but gains a methyl group upon indirect fungal contact. Also, in many cases, proteins were methylated in both control and test roots, but the specific arginine(s) methylated within the protein sequence changed (e.g. HSP80 is methylated at R329 and R375 in axenic root samples, but at R98 during the 48-hour pre-symbiosis stage with *P. albus*).

Thus, the presence of *P. albus* causes alterations not only in the transcription of PRMTs, but in the methylation pattern on different proteins expressed in the plant roots. It should be noted that mass spectrometry cannot differentiate between asymmetric and symmetric arginine dimethylation, thus this table represents activity of both type I and II PRMTs.

Chemical inhibition of PRMT activity and transgenic variation of PRMT gene expression alters colonization of *E. grandis* roots by *P. albus*

To determine the effect of PRMT activity on *E. grandis* root colonization by *P. albus,* we used a combination of chemical inhibitors of PRMT activity and transgenic roots differentially expressing EgPRMT1 and EgPRMT10. All inhibitors of PRMT activity (adenosine dialdehyde (AdOx; general methylation inhibitor), arginine methyltransferase inhibitor 1 (AMI-1) and 2,3-dimethoxynitrostyrene (DMNS; PRMT1 inhibitor)) applied to *E. grandis* roots in contact with *P. albus* caused the percentage of mycorrhizal root tips to significantly decline to approximately half the number found on the control treated plants (Figure 2A). As PRMT inhibitors can equally affect the plant roots and the colonizing fungus, these experiments were complemented with the expression of *E. grandis* PRMTs modified transgenically. Similar to the inhibitor experiments, transgenic repression of *EgPRMT1* resulted in a significant reduction in the percentage of lateral roots colonized by *P. albus.* Elevated expression of *EgPRMT1* resulted in an increase in the number of roots colonized by *P. albus* (Figure 2B). Interestingly, *35S::EgPRMT10* roots showed lower levels of colonization and *EgPRMT10* RNAi roots had elevated percent of mycorrhizal root tips as compared to controls (Figure 2B). While this may seem to contradict the results from the inhibitor experiments, AdOx and AMI-1 are general inhibitors and the resulting repression of other PRMTs may be masking the effect of EgPRMT10 repression. While previous reports on *E. grandis* have demonstrated that transgenic knock down of either EgPRMT1 or EgPRMT10 results in shorter roots with fewer lateral roots (Plett et al. 2017), contact with *P. albus* caused both EgPRMT1 RNAi and EgPRMT10 RNAi roots to grow and branch such that, at harvest, they did not contain significantly fewer lateral roots than PRMT over-expressors or controls (based on students t-test, $p>0.05$ in all cases). Data from all transgenic roots are presented as an average (Figure 2B), however, results from individual roots and lateral rooting data can be found in Supplementary Figure S3.

EgPRMT1 or *EgPRMT10* affect the expression of different functional gene categories in *P. albus* colonized roots

RNA sequencing analysis of transformed mycorrhized roots (*35S::EgPRMT1, EgPRMT1* RNAi, *35S::EgPRMT10* and *EgPRMT10* RNAi) revealed a set of 2089 genes that exhibited differential expression when compared to mycorrhized wild type controls (>5-fold change in gene expression; $p<0.001$; Figure 3). A large number of these genes encode kinases, disease resistance proteins (including TIR-NBS-LRR and NB-ARC), cytochrome P450s, various enzymes (ex. hydrolases, peptidases etc.), DNA binding proteins or transcription factors.

This list of genes was further filtered to produce two gene groupings: those regulated in an opposite manner between 35S::EgPRMT1 and EgPRMT1 RNAi transgenic roots (Group A, 143 genes total; Supplementary Table S2) and those regulated in an opposite manner between 35S::EgPRMT10 and EgPRMT10 RNAi transgenic roots (Group B, 467 genes total; Supplementary Table S3). Genes without at least a five times change in expression in at least one of the considered conditions were discarded. There is some overlap between the two groups (see Figure 3). Group A (EgPRMT1 affected) does not contain as many genes as Group B, nor does it contain unique gene families that are not represented in some form in Group B. EgPRMT10 affects the expression of a number of genes found in hormone biogenesis and signaling pathways not similarly found in EgPRMT1 transformants. Table 2 lists some of the most notable genes in these hormone pathways while the complete list of all Group B EgPRMT10 regulated genes is in Supplementary Table S3.

PFAM enrichment analysis of the genes included in Groups A and B gives an extensive list of enriched PFAM families in the areas of biological processes (see Supplemental Tables 4-7). Those PFAM families that are within the top ten of each data set (based on Z-score) that also pertain to defense or symbiosis responses or hormone signaling are listed in Table 3. Both Group A and B show enrichment in defense responses to a fungus, the flavonoid and terpenoid pathways and gibberellin biosynthetic pathways. Group B genes (EgPRMT10 affected) are also enriched in genes pertaining to responses to general hormones and jasmonic acid. To ensure that these were meaningful results, we performed the same PFAM enrichment analysis on genes up-regulated in all four conditions (446 genes) or down-regulated in all four

conditions (537 genes) and found none of the same enriched PFAM domains, except for one instance of 'response to hormone' where genes were up-regulated in all four conditions.

EgPRMT10 expression is correlated to the expression of key genes in plant hormone pathways

As the transcriptomic data indicates that EgPRMT10 may affect plant hormone pathways, we treated plant roots with dsiRNA designed to specifically reduce the expression of *EgPRMT10* and considered the effect on the expression of genes involved in hormone response. dsiRNA was shown to enter plant cells within 2 hours through application of a fluorescently labeled control (Figure 4A) and to persist in the roots for the duration of the experiment. Application of the test EgPRMT10 dsiRNA caused a significant reduction in the expression of *EgPRMT10* within 4 hours of exposure as compared to a scrambled control dsiRNA treatment (Figure 4B). After 8 hours, however, expression levels of *EgPRMT10* began to return to normal, or to be over-expressed compared to controls, possibly due to the depletion of the dsiRNA construct or to plant feedback. qPCR analysis demonstrated that a number of hormone responsive genes were affected by the reduction in *EgPRMT10* expression, including genes involved in jasmonic acid and gibberellin pathways complementing the transcriptomic data sets (Figure 4C). These genes showed a strong, significant positive correlation between their relative expression levels and that of *EgPRMT10*. Thus, the alteration in *EgPRMT10* expression simultaneously affected gene expression in multiple hormone pathways.

Discussion

E. grandis is an economically important tree in forestry and bioenergy sectors and a useful model organism given its fully sequenced genome (Myberg et al. 2014). In natural settings, its key ECM associates are *P. albus* and the closely related *P. microcarpus*. While many aspects of the signaling and transcriptomic regulation involved in the formation of ectomycorrhizal symbiosis between these partners are known (Duplessis et al. 2005; Plett et al. 2015a), the role of post-translational modifications, particularly methylation, has not been investigated. We demonstrate here a novel role of host PRMTs in balancing the symbiotic interaction between *E. grandis* and the mycorrhizal fungus *P. albus*. Expression of EgPRMT1 facilitates mycorrhizal colonization, while expression of EgPRMT10 is inhibitory to colonization, thus EgPRMT1 and EgPRMT10 appear to affect pathways that work antagonistically in the colonization process. Q-PCR analysis of the expression of EgPRMT genes throughout the colonization process shows that EgPRMT1 is generally repressed compared to control roots and EgPRMT10 is overexpressed. Thus, based on our results, the plant host appears to regulate the expression of these PRMTs and any downstream pathways in a way that is antagonistic to fungal colonization. While defenses raised by a plant host against a mycorrhizal fungus are generally much milder than those raised against a pathogen, they are believed to serve as a mechanism to protect the plant from over-colonization by the fungus (Plett et al., 2014b; Plett and Martin, 2017). The regulation of these PRMTs may serve as a protection for the plant as with other defense related gene pathways. Other *E. grandis* PRMTs are also differentially expressed over the time course of colonization and their more specific involvement in the mycorrhization process remains a question for future research.

