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SWEET transporters of *Medicago lupulina* in the arbuscular-mycorrhizal system in the presence of medium level of available phosphorus

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Abstract. Arbuscular mycorrhiza (AM) fungi receive photosynthetic products and sugars from plants in exchange for contributing to the uptake of minerals, especially phosphorus, from the soil. The identification of genes controlling AM symbiotic efficiency may have practical application in the creation of highly productive plant-microbe systems. The aim of our work was to evaluate the expression levels of SWEET sugar transporter genes, the only family in which sugar transporters specific to AM symbiosis can be detected. We have selected a unique "host plant–AM fungus" model system with high response to mycorrhization under medium phosphorus level. This includes a plant line which is highly responsive to inoculation by AM fungi, an ecologically obligate mycotrophic line MIS-1 from black medick (*Medicago lupulina*) and the AM fungus *Rhizophagus irregularis* strain RCAM00320, which has a high efficiency in a number of plant species. Using the selected model system, differences in the expression levels of 11 genes encoding SWEET transporters in the roots of the host plant were evaluated during the development of or in the absence of symbiosis of *M. lupulina* with *R. irregularis* at various stages of the host plant development in the presence of medium level of phosphorus available for plant nutrition in the substrate. At most stages of host plant development, mycorrhizal plants had higher expression levels of *MlSWEET1b*, *MlSWEET3c*, *MlSWEET12* and *MlSWEET13* compared to AM-less controls. Also, increased expression relative to control during mycorrhization was observed for *MlSWEET11* at 2nd and 3rd leaf development stages, for *MlSWEET15c* at stemming (stooling) stage, for *MlSWEET1a* at 2nd leaf development, stemming and lateral branching stages. The *MlSWEET1b* gene can be confidently considered a good marker with specific expression for effective development of AM symbiosis between *M. lupulina* and *R. irregularis* in the presence of medium level of phosphorus available to plants in the substrate.
Key words: arbuscular mycorrhiza; *Medicago lupulina*; *Rhizophagus irregularis*; SWEET; gene expression assessment; sugar transporter genes.

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SWEET транспортеры *Medicago lupulina* в арбускулярно-микоризной системе в условиях среднего уровня доступного фосфора

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Аннотация. Грибы арбускулярной микоризы (АМ) помогают растениям усваивать из почвы минеральные вещества, особенно фосфор, в то время как получают от растений продукты фотосинтеза – сахара. Выявление генов, контролирующих симбиотическую эффективность АМ, может иметь практическое применение при создании высокопродуктивных растительно-микробных систем. Поэтому целью работы была оценка уровней экспрессии генов семейства транспортеров сахаров SWEET – единственного семейства, где могут быть обнаружены специфические для АМ-симбиоза транспортеры сахаров. Нами разработана уникальная высокочувствительная к уровню фосфора и микоризации модельная система «растение-хозяин–АМ-гриб», включающая высокоотзывчивую на инокуляцию АМ-грибами экологически облигатно микотрофную линию MIS-1 люцерны хмелевидной

(*Medicago lupulina*) и штамм АМ-гриба RCAM00320 *Rhizophagus irregularis*, обладающий высокой эффективностью на множестве видов растений. С использованием этой модельной системы в корнях растения оценены изменения в уровне экспрессии 11 генов, кодирующих транспортеры семейства SWEET, при развитии либо при отсутствии симбиоза *M. lupulina* с *R. irregularis* в различные фазы развития растения-хозяина в условиях среднего уровня доступного для питания растений фосфора в субстрате. У микоризованных растений обнаружены более высокие уровни экспрессии в большинстве фаз развития растения-хозяина для *MlSWEET1b*, *MlSWEET3c*, *MlSWEET12*, *MlSWEET13* по сравнению с контролем без АМ. Повышенная относительно контроля экспрессия при микоризации наблюдалась также и для *MlSWEET11* в фазу развития 2-го и 3-го листьев, для *MlSWEET15c* – в фазу стеблевания, для *MlSWEET1a* – в фазу 2-го листа, стеблевания и бокового ветвления. Ген *MlSWEET1b* с уверенностью можно считать хорошим маркером со специфической экспрессией для эффективного развития АМ-симбиоза *M. lupulina* с *R. irregularis* в условиях среднего уровня доступного для растений фосфора в субстрате.
Ключевые слова: арбускулярная микориза; *Medicago lupulina*; *Rhizophagus irregularis*; SWEET; оценка экспрессии генов; гены транспортеров сахаров.

