Abstract: This investigation involved a comparative analysis of the small GTPase superfamily in *S. lycopersicum* super strain B compared to their analogues in leguminous and other non-leguminous species. The small GTPases superfamily members were recognized by tBLASTn searches. The sequences of amino acid were aligned using Clustal Omega and the analysis of phylogeny was performed with the MEGA7 package. Protein alignments were applied for all studied species. Three-dimensional models of RABA2, ROP9, and ROP10 from *Solanum lycopersicum* “Super strain B” were performed. The levels of mRNA of the Rab, Arf, Rop, and Ran subfamilies were detected in aerial tissues vs. roots. Significant divergences were found in the number of members and groups comprising each subfamily of the small GTPases and *Glycine max* had the highest count. High expression of Rab and Arf proteins was shown in the roots of legumes whilst in non-legume plants, the highest values were recorded in aerial tissues. *S. lycopersicum* super strain B had the highest expression of Rab and Arf proteins in its aerial tissues, which may indicate that diazotroph strains have supreme activities in the aerial tissues of strain B and act as associated N-fixing bacteria. The phylogenies of the small GTPase superfamily of the studied plants did not reveal asymmetric evolution of the Ra, Arf, Rop, and Ran subfamilies. Multiple sequence alignments derived from each of the Rab, Arf, and Rop proteins of *S. lycopersicum* super strain B showed a low frequency of substitutions in their domains. GTPases superfamily members have definite functions during infection, delivery, and maintenance of N<sub>2</sub>-fixing diazotroph but show some alterations in their function among *S. lycopersicum* super strain B, and other species.

Keywords: *S. lycopersicum* super strain B; phylogenetic analyses; protein expression; protein sequence alignment; small GTPase superfamily

1. Introduction

*Solanum lycopersicum* L. (tomato) is a vegetable crop cultivated all over the world for its high agro-economic importance [1]. It requires heavy manure and an adequate nitrogen supply to obtain the highest yields [2]. It appears that *S. lycopersicum* obtains its nitrogen from both chemical fertilization with organic and inorganic manure [3] and acetylene reduction performed by diazotrophic bacteria present on the rhizoplane and the rhizosphere soil [4,5].

Various molecular components involved with diazotrophs infection have been highlighted as facilitating intracellular membrane trafficking [6–8], cytoskeleton related-proteins [9,10], and cell-wall degeneration enzymes. Among the proteins related to vesicle membrane trafficking are small GTPases, which have essential contributions to plant growth and development, including in the first diazotroph contagion process, root hair formation,
and in the signaling pathway essential for nodulation in leguminous plants [7,8,11] and in the late symbiosome stage [12,13].

The small GTPases superfamily are commonly classified into five main subfamilies depending on their sequence, structure, and function identities: (1) Ras, (2) Rab, (3) Arf/Sar, (4) Rop/Roh, and (5) Ran [14,15], but only four of them are present in plants. Ras/Rab family members are key regulators that control the cell fate through specification, development, and differentiation [16], but Ras proteins are absent from plant genomes [15]. Arf/Sar are the main GTP-binding proteins that control morphogenesis, microtubule organization, and membrane trafficking joining the endoplasmic reticulum with the Golgi apparatus [16–20]. Individuals of the Rop family, noted as Rho of plants, are Ras homologous and regulate many processes in plants, such as polarized cell development, morphogenesis, cytoskeleton dynamics, hormone signaling, safeguard, and responses of the cell towards external stimuli [16,21,22]. However, Ran members (Ras-related nuclear protein) regulate nuclear import/export across the nuclear pore, mitotic nuclear reassembly, and kinetochore binding with microtubules [16,23].

Since these genes are crucial for cell vitality because of their housekeeping activities, they are well conserved functionally and sequentially among eukaryotes [24,25]. However, some alterations in their functional and expression lineaments have been detected in some plants, indicating the possibility of acquiring linked or even new functions [15,26]. Therefore, transcriptome analyses are essential for collecting all sequence information from available plant species to investigate the degree of gene divergence between species-species and species-progenies [25,27].

The tomato cultivar super strain B is widely cultivated in Saudi Arabia and due to the nature of the soil in Saudi Arabia, which is almost desert, the plant can obtain its nutrients via chemical fertilization or via the degradable materials from microorganisms in the rhizosphere and rhizoplane of the plant. The GTPase family members are involved in nitrogen nutrition, so the present investigation aimed to detect the small GTPase family in the transcriptome of tomato cultivar “strain B” and compare the distinctive expression motif with their analogues in some leguminous and non-leguminous plants obtained from the database.