In addition to demonstrating differential transcription of PRMT genes throughout the colonization process, we used analysis of protein methylation by mass spectrometry to demonstrate that the protein targets of *E. grandis* PRMTs are altered by the presence of *P. albus*. While we have not detected all methylated proteins from the tissues analyzed, it is interesting to note within those proteins found here that there were a number of examples whereby arginine methylation changed depending upon whether the root was grown axenically or if it was exposed to *P. albus*. In many of

these cases, exposure to *P. albus* results in additional methylation at arginine residues, highlighting the specific activity of PRMTs in the plant response to the fungus. In other cases, we show that the original methylation seen in axenically grown roots is no longer detected. For example, the heat shock 70kDa protein-like protein has two arginine residues, R225 and R608, which are only methylated under axenic conditions. Until recently, the presence of an arginine de-methylase protein was highly debated, however, it is now commonly accepted that a Jumonji-domain containing protein (JMJD6) might have a role in the de-methylation of arginines (Chang et al. 2007; Poulard et al. 2016). Alternatively, protein turnover may describe the effects seen. As old protein is recycled and new protein is transcribed, a new pattern of post-translational modifications can be applied. It will be interesting to determine in future investigations how these differences in methylation sites affect protein function with relation to plant-microbe interactions.

Previous work on the effect of type I PRMTs on root development in *E. grandis* showed that repression of either EgPRMT1 or EgPRMT10 resulted in the same phenotype – a reduction in root growth and lateral rooting (Plett et al. 2017). This raised the question of whether *E. grandis* type I PRMTs acted redundantly, in concert, or affected different parts of the same pathways. Our results show that while EgPRMT1 and EgPRMT10 may alter some pathways in common, they also have distinct roles and pathways that they control as evidenced by the different effect of their expression on mycorrhization. Additionally, these results demonstrate that the mycorrhization phenotype observed is unlikely to be caused entirely by the root growth phenotypes previously reported. The process of mycorrhizal root tip formation is highly dependent on the induction of lateral roots and thus the short root phenotype of the RNAi knock down roots would appear to be inhibitory to colonization. ECM fungi, however, are known to stimulate lateral root formation (Burgess et al. 1996; Felten et al. 2009) and, in our experiments, contact with *P. albus* caused both EgPRMT1 RNAi and EgPRMT10 RNAi roots to grow and branch such that, at harvest, they were similar in appearance to controls. Transcriptomic analysis of ECM colonized roots differentially expressing *EgPRMT1* or *EgPRMT10* (both *35S::* and RNAi) was conducted to indicate potential pathways that were being affected to cause the observed phenotype. The total of 2089 genes significantly ($p<0.001$) differentially expressed across these transgenic roots highlights the large impact that

PRMTs have on cellular processes. In *E. grandis* roots differentially expressing *EgPRMT10,* we observed significant regulation of genes associated with plant hormone synthesis and regulation including genes associated with the ethylene, jasmonic acid and auxin pathways. Appropriate balance of hormone signaling within plant roots is important for the successful establishment of ECM symbiosis. Ethylene and jasmonic acid production are antagonists to ECM colonization of host tissues (Plett et al. 2014a,b) while auxin improves colonization through modification of root growth and architecture (Vayssieres et al. 2015). Overall, enrichment analysis shows a significant over-representation of genes with PFAM domains associated with hormone stimuli in the Group B transcriptomic data set. This indicates that EgPRMT10 may have a potentially important effect on proteins in or upstream of multiple plant hormone signaling pathways. Alternatively, differential expression of *EgPRMT10* could also affect other physiological pathways in the root that indirectly result in a shift in hormone signaling pathways. To address this possibility, experiments using dsiRNA to reduce the expression of *EgPRMT10* on a shorter time scale demonstrated that the expression of *EgPRMT10* was significantly correlated to the expression of a number of genes within hormone responsive pathways. The high level of correlation to several genes in multiple hormone pathways may be indicative of the rapid cross talk between hormone pathways, or that EgPRMT10 targets a master regulator of these pathways. For example, in *Arabidopsis*, the SWI/SNF chromatin remodeling complex is known to affect the expression of all genes tested in this study (Sarnowska et al. 2016). As PRMT10 has evolved only in plant lineages, there is very little indication of its role or activity in the literature, however, one study has shown that it is able to methylate histones and has a role in flowering time through controlling the expression of FLOWERING LOCUS C (FLC) in *Arabidopsis* (Niu et al. 2007). Like the establishment of ectomycorrhizal symbiosis, flowering time in plants is a process that is highly dependent on hormonal and epigenetic regulation (see recent reviews by Campos-Rivero et al. (2017) and Conti (2017)). It remains to be seen whether the impact of EgPRMT10 activity on hormone signaling is direct – through modification to histones or proteins in hormone pathways – or indirect – via the methylation of a transcriptional regulator.

Overall, here we present a framework, identifying unique and opposing effects of *E. grandis* PRMT1 and 10 homologues in mutualistic symbiosis. Additionally, we

suggest a potential role for EgPRMT10 in the regulation of plant-based hormones. This work highlights the evolutionary importance of post-translational modifications like arginine methylation in biotic interactions and paves the way for future work on the specific mechanisms of control.

Materials and Methods

Mycorrhization time course and PRMT expression

E. grandis seedlings were grown from sterile seed on 1% agar media for one month followed by growth on ½ MMN (Modified Melin-Norkrans media; 1 g/L glucose) on a sterile cellophane membrane for one further month. Two-week old colonies of *P. albus* (strain SI-12; Plett et al. 2015a), grown on ½ MMN media were placed on top of the plant roots and left for two weeks (16:8 h light cycle; 25^{o}C). Control seedlings were placed on ½ MMN without any fungus. For the mycorrhization q-PCR timecourse, three biological replicates of both test and control seedlings were harvested at 24 hours, 48 hours, 1 week and 2 weeks post contact. These samples were immediately frozen in liquid nitrogen upon harvest and RNA was extracted using Qiagen RNeasy Plant Mini kits as per manufacturer's instructions. RNA was used as a template for cDNA synthesis using the iScript cDNA Synthesis Kit (Bio-Rad) and the SensiFAST SYBR No-ROX kit (Bioline) was used for q-PCR analysis on a Corbett Rotor-gene 6000 RT-PCR cycler. Log2 fold expression change from axenic roots was calculated using three biological replicates for EgPRMT1 (*Eucgr.C03665*), EgPRMT10 (*Eucgr.C01117*), EgPRMT3 (*Eucgr.G03214*), EgPRMT4 (*Eucgr.B01318*), EgPRMT5 (*Eucgr.D02618*), EgPRMT6 (*Eucgr.J00342*) and EgPRMT7 (*Eucgr.D02075*). Q-PCR gene expression results were normalized using *Eucgr.B03031* and *Eucgr.B02502* as control genes (Cassan-Wang et al. 2012; see Supplementary Table S1 for primer sequences).

Protein extraction and analysis

For the production of protein samples, *E. grandis* seedlings were grown as above. The seedlings were either put in indirect contact with *P. albus* SI-12 for 48-hours (accomplished using a sterile cellophane membrane between the plant and fungal colony to allow the passage of nutrients and small molecules, but not roots or hyphae), put in direct contact with SI-12 for two weeks, or left in axenic culture (harvested at 48 hour and 2 week time points). All cultures were grown on ½ MMN. Roots from 3-5 seedlings from each of these three treatments were pooled to generate sufficient tissue for protein extraction and treated as one biological replicate. Results presented are from three biological replicates (i.e. three independent sets of pooled

samples) for all conditions. After extraction, proteins were separated on a SDS-PAGE gel and analyzed by mass spectrometry according to the methods of Plett et al. (2017).

Mycorrhization of inhibitor treated roots

E. grandis seedlings were grown as above and placed in contact with a 2-week old colony of SI-12. They were treated with water, 5 μM adenosine dialdehyde (AdOx), 50 μM arginine methyltransferase inhibitor 1 (AMI-1) (Cheng et al. 2004), 1% DMSO or 30 μM 2,3-dimethoxynitrostyrene (DMNS; in 1% DMSO; Dillon et al. 2012) for the inhibition trials (n=9-17 plants per treatment). Inhibitor treatment was delivered via aerosol (to avoid over-wetting the fungus in one place) to the root system using a sterilized atomizer. Approximately 1 mL of liquid was added each time, and treatments were administered six times over the two-week fungal contact period at regular intervals. At the end of the two weeks, percent fungal colonization on each root system was scored (number of colonized lateral roots/total lateral roots x 100%). Determination of whether a root tip is colonized or not is assessed visually based on morphological changes in the shape and color of the root tip.