Introduction

Plant sugar transporters belong to three key families: Sucrose Transporters (SUT = SUC), Monosaccharide Transporters (MST, including subfamilies STP, TMT, PMT, VGT, pGlct/SGB1, ESL, INT) and Sugars Will Eventually be Exported Transporters (SWEET). SUT transporters are involved in loading the phloem with sucrose and its long-distance transport from the leaves to other parts of the plant. There sugars are broken down into monosaccharides and transported to the cells by MST proteins.

The least studied of these groups is the SWEET family of transporters. These are non-volatile and bidirectional transmembrane transporters of various sugars in all plant organs and tissues. L.Q. Chen's was first to account for this family of transporters in 2010. It is in the SWEET family that the proteins particular to AM-symbiosis could be identified, while ones in the other two families had not yet been found (Chen et al., 2010; Doidy et al., 2019). Currently, it is believed that SWEET transporters are found in all living organisms (Feng et al., 2015). At the same time, it is noted that the number of isoforms of these transporters differs even in closely related species. The numbering of new SWEET proteins and their isoforms in other organisms is executed according to gene orthology principals as with the proteins in *Arabidopsis thaliana.*

As a result of early studies of transporters, it turned out that the *SWEET* plant genes, despite their low homology, are grouped into four clades (Chen et al., 2015). Representatives of each of the clades are observed in almost all terrestrial plants. It is believed that the representatives of the four clades are phylogenetically and functionally distinct. Thus, it is noted that representatives of clades I and II transport hexoses, clade III, mainly sucrose, and clade IV, mainly fructose (Chen et al., 2012; Feng et al., 2015).

SWEET proteins are involved in a variety of processes. In addition to the transport of sugars, they apparently participate in the transport of other substances, for example, gibberellins, as was shown for Arabidopis (Kanno et al., 2016). A great deal of data in the literature deals with the functions of SWEET proteins in different plant species. For example, the MtSWEET1b transporter may supply glucose to AM fungi (An et al., 2019), LjSWEET3 mediates sucrose transport (Sugiyama et al., 2017) to nodules of *Lotus japonicus*. SWEET clade I transporters are probably involved in the supply of sugars to symbiotic systems (Doidy et al., 2019). Rhizosphere pathogens can cause increased synthesis of clade III proteins. This, in turn, leads to additional sucrose transport to roots and contributes to the nutrition of microorganisms in the rhizosphere (Doidy et al., 2019). In 2010, L.Q. Chen et al. demonstrated that pathogenic bacteria, for example, of the genus *Xanthomonas*, can enter tissues of the host plant and induce expression of the *SWEET* genes. These encode transporters from clade III (primarily *SWEET11* and *SWEET14*) in order to produce sugars. Like symbiotic AM fungi, pathogenic fungi induce gene expression to produce sugars (Chen et al., 2010).

J. Manck-Gotzenberger and N. Requena (2016) note that the genes of many transporters have a significant level of expression in AM symbiosis, but are not necessarily particular to it. The work of A. Kafle shows that the orthologs of *SWEET1 – MtSWEET1.2* and *PsSWEET1.2* – can be expressed both in mycorrhizal roots and in root nodules (Kafle et al., 2019). Therefore, the orthologs of the transporters of clades I (MtSWEET1-MtSWEET3) and III (MtSWEET9-MtSWEET15) are primarily considered as active participants in the symbiotic relationship "plant–AM fungus" (Kryukov et al., 2021).

The study of the role of SWEET transporters in the formation of symbiotic relationships has never been specifically directed toward PMS. In this regard, the aim of this work was to evaluate the expression of the *SWEET* genes in PMS models during mycorrhization and its respective absence at different stages of plant development.

Materials and methods

Plant and fungal material. Black medick (*Medicago lupulina* L. subsp. *vulgaris* Koch) is a widespread species of the genus *Medicago*, a self-pollinating diploid. In the present study, the authors selected the MlS-1 line as being highly responsive to mycorrhization from the black medick cultivar-population VIK32 (Yurkov et al., 2015). Barring inoculation by AM-fungus, and with low level of available inorganic phosphorus (P_i) in the soil, this line exhibits signs of dwarfism (Yurkov et al., 2015, 2020). An effective strain RCAM00320 of the AM fungus *Rhizophagus irregularis* (formerly known as *Glomus intraradices* Shenck & Smith) was used for inoculation (CIAM8 from the All-Russia Research Institute for Agricultural Microbiology (ARRIAM) collection). An accurate identification of the strain was carried out by the authors (Kryukov, Yurkov, 2018). Since AM-fungi are obligate symbionts, the strain is maintained in a cumulative culture of *Plectranthus* sp. (exact species identification is currently being

undertaken by the authors) under standard conditions in the ARRIAM Laboratory of ecology of symbiotic and associative microorganisms (Yurkov et al., 2010).