2. Results and Discussion

The count of each small GTPases subfamily member in S. lycopersicum “super strain B” compared to the non-leguminous and leguminous species is represented in Table 1. The table showed high divergence in the RAB number present in legume and non-legume species, where G. max recorded the highest member of RAB members (94). L. japonicus and O. sativa possessed the lowest number of members in the RAB subfamily. Between the highest and the lowest RAB number, the other species were 64 members in M. truncatula, followed by 57 in A. thaliana, 53 in Z. mays, 50 in P. vulgaris, and 46 in S. lycopersicum “super strain B”. Regarding the ARF subfamily, the maximum numbers were in G. max (41) and the least were in L. japonicus (13). The other species comprised nearly half the number of members in G. max (19–21), except in case of Z. mays, which has slightly higher numbers of members (23). Concerning the ROP subfamily, G. max still had the highest number of members (20) and the other legumes and non-legumes species had fewer. P. vulgaris and A. thaliana had 11 members, S. lycopersicum had 10, Z. mays and S. lycopersicum “super strain B” had 9, L. japonicas and O. sativa had 8, and finally, M. truncatula had the least members (7). Members of the RAN subfamily were higher in G. max (7) and lower in both L. japonicus and O. sativa (2). The other legumes and non-legume species possessed around three to four RAN members (Table 1). The results indicated the presence of significant variations in the score of members representing each subfamily of the small GTPases superfamily found in leguminous and non-leguminous species, in which G. max scored the highest number of members. According to Singh and Hymowitz [28], the drastic number of soybean GTPases subfamilies may refer to its genomic nature as a partially diploidized tetraploid species.
Table 1. Number of each small GTPases subfamily members expressed in Solanum lycopersicum super strain “B” compared with some non-leguminous and leguminous plants retrieved from the data base.

| GTPases | S. lycopersicum Super Strain “B” | Non-Legumes | Legumes |
|---------|----------------------------------|-------------|--------|
| RAB     | 46                              | 57          | 90     |
| ARF     | 21                              | 21          | 25     |
| ROP     | 9                               | 11          | 8      |
| RAN     | 4                               | 4           | 3      |
| Total   | 80                              | 90          | 53     |

Table 2 illustrates the number of members in each group of the small GTPases Rab subfamily. Group A of the Rab subfamily had the highest number of members along with S. lycopersicum super strain B (186), in which the leguminous plants has more Rab participants (99) than the non-leguminous plants (87), despite the presence of the highest representatives in G. max (41). The other groups of the Rab subfamily acquired less than a quarter of the total members present in group A. From all groups, group B had the lowest total number of members (26), with nearly equal numbers of representatives in both legumes and non-legume species. Table 2 also reveals that the total number of members of Rab Group C was asymmetrically split between the legume and non-legume plants, in which the members in legumes (26) were about triple those in non-legumes species (9).

Table 2. Number of the small GTPases Rab subfamily members expressed in Solanum lycopersicum super strain “B” compared with some non-leguminous and leguminous plants retrieved from the data base.

| Group | S. lycopersicum Super Strain “B” | Non-Legumes | Legumes |
|-------|----------------------------------|-------------|--------|
| A     | 21                              | 26          | 23     |
| B     | 2                               | 3           | 4      |
| C     | 3                               | 3           | 4      |
| D     | 5                               | 4           | 6      |
| E     | 5                               | 5           | 5      |
| F     | 4                               | 3           | 5      |
| G     | 3                               | 8           | 5      |
| H     | 3                               | 5           | 2      |
| Total | 46                              | 57          | 53     |

The number of members in each group of the small GTPases Arf subfamily is presented in Table 3. Group (B + C + D) had the highest number of members (58) among all studied species. Although the non-leguminous species had the same number of participants in the group (A + B + C), G. max still had the highest number of individuals (12). Group (ARLC) was the minor group of members, which consisted of a total of 9 members nearly equal distributed between leguminous and non-leguminous species. The majority of the Arf members in groups (A, ARLA, and ARLB) were from the non-leguminous plants as compared to the leguminous ones. However, the opposite was recorded in group SARA, where the highest total Arf numbers of individuals were detected in leguminous plants (19), which were mainly from G. max (10), Table 3.
Table 3. Number of small GTPases Arf subfamily members expressed in *Solanum lycopersicum* super strain “B” compared with some non-leguminous and leguminous plants retrieved from the data base.

| Group   | *S. lycopersicum* Super Strain “B” | Non-Legumes | Legumes |
|---------|------------------------------------|-------------|---------|
|         | A. thaliana                        | O. sativa   | Z. mays | L. japonicus | M. truncatula | P. vulgaris | G. max |
| A       | 5                                  | 6           | 6       | 6           | 4             | 5           | 4      | 10     |
| B + C + D | 8                              | 6           | 6       | 9           | 4             | 7           | 6      | 12     |
| ARLA    | 3                                  | 4           | 2       | 4           | 1             | 3           | 4      | 5      |
| ARPB    | 1                                  | 1           | 2       | 2           | 1             | 0           | 1      | 2      |
| ARPC    | 1                                  | 1           | 1       | 1           | 1             | 1           | 1      | 2      |
| SARA    | 3                                  | 3           | 4       | 3           | 2             | 3           | 4      | 10     |
| Total   | 21                                 | 21          | 21      | 25          | 13            | 19          | 20     | 41     |