Production and mycorrhization of roots mis-expressing *EgPRMT1* or *EgPRMT10*

EgPRMT1 (*Eucgr.C03665*) and *EgPRMT10* (*Eucgr.C01117*) *35S::* and RNAi constructs were cloned from cDNA synthesized using iScript (Bio Rad) from total RNA extracted from *E. grandis* roots using the RNeasy Plant Mini RNA extraction kit according to manufacturer's instructions (Qiagen). The amplified fragments were gel purified and ligated into pDONR222 and sequence verified. Positive inserts were then transferred to pH2GW7 (*35S::*) or to pH7GWIWG2(II) (RNAi) vectors using Gateway Gene Cloning (Life Technologies) and transformed into *Rhizobium rhizogenes* isolate K599. *E. grandis* seedlings were grown from seed to one month old on 1% agar media. The root system of the plant was cut off and the resulting wound was dipped in freshly grown *R. rhizogenes* expressing the plasmid of choice, or wild type *R. rhizogenes* as a control. Dipped plants were embedded in MS media and left for one week, placed upside down, in a growth cabinet with a constant temperature of 25^{o}C and a 16 hour photoperiod. Plants were then transferred to fresh MS media supplemented with 150 μg/mL Timentin and grown upright under the same conditions (Macrae and van Staden, 1993). Transformed roots typically emerged

within one or two weeks and were allowed to grow for another month and were then transferred to ½ MMN media, covered with a sterile cellophane membrane. Successful transformation of the roots was confirmed by verifying alteration to the expression of the transgene using the RNA sequencing (Supplementary Figure S1). We also used our RNAseq data set to determine the expression of genes previously reported as positively (+) or negatively (-) co-regulated with *PRMT1* and *PRMT10* (based on *E. grandis* publicly available transcriptomic data sets [www.phytozome.org] and based on *Arabidopsis thaliana* publicly available transcriptomic data sets [http://bar.utoronto.ca/ntools/cgi-bin/ntools_expression_angler.cgi]). The logic behind this test was that genes positively co-regulated by PRMT1 or PRMT10 should also be increased in expression in their respective *35S::PRMT* transgenic roots vs. control and more repressed in PRMT RNAi transgenic roots vs. control (and vice versa for negatively co-regulated genes). Those that exhibited this pattern of co-expression with the expression of the PRMT transgenes are shown in supplementary figure S1. Given the fact that these two lines of evidence were complementary to each other, we concluded that our transgenic roots were (i) properly transformed and (ii) that PRMT activity was being affected in the expected manner as determined by the expression patterns of previously described co-regulated genes.

For mycorrhization trials, two-week old colonies of *P. albus* (strain SI-12) grown on ½ MMN media were placed on top of the plant roots and left for two weeks (16:8 h light cycle; 25^{o}C) before percent mycorrhization was scored.

Transcriptomic analysis

Three independent transformed (*35S::EgPRMT1*; *35S::EgPRMT10*; *EgPRMT1* RNAi; *EgPRMT10* RNAi) or control (wild type *R. rhizogenes*) mycorrhized root systems were harvested at the two week time point and frozen immediately in liquid nitrogen and RNA was extracted using Qiagen RNeasy Plant Mini kits as per manufacturer's instructions. Transcriptional analyses of all tissues were performed using RNA-seq via conventional poly-A library preparation for Illumina sequencing. Library construction and 100-bp paired-end reads sequencing was performed by the Western Sydney University Next Generation Sequencing Facility. The samples were indexed and run on a high-output lane of an Illumina Hi-Seq 2000 flow-cell. Raw reads were trimmed for quality and aligned to the primary transcripts of the *E. grandis*

genome taken from www.phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Egrandis (accessed November 2015; Myburg et al. 2014) using CLC Genomics Workbench 7. For mapping, the minimum length fraction was 0.9, the minimum similarity fraction 0.8, and the maximum number of hits for a read was set to 10. The unique and total mapped reads number for each transcript were determined, and then unique reads were normalized to reads per kilobase of exon model per million mapped reads (RPKM). Variation in the expression of the transgene between transformed mycorrhized root systems was checked to ensure sufficient correlation of the data generated. Based on RPKM, the standard error in EgPRMT1 or 10 expression was between 2.6 and 6.7% for transgenic roots, only slightly higher than, or equivalent to, 1.4-2.6% for wild type roots. Genes listed as significantly regulated (based on a students t-test with false discovery rate (FDR) Benjamini-Hochberg multiple testing correction applied to the data) were differentially regulated a minimum of 5 times as compared to wild-type control roots in at least one of the four sets of transformants. Group A and B gene lists were generated from this larger group of genes by considering only genes oppositely regulated in 35S: and RNAi pairs (EgPRMT1 for Group A and EgPRMT10 for Group B) and having a differential expression of at least five times from control roots in the 35S: or RNAi roots or both.

PFAM enrichment analysis of PFAM domains associated with Group A and Group B genes was done using the online program dcGO Enrichment (http://supfam.org/SUPERFAMILY/cgi-bin/dcenrichment.cgi; accessed December, 2017; Fang and Gough, 2013). PFAM families were assessed using the full ontology function and a FDR of <0.001. All domains that were enriched in the area of "Biological Function" are listed in Supplemental Tables 4-7.

dsiRNA treatment of *E. grandis* roots and hormone analysis

Two-month old *E. grandis* seedlings grown as above were treated with solutions of custom synthesized double stranded interfering (dsi) RNA (Integrated DNA Technologies). Roots were treated with dsiRNA designed to reduce expression of EgPRMT10 (complementing GATATGAGTATCTTGACCAAACCAT sequence in gene transcript), a scrambled control sequence, or a Cy® 3 fluorescently labeled cell entry control. All dsiRNA was diluted to a concentration of 2 nM in liquid ½ MMN

and roots were immersed in these solutions for 4 or 8 hours. At each harvest time point, roots were removed from the solution, rinsed in fresh ½ MMN, blotted dry and snap frozen in liquid nitrogen. Fluorescently labeled dsiRNA treated roots were harvested into 4% paraformaldehyde in 1xPBS (phosphate buffered saline) for microscopy. Roots for microscopy were rinsed three times in 1xPBS and observed using a confocal microscope (Leica TCS SP5). The presence of the dsiRNA inside the root cells was confirmed by Cy® 3 fluorescence emission at 570 nm, as compared to non-treated control roots. RNA was extracted from five to six biological replicates of roots (over two independent experiments) treated with either test or scrambled dsiRNA and qPCR analysis was conducted. RNA was used as a template for cDNA synthesis using the SensiFAST cDNA Synthesis Kit (Bioline) and the SensiFAST SYBR No-ROX kit (Bioline) was used for q-PCR analysis on a BioRad CFX96 Touch RT-PCR cycler. Log2 fold expression change from control roots treated with scrambled dsiRNA was calculated for EgPRMT10 (*Eucgr.C01117*), and the closest *E. grandis* homologues to the known Arabidopsis hormone responsive genes: GA3ox1 (*Eucgr.F02568*), Myc2 (*Eucgr.E00277*), VSP2 (*Eucgr.J02927*), ARR16 (*Eucgr.G03141*), ARR6 (*Eucgr.B02571*), PIN3 (*Eucgr.B02902*), PIN2 (*Eucgr.C00078*) and ABI3 (*Eucgr.H00815*). Q-PCR gene expression results were normalized using *Eucgr.C00350* and *Eucgr.K02046* as control genes (see Supplementary Table S1 for primer sequences).

Phylogenetic tree construction

To confirm that PRMT10 homologues are only present in plant genomes a phylogenetic tree was constructed (Supplementary Figure S2). PRMT protein sequences from human (*Homo sapiens*; Venter et al. 2001), mouse (*Mus musculus*; Dietrich et al. 1996), cow (*Bos taurus*; Fries et al. 1993), Xenopus (*Xenopus laevis*; Roe et al. 1985), zebrafish (*Danio rerio*; Woods et al. 2000), fruit fly (*Drosophila melanogaster*; Adams et al. 2000), yeast (*Saccharomyces cerevisiae*; Mewes et al. 1997), *Arabidopsis thaliana* (Lamesch et al. 2012), rice (*Oryza sativa*; Ouyang et al. 2007) and Eucalyptus (*E. grandis*; Myberg et al. 2014) were retrieved using the PANTHER protein classifications PTHR11006 and PTHR10738. A phylogenetic tree was constructed using the online tool 'Phylogeny.fr' (Dereeper et al. 2008). The sequences were aligned using MUSCLE (default settings) and the phylogenetic tree was reconstructed using the maximum likelihood method (PhyML program v3.1/3.0

aLRT). All of the PRMT-like protein sequences were downloaded from the Phytozome database (Phytozome v10.3: phytozome.jgi.doe.gov: accessed 27/02/2018) for plant sequences and Uniprot (www.uniprot.org: accessed 27/02/2018) for all other sequences.