Vegetative method. The procedure for the method is described in the work of A.P. Yurkov et al. (Yurkov et al., 2015). Optimal conditions were provided for the development of AM while preventing spontaneous infection with nodule bacteria and other microorganisms. A mixture of soil and sand in a ratio of 2:1 was autoclaved twice at 134 °C, 2 atm for 1 hour with repeated autoclaving two days later; no signs of toxicity appeared after such treatment. Specimens were planted with two seedlings per one vessel filled with a soil-sand mixture (210 g). Agrochemical characteristics of the soil are given by (Yurkov et al., 2015). The content of P_2O_5 in the soil was 23 mg/kg of soil (as according to Kirsanov). Before the experiment, 0.5 doses of phosphorus were added in the form of CaH_2PO_4*$2H_2O$ (86 mg/kg of soil) according to the prescription of D.N. Pryanishnikov (Klechkovsky, Petersburgsky, 1967). The final phosphorus content in the soil-sand mixture was 109 mg/kg and corresponded to the average P_i level; i. e. the availability of mobile phosphates in the soil in terms of the content in the Kirsanov extract according to (Sokolov, 1975); pH_{KCl} – 6.44. The first measurement of plants was carried out 21 days after sowing and inoculation, followed by measurement at key stages of black medick ontogenesis. There was a total number of 7 measurements (Supplementary Material 1)[1].

The specimens involved *Plectranthus* roots inoculated and uninoculated with *R. irregularis* strain RCAM00320. During collection, the material was frozen in liquid nitrogen and stored for up to 6 months at –80 °C.

RNA isolation and evaluation of gene expression. The selection of genes of interest was carried out based on the results of *M. truncatula* transcriptome analysis (*MtSWEET1a* = Medtr1g029380, *MtSWEET1b* = Medtr3g089125, *MtSWEET2a* = Medtr8g042490, *MtSWEET2b* = Medtr2g073190, *MtSWEET2c* = Medtr6g034600, *MtSWEET3a* = Medtr3g090940, *MtSWEET3b* = Medtr3g090950, *MtSWEET3c* = Medtr1g028460, *MtSWEET4* = Medtr4g106990, *MtSWEET5a* = Medtr6g007610, *MtSWEET5b* = Medtr6g007637, *MtSWEET5c* = Medtr6g007623, *MtSWEET5d* = Medtr6g007633, *MtSWEET6* = Medtr3g080990, *MtSWEET7* = Medtr8g099730, *MtSWEET9a* = Medtr5g092600, *MtSWEET9b* = Medtr7g007490, *MtSWEET11* = Medtr3g098930, *MtSWEET12* = Medtr8g096320, *MtSWEET13* = Medtr3g098910, *MtSWEET14* = Medtr8g096310, *MtSWEET15a* = Medtr2g007890, *MtSWEET15b* = Medtr5g067530, *MtSWEET15c* = Medtr7g405730, *MtSWEET15d* = Medtr7g405710, *MtSWEET16* = Medtr2g436310; sequence numbers from the database: https://phytozome.jgi.doe.gov/pz/portal.html) with subsequent selection of primer sequences for the genes of interest.

Three pairs of primers were tested for each gene. The absence of the second product was estimated based on electrophoresis and melting curves. The effectiveness of primers was calculated on the basis of real-time PCR (quantitative polymerase chain reaction in real time) serial dilutions of the cDNA matrix. Only primers with efficiency equal to or close to 100 % were used. The primer test was carried out for several measurement periods (Supplementary Material 2).

In 2022, the conformity and quality of primers were verified using *M. lupulina* MlS-1 transcriptomic data (MACE sequencing). Total RNA from plant material was isolated using the trizole method with modifications (MacRae, 2007). The quality of DNAase treatment was tested by PCR for RNA with the reference gene, actin tested immediately before cDNA synthesis. cDNA synthesis was carried out using the Maxima First Strand cDNA Synthesis Kit with dsDNase in accordance with the manufacturer's instructions (Thermo Scientific, USA). ~1 mcg of total RNA was selected for cDNA synthesis. cDNA quality was tested with a ubiquitin test.