Gene expression of the GTP protein families often appears to vary in spatio-temporal control between different species. Table 4 delineated the amounts of mRNA expressed in the members of each small GTPases subfamily of *S. lycopersicum* super strain B, and other non-legume and legume plants, present in aerial tissue vs. those of root. Using root samples as a reference, normalized values were derived for cross referencing to other tissues. In all species in this study, GTPases were almost accumulated at higher levels in the roots compared to aerial tissues. The members of the Rab and Rop subfamilies of *S. lycopersicum* super strain B showed a higher mRNA level in their aerial tissues compared to the roots, consistent with those in the other species, except in *P. vulgaris* and G. max, where Rops also demonstrated increased levels of mRNA in its aerial tissues (Table 4). Only one member of Ran subfamily in both *O. sativa* and *L. japonicus* had high mRNA while the other species had none. All species have members of the Rab, Arf, and Rop subfamilies with consistent amounts of mRNA levels in their aerial tissues, in which G. max had higher levels (Table 4). The results of analyzing the expression of small GTPases subfamily members in both leguminous and non-leguminous species indicated that a higher number of Rab and Arf members were upregulated in aerial tissues than roots in non-leguminous plants, especially in *S. lycopersicum* super strain B. However, the members of each subfamily (Rab, Arf, Rop, and Ran) with unchanged levels of mRNA in aerial tissues were comparable in leguminous and non-leguminous species of the study. In addition, the highest downregulation of each small GTPases subfamily member was observed in G. max. The high accumulation of mRNA of both Rab and Arf proteins in the roots of legumes may indicate that they are the main proteins involved in the symbiotic relation between legumes and rhizobia. Probable tissue-specific functionalization of Rab/Arf small-GTP binding genes/proteins was suggested to participate in the genesis, development, and maintenance of nodulations in the roots of legume plant as reported by several investigators of Rab in soybean and Vigna aconitifolia [29], Lotus japonicus [30], soybean [31], Medicago sp [12,32], kidney bean [6], and Rab/Arf in Medicago truncatula [13]. Concerning non-leguminous plants, the high expression of Rab and Arf proteins in the aerial tissues, especially in *S. lycopersicon* strain B, may disclose the presence of another mechanism different from nodulations that involve N₂ uptake and fixation. In this context, Mohandas [33] revealed the domestication of some rhizobacteria in the roots and leaves and on the rhizoplane and phylloplane of tomato (*L. esculentum* Mill “Pusa Ruby”) as associated N₂-fixing bacteria. In addition, Dent and Cocking [34] reported that diazotroph strains can intracellularly colonize, under specific conditions, the roots (or root hairs) and shoots of non-legume plants without nodulation in cereals, such as wheat, maize, and rice, in addition to some crops, such as potato, oilseed rape, and tomato. Moreover, Collavino et al. [35] reported that the diazotrophic populations inside the stem and root of tomato plants play a critical function in the early growth phases and are distinctly influenced by N fertilization. From all the above, we can speculate that the high gene expressions may mean that diazotroph strains are colonized more in the aerial tissues than the roots (either inside or on the surface) of *S. lycopersicum* super strain B.
Table 4. The number of each GTPase subfamily member that shows no change (N), reduced (−), or increased (+) levels of mRNA in *Solanum lycopersicum* super strain “B” compared with some non-leguminous and leguminous plants retrieved from the database in aerial tissue vs. the root.

| Plant Species                        | Rab | Arf | Rop | Ran |
|--------------------------------------|-----|-----|-----|-----|
|                                      | N + | −   | N + | −   |
| *S. lycopersicum* super strain “B” a | 8   | 24  | 14  | 8   |
| *A. thaliana* a                      | 16  | 36  | 5   | 6   |
| *O. sativa* b                        | 14  | 20  | 2   | 4   |
| *Z. mays* c                          | 17  | 34  | 0   | 5   |
| *L. japonicus* d                     | 1   | 29  | 0   | 0   |
| *M. truncatula* a                    | 17  | 26  | 2   | 5   |
| *P. vulgaris* a                      | 14  | 28  | 3   | 5   |
| *G. max* a                           | 50  | 42  | 2   | 19  |

Note: a compared to leaf; b compared to shoot (2-week-old); c compared to stem; d compared to leaf (6-week-old).

Monomeric GTPase sequences of amino acids from tBLASTn searches were applied to recognize the individuals of the small GTPases superfamily of *S. lycopersicum* super strain B and those retrieved from the genomic databases (*O. sativa*, *A. thaliana*, *S. lycopersicum*, *L. japonicus*, *Z. mays*, *M. truncatula*, *G. max*, and *P. vulgaris*). Amino acid sequences of those proteins were employed to create phylogenetic trees of those species, permitting their categorization using small GTPases subfamilies into Rab (green), Arf (blue), Rop (Pink), and Ran (violet), (Figure 1). The phylogenetic inspection of the small GTPase superfamily of the studied leguminous and non-leguminous plants did not reveal asymmetric evolution of the Ra, Arf, Rop, and Ran subfamilies. These results were in accordance with those of Flores et al. [27].