Statistical methods

Three or more independent biological replicates were performed for each test outlined. A biological replicate is represented by one seedling root system except in the protein extraction experiments, where each biological replicate consists of 3-5 pooled root systems to generate sufficient sample for each of three extractions. For all transgenic experiments, a minimum of three independent transgenic root systems were used. A Student's two-tailed independent *t*-test was calculated to determine the statistical significance of the differences observed ($p < 0.05$) unless otherwise noted. Correlations between EgPRMT10 and test gene expression in the dsiRNA treatment experiment were determined by calculating the Pearson's correlation coefficient (r) and the correlation was deemed as significant for $p < 0.05$.

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Data Availability Statement

The transcriptomic datasets generated and analyzed in this study are available at http://hie-pub.westernsydney.edu.au/47b96970-1b57-11e8-a446-525400daae48/.

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Tables

Table 1 Identification of arginine methylation sites from total protein extracted from 48-hour (n=3) or two-week axenic controls (n=3), 48-hour indirect fungal contact (n=3), or 2-week mycorrhized (n=2) E. grandis roots. As specific proteins were detected multiple times in each sample, the mean percentage detected as methylated is given to indicate the consistency of the methyl modification across all proteins detected (+/- S.D.). Underlined arginine residues are mono-methylated while bolded arginine residues are di-methylated. Those methylated residues that are superscripted with an * are unique to that given sample condition as the residue was detected and confirmed as not being methylated in other sample conditions. N.D indicates that the protein was not detected by mass spectrometry. N.M. indicates that a portion of the protein was detected, but no methylation was detected.

Table 2 Selected genes pertaining to hormone synthesis or signalling from those that are significantly differentially regulated in mycorrhized roots with modified EgPRMT10 expression as compared to wild type mycorrhized roots (Group B genes).

Table 3 List of the over-represented PFAM families that pertain to defense, symbiotic responses or hormone signaling from the Group A and Group B datasets that are within the top ten most enriched families (based on Z-score) within the dataset.

841 **Figure Legends**

842

843 **Figure 1 Change in expression of PRMT genes in *E. grandis* roots during**
844 **colonization by the ectomycorrhizal fungus *P. albus.*** (**A**) *E. grandis* undergoing
845 colonization by *P. albus* (two weeks post contact). (**B**) Close up of mature
846 mycorrhizal root tips (two weeks post contact). (**C**) The relative expression (log2) of
847 PRMTs encoded by *E. grandis* in roots undergoing colonization as compared to
848 axenically growing roots. Data is presented over the timecourse of colonization: 24hr
849 (white), 48hr (dark grey), 1 week (black) and 2 weeks (light grey) post contact and is
850 the average of three biological replicates ± SE; * = significant expression change as
851 compared to axenically grown roots ($p<0.05$).

852

853 **Figure 2 Effect of PRMT inhibition or transgenic mis-expression on the percent**
854 **of *E. grandis* lateral roots colonized by *P. albus.*** (**A**) Percent colonization of *E.*
855 *grandis* roots after two weeks of contact with *P. albus* strain SI-12 when treated with
856 water (control; n=9), AdOx (n=12), AMI-1 (n=11), 1% DMSO (DMSO control;
857 n=10) or 2,3-dimethoxynitrostyrene (DMNS; n=17) ± SE; (**B**) Percent colonization
858 of *R. rhizogenes* transformed control (n=9) and test (*35S::EgPRMT1* (n=7)*;*
859 *EgPRMT1* RNAi (n=3); *35S::EgPRMT10* (n=6)*; EgPRMT10* RNAi (n=6)) roots after
860 two weeks of contact with *P. albus* strain SI-12, ± SE; * = significant change in the
861 percentage of lateral roots colonized as compared to the control treatment ($p<0.05$).

862

863 **Figure 3 Heat map representation of differentially expressed genes in**
864 **mycorrhized *E. grandis* roots mis-expressing either EgPRMT1 or EgPRMT10.**
865 Log2-transformed heat map of the 2089 significantly regulated genes ($p<0.001$;
866 excepting those which are similarly up or down regulated in all four cases) in
867 *35S::EgPRMT1*, *EgPRMT* RNAi, *35S::EgPRMT10*, *EgPRMT10* RNAi mycorrhized
868 roots as compared to mycorrhized controls. Data is the average of three biological
869 replicates. Genes used in the assembly of Group A and B gene lists are indicated.

870

871 **Figure 4 Uptake of *EgPRMT10* targeting dsiRNA into plant cells and effect on**
872 **gene expression.** (**A**) Merged brightfield and fluorescent confocal microscopy
873 images of control *E. grandis* root tip or *E. grandis* root tip after 2 hours exposure to
874 Cy®3-labeled dsiRNA. Scale bar = 100 μm. (**B**) Relative expression (log2) of

EgPRMT10 in EgPRMT10 dsiRNA treated roots as compared to scrambled control dsiRNA treated roots after 4 and 8 hours of exposure. n=5 ± SE; * = significant change ($p<0.05$). (**C**) Correlations between the relative expression of *EgPRMT10* in dsiRNA treated root tissues (log2(fold change), x-axis) and the expression of hormone responsive genes (log2(fold change), y-axis) for individual replicates as compared to scrambled control dsiRNA treated roots. Genes chosen are indicative of auxin, jasmonic acid (JA), cytokinin (CK), gibberellin (GA) or abscisic acid (ABA) responses. Light grey diamonds represent 8 hour time-point replicates, while dark grey diamonds represent 4 hour time-point replicates. The gene considered, Pearson's correlation coefficient (r) and significance of the correlation are indicated on each plot.

888 **e-Xtra Supplementary Tables**

889

890 **Supplementary Table S1** Sequences of primers used in q-PCR analysis and cloning.

891

892 **Supplementary Table S2** Fold change expression, PFAM classification and
893 annotation of *E. grandis* genes opposingly regulated in *35S::EgPRMT1* and
894 *EgPRMT1* RNAi transformed mycorrhizal roots (Group A).

895

896 **Supplementary Table S3** Fold change expression, PFAM classification and
897 annotation of *E. grandis* genes opposingly regulated in *35S::EgPRMT10* and
898 *EgPRMT10* RNAi transformed mycorrhizal roots (Group B).

899

900 **Supplementary Table S4** List of PFAM domains enriched within the Group A genes
901 upregulated in *35S::EgPRMT1* mycorrhized roots or downregulated in *EgPRMT1*
902 RNAi mycorrhized roots as compared to controls.

903

904 **Supplementary Table S5** List of PFAM domains enriched within the Group A genes
905 downregulated in *35S::EgPRMT1* mycorrhized roots or upregulated in *EgPRMT1*
906 RNAi mycorrhized roots as compared to controls.

907

908 **Supplementary Table S6** List of PFAM domains enriched within the Group B genes
909 upregulated in *35S::EgPRMT10* mycorrhized roots or downregulated in *EgPRMT10*
910 RNAi mycorrhized roots as compared to controls.

911

912 **Supplementary Table S7** List of PFAM domains enriched within the Group B genes
913 downregulated in *35S::EgPRMT10* mycorrhized roots or upregulated in *EgPRMT10*
914 RNAi mycorrhized roots as compared to controls.

915

e-Xtra Supplementary Figures

Supplementary Figure S1: EgPRMT transgenic roots show differential expression of the target gene as well as previously defined co-regulated genes. Heatmaps of average fold-change in gene expression of three independent *EgPRMT1* transgenic roots (**A**) and *EgPRMT10* transgenic roots (**B**) after two weeks of contact with *P. albus* as determined by RNA sequencing. Expression of genes known to be positively (denoted as genes with "+" in the figure) or negatively (denoted as genes with "-" in the figure) co-regulated with *PRMT1* and *PRMT10* were also found to be differentially expressed in the expected manner (e.g. positively co-regulated genes should also be increased in expression in *35S::PRMT* roots vs. control and more repressed in PRMT RNAi roots vs. control).

Supplementary Figure S2 Phylogenetic tree of PRMT protein sequences from human (Homo_sapiens), mouse (Mus_musculus), cow (Bovin), Xenopus (Xenopus), zebrafish (Danio_rerio), fruit fly (Drosophila), yeast (Yeast), *Arabidopsis thaliana* (AT), rice (LOC_Os) and Eucalyptus (Eucgr). Annotations of known PRMTs are indicated and the group for PRMT10 is indicated by a red box. Branch length indicates extent of sequence divergence (scale bar represents 3 substitutions per site) and red numbers indicate the confidence estimate. The phylogenetic tree was constructed using the online tool 'Phylogeny.fr' using the maximum likelihood method and bootstrapped 100 times.

Supplementary Figure S3 (**A**) Percent lateral root colonization of all independent transformed roots differentially expressing EgPRMT1 or EgPRMT10 along with the average of wild type values (n=9; +/-SE). (**B**) Number of lateral roots across all independent transformed root systems differentially expressing EgPRMT1 or EgPRMT10 along with the average of wild type values (n=9; +/-SE).