Changes in gene expression were evaluated using the RT-PCR method employing the BioRad CFX-96 real-time thermal cycler (Bio-Rad, USA) and using a set of reagents for RT-PCR in the presence of the SYBR Green I dye. The parameters of the amplification cycles were as follows: 95 °C, 5 min, 1 cycle; 95 °C, 15 s, 60 °C, 30 s, 72 °C, 30 s, 40 cycles. The specificity of amplification was evaluated using melting curve analysis. Changes in the expression level of the gene under examination were compared with the expression level of the same gene in the control. Analysis was carried out using the $2^{-\Delta\Delta CT}$ method. The levels of gene expression were normalized with the selected reference gene, actin according to (Yurkov et al., 2020). The PCR mix (10 ml) contained: 1 ml of 10x B + SYBR Green buffer, 1 ml of 2.5 mM dNTP, 1 ml of $MgCl_2$ (25 mM), 0.3 ml of each of a pair of primers (10 mM for each primer), 0.125 ml (0.625 units) SynTaq DNA polymerase (manufacturer of mix components – Synthol, Russia), 4.275 µl ddH_2O, 2 µl cDNA sample. The relative values of the cDNA gene expression level for each sample were evaluated (experiment with AM, control without AM). The biological repeatability is 3, the technical repeatability is 4 measurements.

Evaluation of parameters of symbiotic efficiency and activity. J.M. Philips and D.S. Hayman's trypan blue staining method was used for root samples (Phillips, Hayman, 1970). The parameters of AM fungus activity in the root, the mycorrhization indices, are calculated according to (Vorob'ev et al., 2016) as: *a* and *b* (abundance of arbuscules and vesicles in mycorrhized parts of the roots, respectively), and *M* (intensity of AM development in the root). They have the following calculation formulas:

$$M = \frac{(95n_5 + 70n_4 + 30n_3 + 5n_2 + 1n_1)}{N}\%, \quad (1)$$

where n_5 is the number of visual fields with a mycorrhiza density class – $M = 5$; n_4 – with $M = 4$; n_3 – with $M = 3$, etc.; M is estimated from 1 to 5 points: 1 evaluation score: 0–1 % mycorrhiza at the root in the field of view of the microscope; 2 evaluation scores: 2–10 %; 3 scores: 11–50 %; 4 scores: 51–90 %; 5 scores: 91–100 % mycorrhiza in the root.

$$a = \frac{(100mA_3 + 50mA_2 + 10mA_1)}{100}\%, \quad (2)$$

where $mA_i =$

$$= \frac{(95n_5mA_i + 70n_4mA_i + 30n_3mA_i + 5n_2mA_i + 1n_1mA_i)F}{M(N - n_0)}\%, \quad (3)$$

[1] Supplementary Materials 1 and 2 are available in the online version of the paper: http://vavilov.elpub.ru/jour/manager/files/Suppl_Kryukov_Engl_27_3.pdf.

where $n_i mA_j$ is the number of visual fields with $M = i$, $A = j$, F is the incidence of mycorrhizal infection (the proportion of visual fields with AM relative to the total number of visual fields in one root sample), N is the total number of viewed visual fields, n_0 is the number of visual fields without AM, n_i is the number of visual fields with a mycorrhiza density class from 1 to 5, and A_i is the arbuscule density class from 1 to 3.

$$b = \frac{(100mB_3 + 50mB_2 + 10mB_1)}{100}\%, \quad (4)$$

where $n_i mB_j$ is the number of visual fields with $M = i$ and B (vesicle density class) $= j$ is calculated similarly to the calculation for arbuscules (3); B_j is the density class of arbuscules from 1 to 3.

The symbiotic efficiency of AM was estimated as the difference in productivity index (crude weight of aboveground parts) between the variant with AM inoculation ("+AM") and the control without AM ("without AM"), divided by the value in the variant "without AM" as a standard calculation MGR (mycorrhizal growth response) (Kaur et al., 2022). Biological repeatability in assessing the parameters of the effectiveness and activity of AM in each variant was equal to 8 plants.

Statistical analysis. ANOVA and Tukey's HSD test ($p < 0.05$) were used as a post-hoc test to compare the differences in all indicators; Student's t-test ($p < 0.05$) was also used to assess the significance of differences in the average values of gene expression levels between the "+AM" and "without AM" variants.

Results

The results of the evaluation of the symbiotic efficacy and parameters of mycorrhization showed that the studied symbiotic test system *Medicago lupulina* + *Rhizophagus irregularis* should be considered highly effective (with high MGR) and symbiotically active. Symbiotically active refers to the presence of active mycorrhization of roots by mycelium, arbuscules and vesicles under conditions of an average level of phosphorus available to plants in the substrate.

Analysis of *M. lupulina* mycorrhization by AM fungus *R. irregularis* showed that the intensity of mycorrhization (M; Fig. 1, *b*) and the abundance of vesicles (b, see Fig. 1, *d*) significantly decreased at the initiation of lateral branching stage (48 days). However the abundance of arbuscules (a, see Fig. 1, *c*), the principal symbiotic structures of AM, were maintained at a high level along with the symbiotic efficiency (MGR) calculated for the fresh weight of the aboveground parts (see Fig. 1, *a*).