Figure 1. Phylogenetic analysis of the small GTPase superfamily in *S. lycopersicum* super strain B and other legume and non-legume plants. Amino acid sequences corresponding to small GTPases from *O. sativa*, *A. thaliana*, *S. lycopersicum*, *L. japonicus*, *Z. mays*, *M. truncatula*, *G. max*, and *P. vulgaris* were restored from genomic databases. Unrooted neighbor-joining trees were obtained using the Mega 7 software. Subfamilies were recognized for each species: Rab (green), Arf (blue), Rop (Pink), and Ran (violet).
Multiple sequence alignments of RABs proteins of *S. lycopersicum* super strain B, *O. sativa*, *A. thaliana*, *S. lycopersicum*, *L. japonicus*, *Z. mays*, *M. truncatula*, *G. max*, and *P. vulgaris* are illustrated in Figure 2. *S. lycopersicum* super strain B showed 2 RABs strong amino acid conserved domains, which was similar to those of the other legume and non-legume species. RABA2 proteins showed conserved substitutions in the position 177 (out of domains), whereas a valine residue (V) was found in 4 leguminous species (*P. vulgaris*, RABA2, *M. truncatula*, *L. japonicus*, and *G. max*) and a isoleucine (I) in *S. lycopersicum* super strain B and the other non-legume species (*A. thaliana*, *O. sativa*, *S. lycopersicum*, *Z. mays*) (Figure 2).

All alignments of ROP9 proteins manifested powerful amino acid sequence conservation across the studied plants, which were clarified by the presence of 7 domains (Figure 3). Three positions (amino acids 53, 129, and 130) showed conserved substitutions in the domains of ROP9 proteins, and 3 other substitutions were out of it (amino acids 151, 164, 175). The former substitution was at the border of II, where isoleucine in leguminous species was switched with threonine (T) in non-leguminous ones. The second and the third ones were in the mid-region of the domain number V. In the second substitution, cysteine (C) was found in leguminous plants and phenylalanine (F) in non-leguminous ones. Interestingly, the third substitution was variable, in which a valine residue was recorded in only 4 non-leguminous plants (*O. sativa*, *S. lycopersicum*, *Z. mays*, *S. lycopersicum* super strain B). *A. thaliana*, however, differed from its non-legume species and has isoleucine residue like the leguminous ones except for *P. vulgaris*, which has leucine (L) instead (Figure 3).
M. truncatula (Medtr4g064897), P. vulgaris (Phvul.002G106600), and G. max (Glyma01g36880). Black boxes represent the identical residues while gray ones represent conservative substitutions. Alignments were performed with Clustal Omega in MEGA7 followed with Boxshade. The red arrow designates a conservative amino acid substitution in legume against non-legume sequences. The conserved domains of Rab were indicated by green lines. The conserved domains of ROPs were indicated by green lines.

By aligning the sequence of ROP10 proteins of all the species studied, we found the presence of 4 domains. ROP10 protein alignments also possessed substitutions in legume species (Figure 4), but only one of those swaps affects the ROP domain. This substitution was at the beginning of III, where leucine was found in P. vulgaris and G. max, valine in M. truncatula, L. japonicus, S. lycopersicum and Z. mays, and isoleucine in A. thaliana, O. sativa and S. lycopersicum super strain B.

Figure 4. Multiple sequence alignments and the proteins of S. lycopersicum super strain B (ROP10) and those retrieved from the genomic databases: A. thaliana (At1g07410, O. sativa (LOC_Os03g60870), S. lycopersicum (Solyc06g076450), Z. mays (GRMZM2G473906), L. japonicus (chr3.CM0792.300.r2.d), M. truncatula (Medtr4g064897), P. vulgaris (Phvul.011G061100), and G. max (Glyma11g14360). Black boxes represent identical residues while gray ones represent conservative substitutions. Alignments were performed with Clustal Omega in MEGA7 followed with Boxshade. Red arrows designate amino acid substitutions in legumes against non-legumes. The conserved domains of ROP are indicated by green lines.