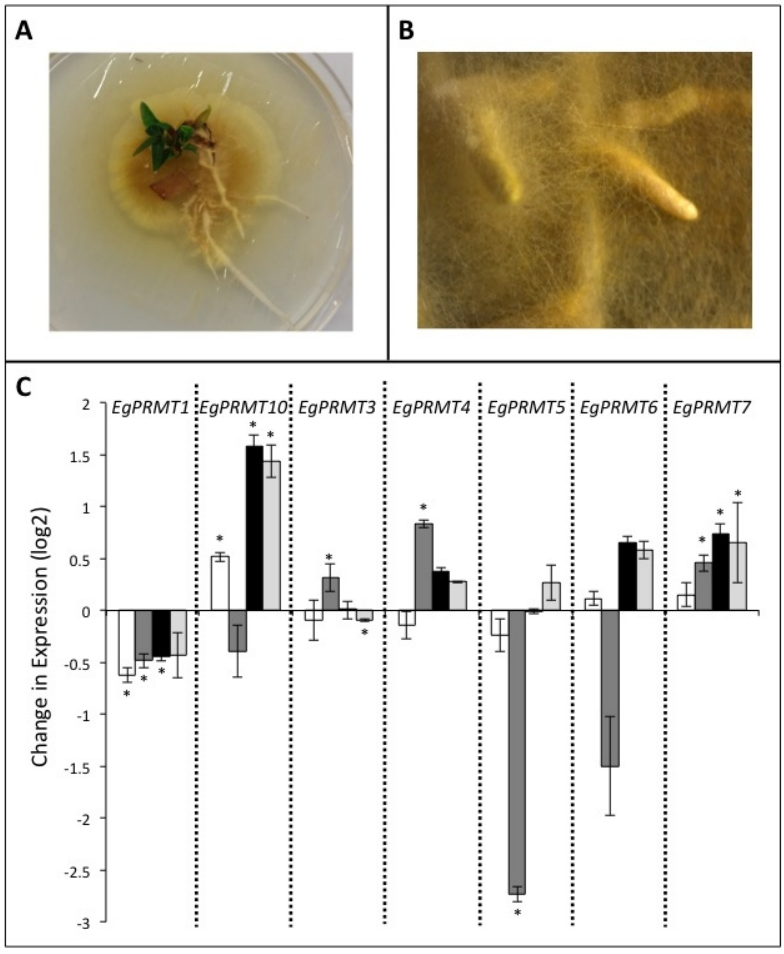

Figure 1 Change in expression of PRMT genes in E. grandis roots during colonization by the ectomycorrhizal fungus P. albus. (A) E. grandis undergoing colonization by P. albus (two weeks post contact). (B) Close up of mature mycorrhizal root tips (two weeks post contact). (C) The relative expression (log2) of PRMTs encoded by E. grandis in roots undergoing colonization as compared to axenically growing roots. Data is presented over the timecourse of colonization: 24hr (white), 48hr (dark grey), 1 week (black) and 2 weeks (light grey) post contact and is the average of three biological replicates + SE; * = significant expression change as compared to axenically grown roots ($p<0.05$).

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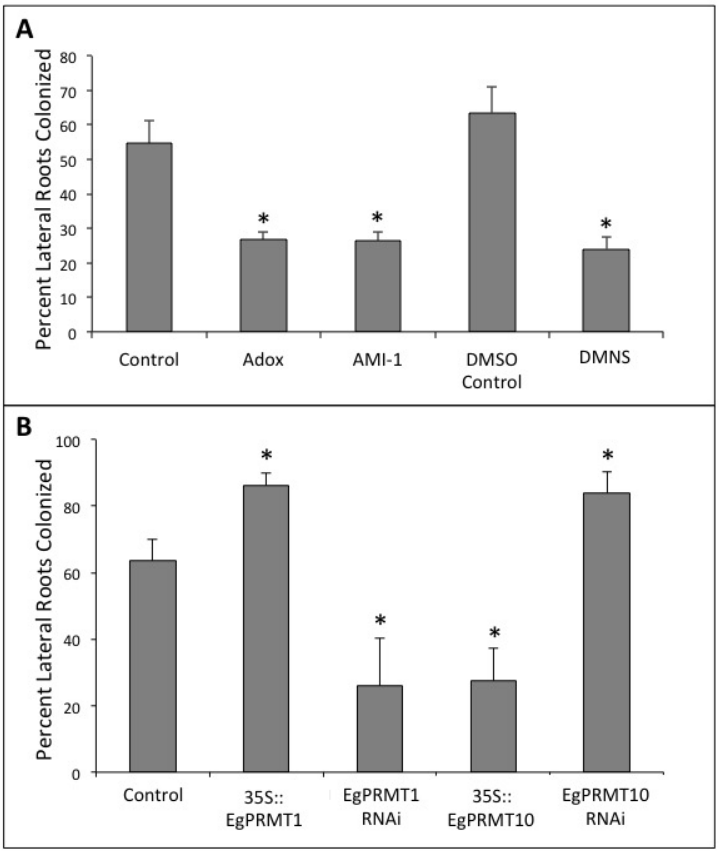

Figure 2 Effect of PRMT inhibition or transgenic mis-expression on the percent of E. grandis lateral roots colonized by P. albus. (A) Percent colonization of E. grandis roots after two weeks of contact with P. albus strain SI-12 when treated with water (control; n=9), AdOx (n=12), AMI-1 (n=11), 1% DMSO (DMSO control; n=10) or 2,3-dimethoxynitrostyrene (DMNS; n=17) + SE; (B) Percent colonization of R. rhizogenes transformed control (n=9) and test (35S::EgPRMT1 (n=7); EgPRMT1 RNAi (n=3); 35S::EgPRMT10 (n=6); EgPRMT10 RNAi (n=6)) roots after two weeks of contact with P. albus strain SI-12, + SE; * = significant change in the percentage of lateral roots colonized as compared to the control treatment ($p<0.05$).

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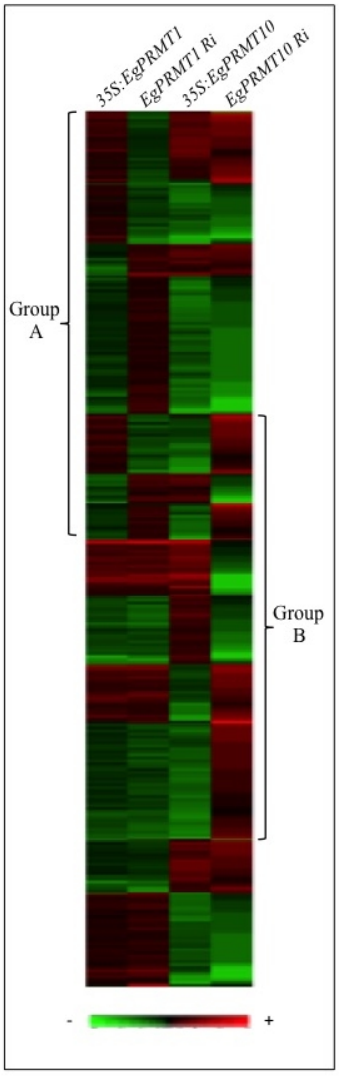

Figure 3 Heat map representation of differentially expressed genes in mycorrhized E. grandis roots mis-expressing either EgPRMT1 or EgPRMT10. Log2-transformed heat map of the 2089 significantly regulated genes ($p<0.001$; excepting those which are similarly up or down regulated in all four cases) in 35S::EgPRMT1, EgPRMT RNAi, 35S::EgPRMT10, EgPRMT10 RNAi mycorrhized roots as compared to mycorrhized controls. Data is the average of three biological replicates. Genes used in the assembly of Group A and B gene lists are indicated.