The obtained data on microscopy and MGR evaluation indicate that highly effective and active PMS with an early and prolonged response can be used as a genetic model for the search and analysis of marker genes for the development of effective AM symbiosis. It can be accessed from the early stage (2nd leaf stage) to the late stage of the fruiting initiation in conditions of an average P_i level in the substrate. To this end, the expression of 11 genes of the SWEET family was evaluated. The relative level of transcripts (normalized values of $2^{-\Delta\Delta Ct}$) in the roots of *M. lupulina* with normalization to control without AM was evaluated (Fig. 2).

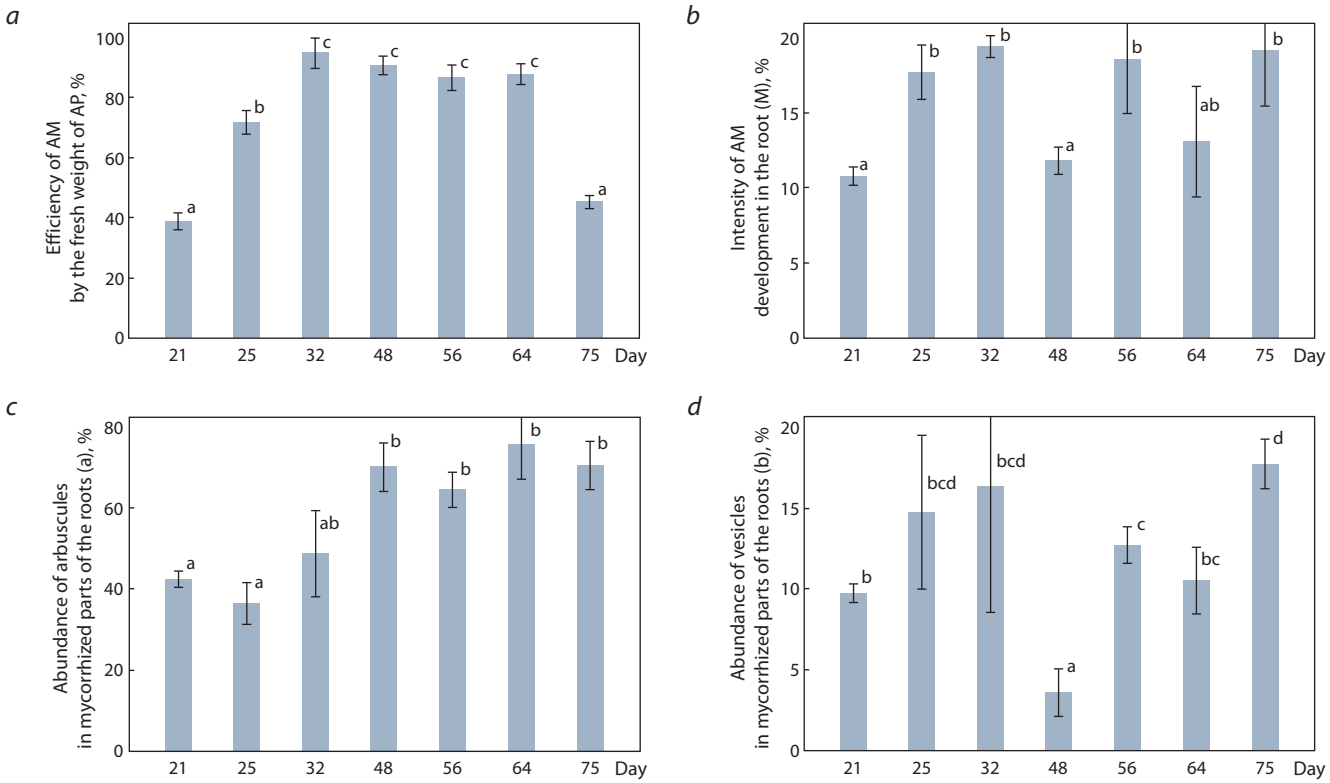

Fig. 1. The symbiotic efficiency of AM calculated from the fresh weight of the aboveground parts (*a*), the intensity of mycorrhization M (*b*), the abundance of arbuscules (*c*) and the abundance of vesicles (*d*) formed by *R. irregularis* in the roots of *M. lupulina*.

The average values with the error of the average are presented; the LF is the aboveground parts; "day" is the day after sowing and inoculation. Values with significant ($p < 0.05$) differences are marked with different letters (a, b, c, d).