By comparing the sequence alignment of the RABA2, ROP9, and ROP10 proteins of S. lycopersicum super strain B to their analogues in non-legume and legume plants, we found that RABA2 has a single conserved substitution while ROP9 and ROP10 have three (Figures 2–4). Those substitutions were affirmed by the predicted 3D configurations of RABA2, ROP9, and ROP10 proteins of S. lycopersicum super strain B (Figure 5). Multiple sequence alignments that were obtained for each of Rab, Arf, and Rop proteins of S. lycopersicum super strain B and their analogues in non-legume and legume plants as illustrated in Figures 2–4 showed a low frequency of substitutions in their domains. This may indicate the strong conservation of amino acid sequence across the leguminous and non-leguminous plants analyzed and it is proposed that those proteins were put through powerful discriminatory pressure as reported by Flores et al. [27]. Multiple sequence alignments of the leguminous plants’ proteins revealed that the domains of Rab proteins had no conserved substitutions while both ROP9 and ROP10 had one each. ROP9 showed a conserved substitution (in amino acid 130) in domain V where leucine was in P. vulgaris (Phvul.002G106600) and isoleucine was in the analogue of the other three legumes [L. japonicus (chr2.CM0272.860.r2.m), M. truncatula (Medtr4g064897), and G. max (Glyma01g36880)]. Moreover, ROP10 had conserved residues (in amino acid 115) in domain III where leucine was found in kidney bean (Phvul.009G180800) and soybean (Glyma04g35110) while valine in M. truncatula ROP10 (Medtr3g078260) and L. japonicus (chr1.CM0166.830.r2.m). Jiang and Ramachandran [24] and Yuksel and Memon [36] reported that small GTP-binding proteins in most plants are functionally very well conserved, but some could follow functional variations in divergent lineages to regulate some lineage-specific functional roles, such as nodulation in legume plants. So, the variations in the conserved residues in those
legume plants may reflect the specific contribution of those proteins in the legumes–rhizobia symbiosis relationship [27].

Figure 5. Three-dimensional models of RABA2, ROP9, and ROP10 from *Solanum lycopersicum* “Super strain B”. Arrows and yellow boxes indicate the position of the substitutions noticed in super strain B versus other legume and non-legume species.

3. Materials and Methods
3.1. Identification of Small GTPases from Different Species

The small GTPases superfamily members were recognized by tBLASTn searches [37] using the amino acid sequence of all small GTPase family individuals that were previously described and categorized in *Arabidopsis* [15,26]. These genes were selected and categorized manually following a systematic phylogenetic analysis.

3.2. Phylogenetic Analysis

The sequences of amino acids were aligned using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo (accessed on 15 March 2020)) [38] and the analysis of phylogeny was carried out with the MEGA7 package (http://www.megasoftware.net (accessed on 15 March 2020)) [39] using the method of neighbor-joining [40]. The distances of evolution were computed by the difference’s method [41]. All positions of gaps and data missing were omitted from the dataset.

3.3. Protein Alignments

Small GTPases amino acid sequences that participated in the initiation of the symbiotic relation between rhizobia and legumes were applied to identify members from other species by BLASTP. *Tc PvRabA2* and *PvArfA1* were selected from *Phaseolus vulgaris* (common bean), *LjRop6* from *Lotus japonicus*, and *MtRab7*, *MtRop9*, and *MtRop10* from *Medicago truncatula* as queries. The sequences with the lowest E value were applied to create multiple
alignments by Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo accessed on 15 March 2020) and organized with Boxshade (http://embnet.vital-it.ch/software/BOX_form.html accessed on 15 March 2020)).

3.4. D Modeling

Swiss Model [42] (https://swissmodel.expasy.org/ (accessed on 15 March 2020)) was used for protein modeling and the 3D structure viewer iCn3D (https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html (accessed on 15 March 2020)) for analysis.

3.5. Genomic Datasets

Sequences of Arabidopsis thaliana (TAIR10), Glycine max (Wm82.a2.v1), Medicago truncatula (M4.0v1), Oryza sativa (v7_JGI), Solanum lycopersicum (iTAG2.3), and Zea mays (Ensembl-18) were obtained from datasets. Lotus japonicus (v2.5) was obtained from Miyakogusa v2.5 (http://www.kazusa.or.jp/lotus (accessed on 15 March 2020)) and Phaseolus vulgaris (v1.0) from Phytozome v11.0 (https://phytozome.jgi.doe.gov/pz/portal.html (accessed on 15 March 2020)).

3.6. Transcriptomic Datasets

Small GTPases gene expression of A. thaliana: expression data were obtained from TraVA (http://travadb.org (accessed on 15 March 2020)) [43], S. lycopersicum cv. Heinz (http://ted.bti.cornell.edu (accessed on 15 March 2020)) (TGC 2012), O. sativa cv. Nippon bare [44], Z. mays from RNAseq transcriptomic analyses [45], M. truncatula cv Jemalong A17 from MtGEA (http://mtgea.noble.org/v3 (accessed on 15 March 2020)) [46], L. japonicus from LjGEA (http://ljgea.noble.org/v2 (accessed on 15 March 2020)) [47], P. vulgaris cv. NAG12 from (http://plantgrn.noble.org/PvGEA (accessed on 15 March 2020)) [48], and G. max from SoyBase (https://www.soybase.org/soyseq (accessed on 15 March 2020)) [49] was retrieved using databases available in the public domain.

3.7. Statistical Analysis of Expression Data

Small GTPase members were retrieved using the public datasets for each species. Their expressions manifested in roots (reference organ) and in other organs of the eight species in study. The values of expression were normalized for each tissue and the statistical analyses were achieved using CuffDiff [50] to identify the expressed genes.