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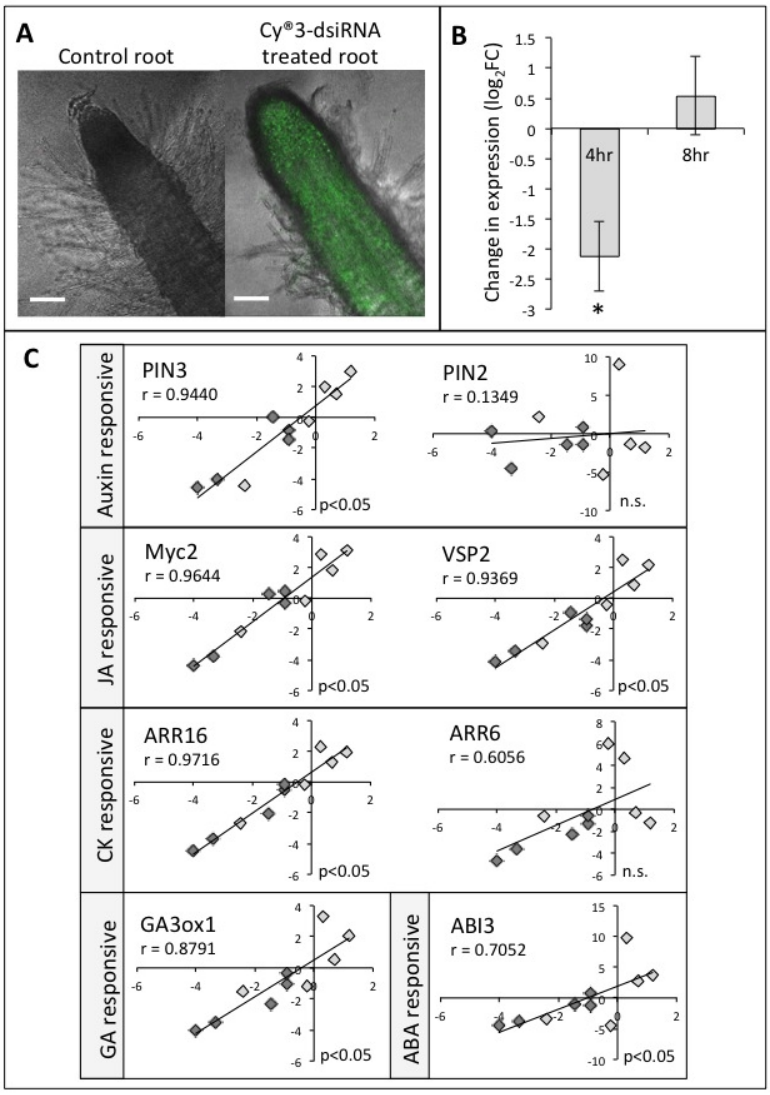

Figure 4 Uptake of EgPRMT10 targeting dsiRNA into plant cells and effect on gene expression. (A) Merged brightfield and fluorescent confocal microscopy images of control E. grandis root tip or E. grandis root tip after 2 hours exposure to Cy®3-labeled dsiRNA. Scale bar = 100 µm. (B) Relative expression (log2) of EgPRMT10 in EgPRMT10 dsiRNA treated roots as compared to scrambled control dsiRNA treated roots after 4 and 8 hours of exposure. n=5 + SE; * = significant change ($p<0.05$). (C) Correlations between the relative expression of EgPRMT10 in dsiRNA treated root tissues (log2(fold change), x-axis) and the expression of hormone responsive genes (log2(fold change), y-axis) for individual replicates as compared to scrambled control dsiRNA treated roots. Genes chosen are indicative of auxin, jasmonic acid (JA), cytokinin (CK), gibberellin (GA) or abscisic acid (ABA) responses. Light grey diamonds represent 8 hour time-point replicates, while dark grey diamonds represent 4 hour time-point replicates. The gene considered, Pearson's correlation coefficient (r) and significance of the correlation are indicated on each plot.

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Table 1 Identification of arginine methylation sites from total protein extracted from 48-hour (n=3) or two-week axenic controls (n=3), 48-hour indirect fungal contact (n=3), or 2-week mycorrhized (n=2) E. grandis roots. As specific proteins were detected multiple times in each sample, the mean percentage detected as methylated is given to indicate the consistency of the methyl modification across all proteins detected (+/- S.D.). Underlined arginine residues are mono-methylated while bolded arginine residues are di-methylated. Those methylated residues that are superscripted with an * are unique to that given sample condition as the residue was detected and confirmed as not being methylated in other sample conditions. N.D indicates that the protein was not detected by mass spectrometry. N.M. indicates that a portion of the protein was detected, but no methylation was detected.

Protein Name	Gene No.	48h Control *E. grandis* root		*E. grandis* root after 48h indirect contact		2 Week Control *E. grandis* root		*E. grandis* root after 2 week mycorrhization	
		Methylated Residues	Mean % Methylated ± S.D	Methylated Residues	Mean % Methylated ± S.D	Methylated Residues	Mean % Methylated ± S.D	Methylated Residues	Mean % methylated ± S.D
187-kDa microtubule-associated protein	Eucgr.E02482	R981	100±0	N.D	N/A	N.M	N/A	**R90**, **R889**	35±21,100±0
30S ribosomal protein S3	Eucgr.J02481	R26	100±0	N.D	N/A	N.M	N/A	N.D	N/A
5-methyltetrahydropteroyltriglutamate- homocysteine methyltransferase	Eucgr.A02126	R447*, R571*	100±0, 46±15	R705, R714*	52±9, 60±14	R705	33±57	N.D	N/A
ATP synthase subunit beta	Eucgr.G02224	R159*, R176	63±32, 33±28	R176	56±38	N.M	N/A	N.M	N/A
Catalase	Eucgr.F01776	N.M	N/A	**R102**, **R146**	53±11, 45±18	N.D	N/A	N.M	N/A
Cell division control protein	Eucgr.H03858	N.D	N/A	**R315**	100±0	N.D	N/A	N.M	N/A
Cell division cycle-associated 7-like protein	Eucgr.H00548	N.D	N/A	R50	100±0	N.D	N/A	N.D	N/A
E3 ubiquitin transferase	Eucgr.J00838	N.D	N/A	R990	100±0	N.D	N/A	N.M	N/A
E3 ubiquitin-protein ligase	Eucgr.E00402	R20	51±8	N.D	N/A	N.M	N/A	N.D	N/A
Elongation Factor 1-alpha	Eucgr.J01121	**R411**	75±25	R166*, **R411**	46±8, 60±15	N.M	N/A	R254	100±0
Elongation Factor 2	Eucgr.F01462	R592, R774	48±13, 83±15	R592, R774	65±31, 70±26	N.D	N/A	N.M	N/A
Fructose-bisphosphate aldolase	Eucgr.A01538	R53	100 ± 0	N.M	N/A	N.D	N/A	N.D	N/A
Fructose-bisphosphate aldolase	Eucgr.K02073	**R249**	66 ± 30	N.M	N/A	N.D	N/A	N.D	N/A
Glyceraldehyde-3-phosphate dehydrogenase	Eucgr.B00144	R169, R293	100±0, 61±13	N.M	N/A	N.M	N/A	**R16, R236**	33±57, 33±57
Heat shock 70kDa protein-like	Eucgr.J00025 Eucgr.F03980	**R145**, **R225***, R608*	100±0, 46±6, 85±13	**R145**	100±0	R145	100±0	**R647**	100±0
Heat shock 70kDa protein	Eucgr.E01024	**R149**	100±0	N.M	N/A	R546, R568	33±57, 100±0	**R256**	100±0
Histone H3	Eucgr.D00584	**R43**	78±20	R64	58±8	R84	33±57	N.D	N/A
Histone H4	Eucgr.J02062	N.D	N/A	R56, **R93**	48±3, 51±8	N.M	N/A	N.M	N/A
HSP80	Eucgr.F03673	R329*, R375*	62±9, 59±14	**R98***	70±26	N.D	N/A	N.M	N/A
Nucleoside triphosphate hydrolase superfamily protein	Eucgr.D01797	N.D	N/A	R303	100±0	N.D	N/A	N.M	N/A
Phosphoenolpyruvate carboxylase	Eucgr.F01229	N.D	N/A	R574	61±10	N.D	N/A	N.D	N/A

Phosphoglycerate kinase	Eucgr.F04463	R99	100±0	N.M	N/A	N.D	N/A	N.D	N/A
Proteasome activator complex subunit	Eucgr.F02001	N.D	N/A	N.D	N/A	N.D	N/A	**R1062**	100±0
Survival motor neuron interacting protein	Eucgr.A00251	N.D	N/A	N.D	N/A	N.D	N/A	R67, R69	100±0
TIR-NBS-LRR type disease resistance protein/ TMV resistance protein	Eucgr.J01296	N.D	N/A	R730	100±0	N.D	N/A	N.D	N/A
Transmembrane protein	Eucgr.F04115	N.D	N/A	N.D	N/A	N.D	N/A	**R273**	65±21
Tubulin alpha	Eucgr.G01186	N.D	N/A	R79	100±0	N.D	N/A	N.D	N/A
Tubulin beta	Eucgr.K00264	N.D	N/A	R77, R262, R276	63± 16, 54±12, 62±62	N.D	N/A	N.D	N/A