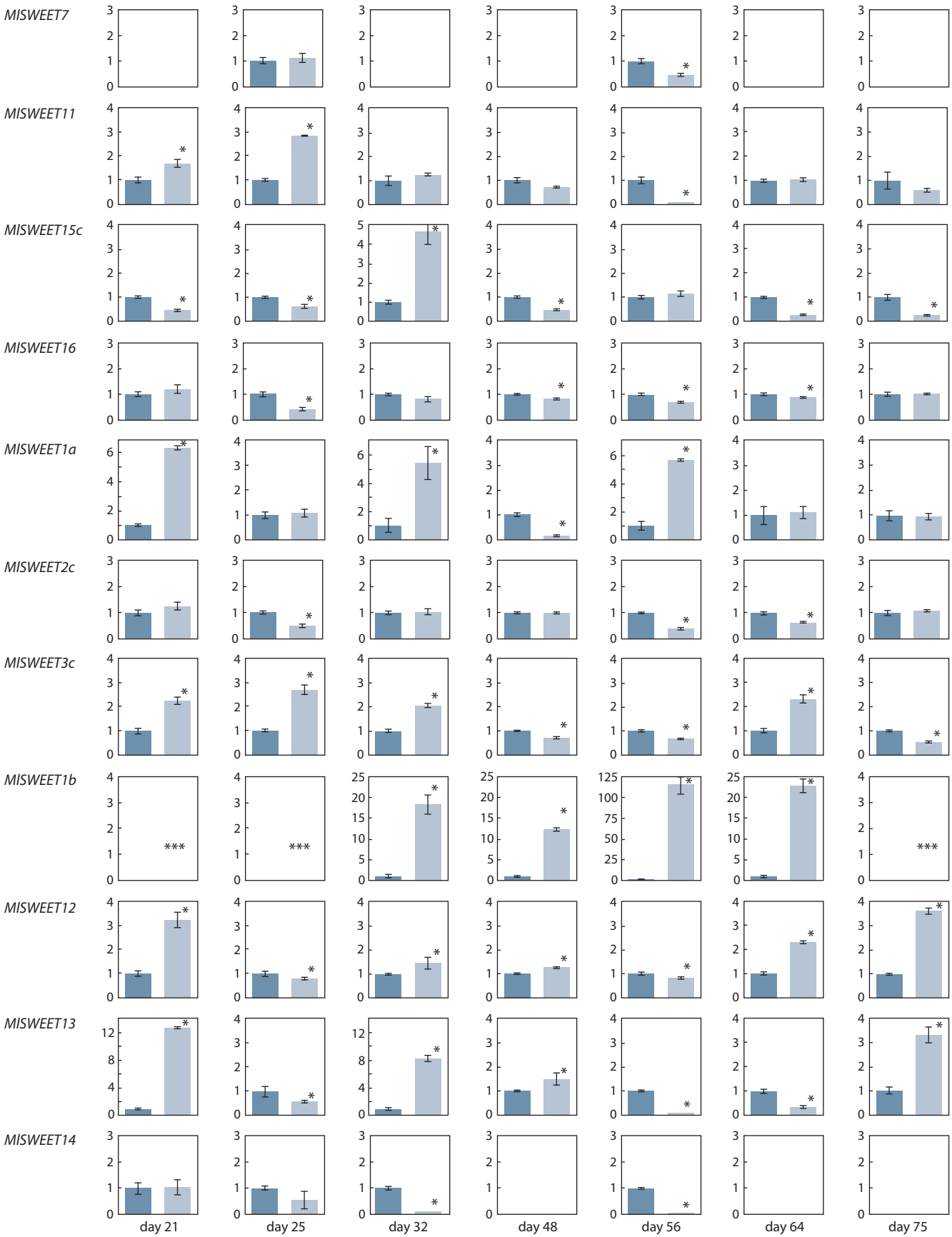

Fig. 2. Relative transcript level (normalized value of $2^{-\Delta\Delta Ct}$) of the SWEET family genes in the roots of *M. lupulina*.

The average values with the error of the average are presented. * The presence of significant ($p < 0.05$) differences between the variant "without" (dark bars) and the variant "+AM" with *R. irregularis* inoculation (light column); empty diagrams – the absence of gene expression in the variants; "day" – day after sowing and inoculation. *** Specific gene expression in the variant "with AM").