4. Conclusions

The highest numbers of Rab and Arf proteins were expressed in tomato super strain B and all the species compared in this study. The levels of Rab and Rop mRNA in aerial tissues were higher than in roots, but in contrast, Arf mRNA levels was higher in roots than in aerial tissues. The Ran subfamily showed the least expression in different tissues of tomato super strain B.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants11050641/s1, Table S1: Expression data of the Arabidopsis thaliana small GTPase superfamily, Table S2: Expression data of the Oryza sativa small GTPase superfamily by RNA sequencing, Table S3: Expression data of the Solanum lycopersicum small GTPase superfamily, Table S4: Expression data of the Zea mays small GTPase superfamily by RNA sequencing, Table S5: Expression data of the Lotus japonicus small GTPase superfamily, Table S6: Expression data of the Medicago truncatula small GTPase superfamily by microarray, Table S7: Expression data of the Phaseolus vulgaris small GTPase superfamily by RNA sequencing, Table S8: Expression data of the Glycine max small GTPase superfamily by RNA sequencing, Table S9: RNA-seq data for M. truncatula small GTPases detected in different regions of the nodule.
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References

1. Al-Daej, M.I. Salt Tolerance of Some Tomato (Solanum lycopersicum L.) Cultivars for Salinity under Controlled Conditions. *Am. J. Plant Physiol.* 2018, 13, 58–64. [CrossRef]

2. Splittstoesser, W.E. Vegetable Growing Handbook, Organic and Traditional Methods, 3rd ed.; Van Nostrand Reinhold: New York, NY, USA, 1990.

3. Fouda, K.F. Effect of Interaction among N Forms and Calcium Sources on Quality and Chemical Composition of Tomato (Lycopersicon esculentum). *Egypt. J. Soil Sci.* 2017, 57, 61–71. [CrossRef]

4. Kloepper, J.W.; Beauchamp, C.J. A review of issues related to measuring colonization of plant roots by bacteria. *Can. J. Microbiol.* 1992, 38, 1219–1232. [CrossRef]

5. Elmerich, C.; Newton, W.E. Associative and Endophytic Nitrogen-Fixing Bacteria and Cyanobacterial Associations; Springer: Dordecht, The Netherlands, 2007.

6. Dalla Via, V.D.; Traubenik, S.; Rivero, C.; Aguilar, O.M.; Zanetti, M.E.; Blanco, F.A. The monomeric GTPase RabA2 is required for progression and maintenance of membrane integrity of infection threads during root nodule symbiosis. *Plant Mol. Biol.* 2017, 93, 549–562. [CrossRef]

7. Ke, D.; Fang, Q.; Chen, C.; Zhu, H.; Chen, T.; Chang, X.; Yuan, S.; Kang, H.; Ma, L.; Hong, Z.; et al. The Small GTPase ROP6 Interacts with NFR5 and Is Involved in Nodule Formation in *Lotus japonicus*. *Plant Physiol.* 2012, 159, 131–143. [CrossRef]

8. Kiirika, L.M.; Bergmann, H.F.; Schikowsky, C.; Wimmer, D.; Korte, J.; Schmitz, U.; Niehaus, K.; Colditz, F. Silencing of the Rac1 GTPase MrROP9 in *Medicago truncatula* Stimulates Early Mycorrhizal and Oomycete Root Colonizations But Negatively Affects Rhizobial Infection. *Plant Physiol.* 2012, 159, 501–516. [CrossRef]

9. Hossain, S.; Liao, J.; James, E.; Sato, S.; Tabata, S.; Jurkiewicz, A.; Madsen, L.H.; Stougaard, J.; Ross, L.; Szczygowski, K. *Lotus japonicus* ARPC1 Is Required for Rhizobial Infection. *Plant Physiol.* 2012, 160, 917–928. [CrossRef]

10. Fournier, J.; Teillet, A.; Chabaud, M.; Ivanov, S.; Genre, A.; Limpens, E.; de Carvalho-Niebel, F.; Barker, D.G. Remodeling of the COPI-Coated Vesicles. *Plant Cell* 2000, 12, 2219–2235. [CrossRef]

11. Oldroyd, G.E.; Downie, J.A. Coordinating Nodule Morphogenesis with Rhizobial Infection in Legumes. *Annu. Rev. Plant Biol.* 2008, 59, 519–546. [CrossRef]

12. Limpens, E.; Ivanov, S.; van Esse, W.; Voets, G.; Fedorova, E.; Bisseling, T. *MedicagoN2*-Fixing Symbiosomes Acquire the Endocytic Identity Marker Rab7 but Delay the Acquisition of Vacuolar Identity. *Plant Cell* 2009, 21, 2811–2828. [CrossRef]

13. Memon, A.R.; Schwager, C.K.; Niehaus, K. Expression of small GTPases in the roots and nodules of *Lotus japonicus* ARPC1 Is Required for Rhizobial Infection. *Plant Physiol.* 2015, 167, 1233–1242. [CrossRef]

14. Kahn, R.A.; Der, C.J.; Bokoch, G.M. The Ras superfamily of GTP-binding proteins: Guidelines on nomenclature. *FASEB J.* 1992, 6, 2512–2513. [CrossRef]

15. Vernoud, V.; Horton, A.; Yang, Z.; Nielsen, E. Analysis of the Small GTPase Gene Superfamily of *Arabidopsis*. *Plant Physiol.* 2003, 131, 1191–1208. [CrossRef] [PubMed]

16. Lundquist, E.A. Small GTPases, WormBook, edn. The *C. elegans* Research Community, WormBook. 2006. Available online: http://www.wormbook.org/chapters/ww_smallGTPases/smallGTPases.pdf (accessed on 9 December 2021).