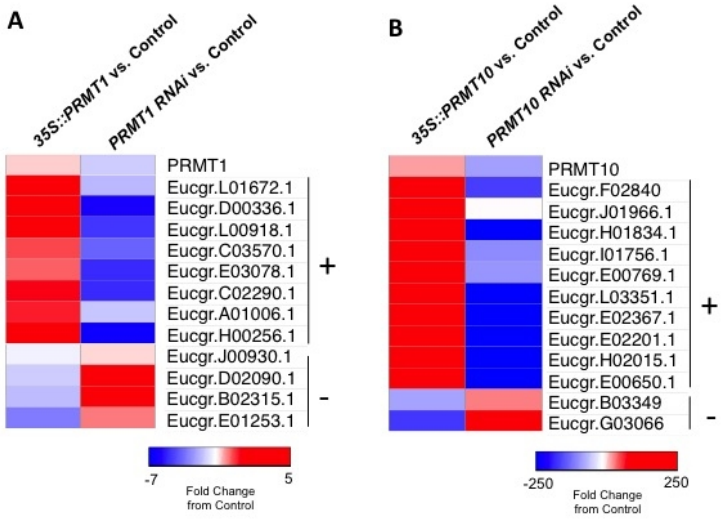
Table 2 Selected genes pertaining to hormone synthesis or signalling from those that are significantly differentially regulated in mycorrhized roots with modified EgPRMT10 expression as compared to wild type mycorrhized roots (Group B genes).

E. grandis gene ID	Arabidopsis homologue	Annotation	Role in hormone signalling	Average fold change in expression in 35S::EgPRMT10 roots	Average fold change in expression in EgPRMT10RNAi roots
Hormone Synthesis Pathways					
Eucgr.C03886.1	AT2G19590.1	ACC oxidase 1	Ethylene biosynthesis	0.13	2.20
Eucgr.K00739.1	AT1G05010.1	Ethylene-forming enzyme	Ethylene biosynthesis	0.20	7.76
Eucgr.K00740.1	AT1G05010.1	Ethylene-forming enzyme	Ethylene biosynthesis	0.02	12.46
Eucgr.K00746.1	AT1G05010.1	Ethylene-forming enzyme	Ethylene biosynthesis	0.12	9.85
Eucgr.F00649.1	AT5G55250.1	IAA carboxylmethyltransferase 1	Auxin conversion	0.11	1.13
Eucgr.H02641.1	AT4G15550.1	Indole-3-acetate beta-D-glucosyltransferase	Auxin conversion	0.52	5.13
Eucgr.F02495.1	AT1G67080.1	Abscisic acid (aba)-deficient 4	Abscisic acid biosynthesis	0.57	14.54
Hormone Responsive Transcription Factors/Complexes					
Eucgr.K03266.1	AT3G23240.1	Ethylene response factor 1	Ethylene signalling	0.12	5.18
Eucgr.B03050.1	AT1G68840.2	Related to ABI3/VP1 2	Abscisic Acid signalling	0.02	2.79
Eucgr.B03545.1	AT5G13220.1	Jasmonate-zim-domain protein 10	Jasmonic Acid signalling	0.08	11.99
Eucgr.G01954.1	AT5G13220.1	Jasmonate-zim-domain protein 10	Jasmonic Acid signalling	0.12	1.64
Expression Regulated by Hormones					
Eucgr.D00773.1	AT5G54490.1	Pinoid-binding protein 1	upregulated by auxin[1]	0.65	6.84
Eucgr.K00884.1	AT5G54490.1	Pinoid-binding protein 1	upregulated by auxin[1]	0.10	2.12
Eucgr.F00588.1	AT1G75750.2	GAST1 protein homolog 1	Giberallin/ABA regulated[2]	0.88	8.62
Eucgr.I02349.1	AT4G09600.1	GAST1 protein homolog 3	Giberallin/ABA regulated[2]	0.03	1.53

[1] Benjamins et al., 2003; [2] Shi and Olszewski, 1995

Table 3 List of the over-represented PFAM families that pertain to defense, symbiotic responses or hormone signaling from the Group A and Group B datasets that are within the top ten most enriched families (based on Z-score) within the dataset.

Group A (EgPRMT1 responsive)		
PFAM family	**Z-Score**	**p-value**
Up-regulated in 35S::EgPRMT1		
Defense response to fungus	21.43	3.24E-14
Gibberellin biosynthetic process	20.85	3.05E-07
Diterpenoid biosynthetic process	18.91	3.11E-09
Response to fungus	18.21	1.24E-13
Gibberellin metabolic process	18.02	1.22E-06
Flavenoid biosynthetic process	18.02	1.22E-06
Down-regulated in 35S::EgPRMT1		
Response to chitin	15.6	3.52E-07
Diterpenoid metabolic process	14.25	8.99E-08
Response to oxidative stress	13.11	1.16E-13
Terpenoid metabolic process	12.67	2.95E-07
Group B (EgPRMT10 responsive)		
PFAM family	**Z-Score**	**p-value**
Up-regulated in 35S::EgPRMT10		
Diterpenoid biosynthetic process	17.72	3.37E-10
Gibberellin biosynthetic process	16.29	1.35E-06
Flavenoid metabolic process	15.27	2.16E-07
Down-regulated in 35S::EgPRMT10		
Defense response to fungus	22.27	4.85E-22
Response to fungus	17.93	8.27E-19
Response to jasmonic acid	17.4	2.56E-13
Response to hormone	17.23	1.06E-31



Supplementary Figure S1: EgPRMT transgenic roots show differential expression of the target gene as well as previously defined co-regulated genes. Heatmaps of average fold-change in gene expression of three independent EgPRMT1 transgenic roots (A) and EgPRMT10 transgenic roots (B) after two weeks of contact with P. albus as determined by RNA sequencing. Expression of genes known to be positively (denoted as genes with "+" in the figure) or negatively (denoted as genes with "-" in the figure) co-regulated with PRMT1 and PRMT10 were also found to be differentially expressed in the expected manner (e.g. positively co-regulated genes should also be increased in expression in 35S::PRMT roots vs. control and more repressed in PRMT RNAi roots vs. control).