It should be noted that some of the genes did not have a pronounced difference in variants with and without mycorrhiza. Nevertheless, results showed the presence of genes that had significantly higher expression at AM for most stages of host plant development: *MlSWEET3c*, *MlSWEET1b*, *MlSWEET12*, *MlSWEET13*. Increased expression relative to the control during mycorrhization was also observed for *MlSWEET11* at the 1st and 2nd stage of analysis (in the development stage of the 2nd and 3rd leaf), for *MlSWEET15c* at the 3rd stage of stemming. Significantly higher expression during mycorrhization was shown by the genes *MlSWEET1a* at the 1st, 3rd and 5th stage (in the stage of the 2nd leaf, stemming and lateral branching, respectively) and *MlSWEET1b* at all 7 stages of measurement. Furthermore, its expression at the 1st, 2nd and 7th stages (in the stage of the 2nd and 3rd leaves and the fruiting initiation stage, respectively) was also dependent upon mycorrhization.

Discussion

According to the results obtained, in the roots of mycorrhizated *M. lupulina*, a higher expression of SWEET family genes was observed (see Fig. 2) in comparison to plants without AM: in six genes at the stage of the second leaf (21 days); in three genes at the stage of the stemming initiation (25 days); in six genes at the stage of stemming (32 days); in three genes at the stage of the lateral branching initiation (48 days); in two genes at the stage of lateral branching (56 days); in three genes at the stage of the flowering initiation (64 days); in three genes at the stage of the fruiting initiation (75 days). Reduced expression in the roots of plants with AM was characterized (see Fig. 2) by: one gene at day 21; five genes at day 25; one gene at day 32; four genes at day 48; eight genes at day 56; four genes at day 64; two genes at day 75.

Based on this, it can be assumed that during the stemming stage (32 days), the formation and maintenance of AM required enhanced transport of sugars. This led to increased expression of the largest number (6) of the SWEET family of genes in AM, and to reduced expression of only one gene during mycorrhization. On the other hand, the stage at the lateral branching initiation (48 days) was characterized by a sharp decrease in expression. Increased expression was observed in only 3 genes with AM and the number of genes with reduced expression increased to 4. The lateral branching stage of stem (56 days) was accompanied by even weaker gene expression. It should be noted that the observed relationship with the development stage of the host plant also took place in our previous studies (Yurkov et al., 2021), in which it was shown that the role of the development stage in the formation of the plant metabolome was higher than the effect of the actual inoculation by an effective AM fungus in conditions of low P_i level in the substrate. This explains why the analysis of gene expression was carried out taking into account the timing of plants entering new stages of development.

This study shows that the symbiotic pair "MlS-1 line of *M. lupulina* + RCAM00320 strain of *R. irregularis*" retains high efficiency in conditions of medium P_i (see Fig. 1, *a*), although this efficiency is still lower than in conditions of low P_i (Yurkov et al., 2020, 2021). Furthermore, the model PMS maintains high mycorrhization parameters throughout the entire development from the 2nd leaf stage to the late stage of the fruiting initiation, apart from a decrease in the parameters of the intensity of mycorrhization M and the abundance of vesicles b at the lateral branching initiation (see Fig. 1, *b*, *d*) (48 days). It is noteworthy that, in comparison with the previous period, only at this stage was a lower relative expression observed in 8 out of the 11 studied genes in AM infected roots: *MlSWEET1a*, *MlSWEET1b*, *MlSWEET3c*, *MlSWEET11*, *MlSWEET12*, *MlSWEET13*, *MlSWEET15c*, *MlSWEET16* (see Fig. 2).

Studies with low P_i level in the substrate also showed significant changes at the initiation of lateral branching, but of a different nature. At this point the development of AM-symbiosis was observed with the greatest efficiency and with significant metabolic rearrangements (Yurkov et al., 2021).

Among the genes of the SWEET family examined, as a rule, only the genes *MlSWEET1b*, *MlSWEET3c*, *MlSWEET12*, *MlSWEET13* were characterized by higher expression during mycorrhization under conditions of medium P_i in the substrate (see Fig. 2).

The literature still offers insufficient and contradictory data about the localization and function of proteins of the SWEET family, but it can be assumed that some of these aspects will be identical for homologues between different plant species. Thus, it is known that VfSWEET1 in *Vernicia fordii* is found in leaves (Cao et al., 2019), SlSWEET1a in *Solanum lycopersicum* in young leaves and flowers (Ho et al., 2019), TaSWEET1b1-1B in *Triticum aestivum* in the stem, StSWEET1h and StSWEET1i in leaves, stems, and root, BrSWEET1b in *Brassica rapa* in the root (Li et al., 2018); MtSWEET1b in *M. truncatula* in seeds and nodules (Hu et al., 2019). It should be noted that the *SWEET1b* gene is fixed in studies mainly in aboveground parts, when, as in our study, its specificity in AM is demonstrated in roots under growth conditions with a medium level of P_i in the substrate.