17. Pimpl, P.; Movafeghi, A.; Coughlan, S.; Denecke, J.; Hillmer, S.; Robinson, D.G. In Situ Localization and in Vitro Induction of Plant COPI-Coated Vesicles. *Plant Cell* 2000, 12, 2219–2235. [CrossRef]

18. Jürgens, G. Membrane Trafficking in Plants. *Annu. Rev. Cell Dev. Biol.* 2004, 20, 481–504. [CrossRef] [PubMed]

19. Memon, A.R. The role of ADP-ribosylation factor and SAR1 in vesicular trafficking in plants. *Biochim. Biophys. Acta* 2004, 1664, 9–30. [CrossRef] [PubMed]

20. Memon, A.R. Transcriptomics and proteomics analysis of root nodules of model legume plants. In *Crop Production for Agricultural Improvement*; Ashraf, M., Ozturl, M., Ahmed, S.M.A., Aksoy, A., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 291–315.
Plants 2022, 11, 641

21.  Etienne-Manneville, S.; Hall, A. Rho GTPases in cell biology. *Nature* 2002, 420, 629–635. [CrossRef]

22.  Craddock, C.; Lavaggi, I.; Yang, Z. New insights into Rho signaling from plant ROP/Rac GTPases. *Trends Cell Biol.* 2012, 22, 492–501. [CrossRef] [PubMed]

23.  Cavazza, T.; Vernos, I. The RanGTP Pathway: From Nucleo-Cytoplasmic Transport to Spindle Assembly and Beyond. *Front. Cell Dev. Biol.* 2016, 3. [CrossRef]

24.  Jiang, S.-Y.; Ramachandran, S. Comparative and evolutionary analysis of genes encoding small GTPases and their activating proteins in eukaryotic genomes. *Physiol. Genom.* 2006, 24, 235–251. [CrossRef]

25.  Yuksel, B.; Memon, A.R. Legume small GTPases and their role in the establishment of symbiotic associations with *Rhizobium spp.* *Plant Signal. Behav.* 2009, 4, 257–260. [CrossRef] [PubMed]

26.  Rutherford, S.; Moore, I. The Arabidopsis Rab GTPase family: Another enigma variation. *Curr. Opin. Plant Biol.* 2002, 5, 518–528. [CrossRef]

27.  Flores, A.C.; Via, V.D.; Savvy, V.; Villagrazia, U.M.; Zanetti, M.E.; Blanco, F. Comparative phylogenetic and expression analysis of small GTP-binding genes of model legume plants and assessment of their roles in root nodules. *Plant J.* 1997, 11, 237–250. [CrossRef] [PubMed]

28.  Singh, R.J.; Hymowitz, T. The genomic relationship between *Glycine max* (L.) Merr. and *G. soja* Sieb. and Zucc. as revealed by pachytene chromosome analysis. *Theor. Appl. Genet.* 1988, 76, 705–711. [CrossRef] [PubMed]

29.  Cheon, C.I.; Lee, N.G.; Siddique, A.B.M.; Bai, A.K.; Verma, D.P.S. Roles of plant homologs of Rabs1 and Rab7p in the biogenesis of the peribacteroid membrane, a subcellular compartment formed *de novo* during root-nodule symbiosis. *EMBO J.* 1993, 12, 4125–4135. [CrossRef] [PubMed]

30.  Borg, S.; Brandstrup, B.; Jensen, T.J.; Poulsen, C. Identification of new protein species among 33 different small GTP-binding proteins encoded by cDNAs from *Lotus japonicus*, and expression of corresponding mRNAs in developing root nodules. *Plant J.* 1997, 11, 237–250. [CrossRef] [PubMed]

31.  Son, O.; Yang, H.S.; Lee, H.J.; Lee, M.Y.; Cheon, C.I. Expression of SRAB7 and SCaM genes required for endocytosis of Rhizobium in root nodules. *Plant Sci.* 2003, 165, 1239–1244. [CrossRef]

32.  Schiene, K.; Donath, S.; Brecht, M.; Pühler, A.; Niehaus, K. A Rab-related small GTP binding protein is predominantly expressed in root nodules of *Medicago sativa*. *Mol. Genet. Genom.* 2004, 272, 57–66. [CrossRef]