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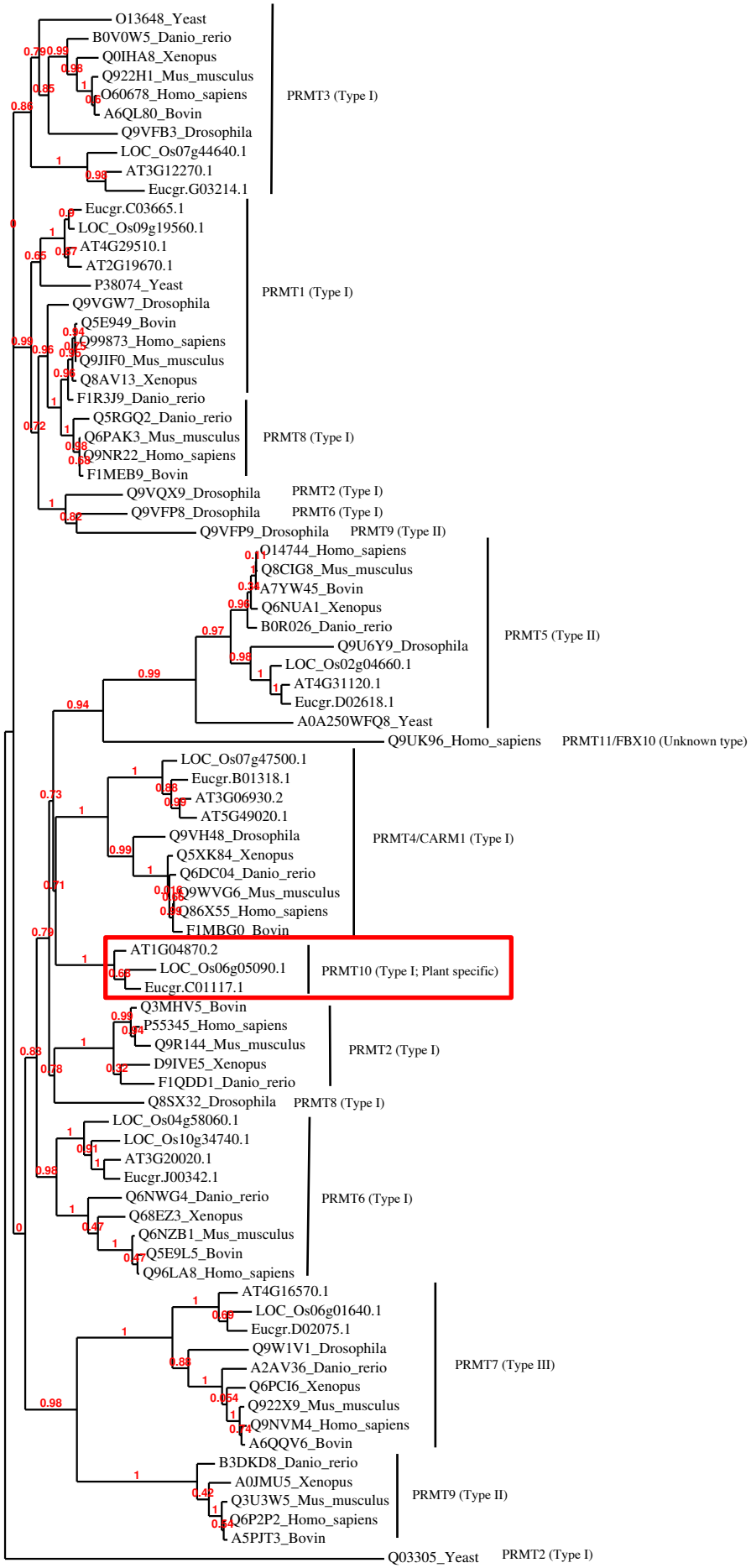
O13648_Yeast
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Q0IHA8_Xenopus
Q922H1_Mus_musculus
O60678_Homo_sapiens
A6QL80_Bovin
Q9VFB3_Drosophila
LOC_Os07g44640.1
AT3G12270.1
Eucgr.G03214.1
PRMT3 (Type I)
Eucgr.C03665.1
LOC_Os09g19560.1
AT4G29510.1
AT2G19670.1
P38074_Yeast
Q9VGW7_Drosophila
Q5E949_Bovin
Q99873_Homo_sapiens
Q9JIF0_Mus_musculus
Q8AV13_Xenopus
F1R3J9_Danio_rerio
PRMT1 (Type I)
Q5RGQ2_Danio_rerio
Q6PAK3_Mus_musculus
Q9NR22_Homo_sapiens
F1MEB9_Bovin
PRMT8 (Type I)
Q9VQX9_Drosophila
PRMT2 (Type I)
Q9VFP8_Drosophila
PRMT6 (Type I)
Q9VFP9_Drosophila
PRMT9 (Type II)
Q14744_Homo_sapiens
Q8CIG8_Mus_musculus
A7YW45_Bovin
Q6NUA1_Xenopus
B0R026_Danio_rerio
Q9U6Y9_Drosophila
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AT4G31120.1
Eucgr.D02618.1
A0A250WFQ8_Yeast
PRMT5 (Type II)
Q9UK96_Homo_sapiens
PRMT11/FBX10 (Unknown type)
LOC_Os07g47500.1
Eucgr.B01318.1
AT3G06930.2
AT5G49020.1
Q9VH48_Drosophila
Q5XK84_Xenopus
Q6DC04_Danio_rerio
Q9WVG6_Mus_musculus
Q86X55_Homo_sapiens
F1MBG0_Bovin
PRMT4/CARM1 (Type I)
AT1G04870.2
LOC_Os06g05090.1
Eucgr.C01117.1
PRMT10 (Type I; Plant specific)
Q3MHV5_Bovin
P55345_Homo_sapiens
Q9R144_Mus_musculus
D9IVE5_Xenopus
F1QDD1_Danio_rerio
PRMT2 (Type I)
Q8SX32_Drosophila
PRMT8 (Type I)
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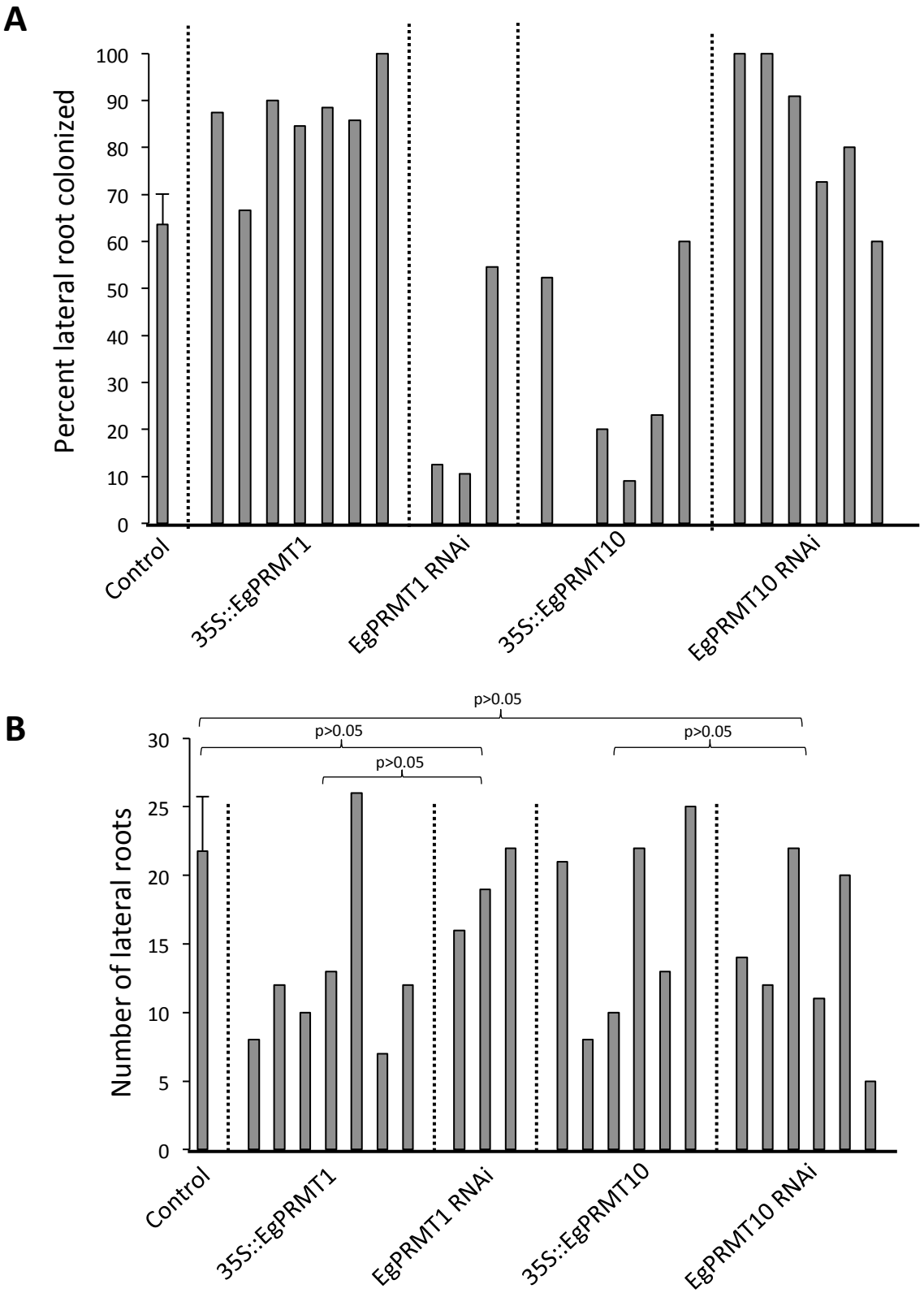
LOC_Os10g34740.1
AT3G20020.1
Eucgr.J00342.1
Q6NWG4_Danio_rerio
Q68EZ3_Xenopus
Q6NZB1_Mus_musculus
Q5E9L5_Bovin
Q96LA8_Homo_sapiens
PRMT6 (Type I)
AT4G16570.1
LOC_Os06g01640.1
Eucgr.D02075.1
Q9W1V1_Drosophila
A2AV36_Danio_rerio
Q6PCI6_Xenopus
Q922X9_Mus_musculus
Q9NVM4_Homo_sapiens
A6QQV6_Bovin
PRMT7 (Type III)
B3DKD8_Danio_rerio
A0JMU5_Xenopus
Q3U3W5_Mus_musculus
Q6P2P2_Homo_sapiens
A5PJT3_Bovin
PRMT9 (Type II)
Q03305_Yeast
PRMT2 (Type I)

3.


A
Percent lateral root colonized
100
90
80
70
60
50
40
30
20
10
0
Control
35S::EgPRMT1
EgPRMT1 RNAi
35S::EgPRMT10
EgPRMT10 RNAi
B
p>0.05
p>0.05
p>0.05
p>0.05
Number of lateral roots
30
25
20
15
10
5
0
Control
35S::EgPRMT1
EgPRMT1 RNAi
35S::EgPRMT10
EgPRMT10 RNAi