The products of the orthologue of the *MlSWEET3c* gene are localized in flowers, leaf petioles, beans, stems, seeds, roots and nodules in *M. truncatula* and transfer glucose (Hu et al., 2019); but, for example, in *Lotus japonicus* they transfer sucrose, and not glucose. Other sources indicate 2-deoxyglucose (Sugiyama et al., 2017). Thus, not only localization but also the functions of the products of the SWEET family proteins are still the subject of discussion.

The products of the orthologs of the *MlSWEET12* and *MlSWEET13* genes carry sucrose in *Solanum lycopersicum* and *Manihot esculenta* (Cohn et al., 2014; Zhao et al., 2018). In *M. truncatula*, MtSWEET13 is found in flowers, leaves, leaf petioles, stems and seeds, MtSWEET12 is found in all organs (Hu et al., 2019). Thus, we observe that MlSWEET12 and MlSWEET13 are polyfunctional. The functions of *MlSWEET12* ortholog products are sucrose outflow from leaves for phloem loading (Chen et al., 2012, 2015) and resistance to pathogens (Cohn et al., 2014; Zhao et al., 2018; Gautam et al., 2019). And the functions of *MlSWEET13* ortholog products are pollen development (Sun et al., 2013), resistance to pathogens (Cohn et al., 2014; Zhao et al., 2018), possible deposition of primexin, phloem loading (Chen et al., 2012; Gautam et al., 2019), and possible transport of gibberellin (Kanno et al., 2016; Jeena et al., 2019).

In our work, *MlSWEET1b* showed high expression during mycorrhization at all measurement periods (see Fig. 2). This gene can be confidently considered a good marker of the effective development of AM-symbiosis of *M. lupulina* with *R. irregularis* under conditions of an average P_i level in the substrate. It should be noted that *MlSWEET1b* had a specific expression in the roots at AM on the 1st, 2nd and 7th measurements (at the stages of development of the 2nd, 3rd leaf, and fruiting) (see Fig. 2). Perhaps in the initial stages (1 and 2), this gene was specifically expressed for the formation of an effective AM symbiosis (for the redistribution of sugars in favor of the mycosymbiont). Then, at a later stage, it was expressed because of a drop in symbiotic efficiency during the transition to fruiting when the host plant again needed to maintain an effective AM symbiosis to ensure seed maturation.

Thus these observations revealed a relationship between the stage of development of the host plant *M. lupulina* and the stage of development of AM symbiosis with *R. irregularis* with the expression of a particular *MlSWEET1b* gene in AM infected plants. Our data are consistent with the results of studies on other plant species. Thus, a recent work from 2016 has also mentioned orthologs of the *MlSWEET1b* gene as being symbiotic with AM in *S. tuberosum* and *M. truncatula* (Manck-Götzenberger, Requena, 2016; An et al., 2019; Doidy et al., 2019; Kafle et al., 2019; Cope et al., 2022). For genes of *MlSWEET3c* orthologs were found in *Lotus japonicus* and *M. truncatula* (Sugiyama et al., 2017; Cope et al., 2022), orthologs of *MlSWEET12*, in *S. tuberosum* and *M. truncatula* (Manck-Götzenberger, Requena, 2016; Hennion et al., 2019; Cope et al., 2022).

Based on the results of this work and analysis of the literature, it can be assumed that under different biotic and abiotic conditions (for example, with different chemical composition of the substrate, moisture, light, etc.), the expression and function of the *SWEET* genes may differ significantly.

Conclusion

The expression of the genes of the SWEET family during the transition of plants from the initiation of one stage of development to another has been practically ignored in the literature. This study has managed to eliminate this drawback. For the first time the analysis of their expression in the roots of an *M. lupulina* line highly responsive to mycorrhization was performed under conditions of average P_i level in the substrate. Results showed that the expression of the *MlSWEET1b* gene specifically increased with a decrease in symbiotic efficiency calculated by the weight of fresh aboveground parts. It is likely that the high expression in AM plants at early stages of development is associated with the active redistribution of sugars during the formation of effective AM. At the fruiting stage, on the other hand, it is a response to the needs of sugars for seed maturation. More than half of the studied genes also showed increased expression, among which genes such as *MlSWEET3c* and *MlSWEET12* should be singled out. The results obtained are consistent with the literature in that AM-specific genes of the SWEET family can be found among the genes of clades I and III.

Given the diversity of orthologs in other plant species, there is reason to believe that not all the genes of the SWEET family have yet been identified, both in the plant we have examined, *M. lupulina*, and in other species of the *Medicago* genus. Research over the coming years will surely expand our conception of what functions *SWEET* transporters perform in *Medicago* plants.

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