33.  MOhandas, S. Nitrogen fixation in tomato (*Lycopersicon esculentum* Mill. ‘Pusa Ruby’). *Plant Soil* 1988, 107, 219–225. [CrossRef]

34.  Dent, D.; Cocking, E.C. Establishing symbiotic nitrogen fixation in cereals and other non-legume crops: The Greener Nitrogen Revolution. *Agric. Food Secur.* 2017, 6, 7. [CrossRef]

35.  Collavino, M.M.; Cabrera, E.V.R.; Bruno, C.; Aguilar, O.M. Effect of soil chemical fertilization on the diversity and composition of the tomato endophytic diazotrophic community at different stages of growth. *Braz. J. Microbiol.* 2020, 51, 1965–1975. [CrossRef] [PubMed]

36.  Yuksel, B.; Memon, A.R. Comparative phylogenetic analysis of small GTP-binding genes of model legume plants and assessment of their roles in root nodules. *J. Exp. Bot.* 2008, 59, 3831–3844. [CrossRef] [PubMed]

37.  Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 1997, 25, 3389–3402. [CrossRef] [PubMed]

38.  Sievers, F.; Wilm, A.; Dineen, D.; Gibson, T.J.; Karplus, K.; Li, W.; Lopez, R.; McWilliam, H.; Remmert, M.; Söding, J.; et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* 2011, 7, 539. [CrossRef] [PubMed]

39.  Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* 2016, 33, 1870–1874. [CrossRef] [PubMed]

40.  Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 1987, 4, 406–425. [CrossRef]

41.  New, A.; Kumar, S. Molecular Evolution and Phylogenetics *Genet. Res.* 2001, 77, 117–120. [CrossRef]

42.  Biasini, M.; Bienert, S.; Waterhouse, A.; Arnold, K.; Studer, G.; Schmidt, T.; Kiefer, F.; Cassarino, T.G.; Bertoni, M.; Bordoli, L.; et al. SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res.* 2014, 42, W252–W258. [CrossRef]

43.  Klepikova, A.V.; Kasianov, A.S.; Gerasimov, E.S.; Logacheva, M.D.; Penin, A.A. A high resolution map of the *Arabidopsis thaliana* developmental transcriptome based on RNA-seq profiling. *Plant J.* 2016, 88, 1058–1070. [CrossRef]

44.  Secco, D.; Jabouineau, M.; Walker, H.; Shou, H.; Wu, P.; Poirier, Y.; Whelan, J. Spatio-Temporal Transcript Profiling of Rice Roots and Shoots in Response to Phosphate Starvation and Recovery. *Plant Cell* 2013, 25, 4285–4304. [CrossRef]

45.  Sekhon, R.S.; Briskine, R.; Hirsch, C.N.; Myers, C.L.; Springer, N.M.; Buell, C.R.; De Leon, N.; Kaeppeler, S. Maize Gene Atlas Developed by RNA Sequencing and Comparative Evaluation of Transcriptomes Based on RNA Sequencing and Microarrays. *PLoS ONE* 2013, 8, e61005. [CrossRef]

46.  Benedito, V.A.; Torres-Jerez, I.; Murray, J.; Andriankaja, A.; Allen, S.; Kakar, K.; Wandrey, M.; Verdier, J.; Zuber, H.; Ott, T.; et al. A gene expression atlas of the model legume *Medicago truncatula*. *Plant J.* 2008, 55, 504–513. [CrossRef] [PubMed]

47.  Høgslund, N.; Radutoiu, S.; Krusell, L.; Voroshilova, V.; Hannah, M.A.; Goffard, N.; Sanchez, D.H.; Lippold, F.; Ott, T.; Sato, S.; et al. Dissection of Symbiosis and Organ Development by Integrated Transcriptome Analysis of *Lotus japonicus* Mutant and Wild-Type Plants. *PLoS ONE* 2009, 4, e6556. [CrossRef] [PubMed]
48. O’Rourke, J.A.; Iniguez, L.P.; Fu, F.; Bucciarelli, B.; Miller, S.S.; A Jackson, S.; E McClean, P.; Li, J.; Dai, X.; Zhao, P.X.; et al. An RNA-Seq based gene expression atlas of the common bean. *BMC Genom.* 2014, 15, 1–17. [CrossRef] [PubMed]

49. Severin, A.J.; Woody, J.L.; Bolon, Y.-T.; Joseph, B.; Diers, B.W.; Farmer, A.D.; Muehlbauer, G.J.; Nelson, R.T.; Grant, D.; Specht, J.E.; et al. RNA-Seq Atlas of Glycine max: A guide to the soybean transcriptome. *BMC Plant Biol.* 2010, 10, 160. [CrossRef]

50. Trapnell, C.; Roberts, A.; Goff, L.; Pertea, G.; Kim, D.; Kelley, D.R.; Pimentel, H.; Salzberg, S.L.; Rinn, J.L.; Pachter, L. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protoc.* 2012, 7, 562–578. [CrossRef] [PubMed]