

Characterization of the transcriptional responses of *Armillaria gallica* 012m to GA3

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Abstract

Gastrodia elata needs to establish a symbiotic relationship with *Armillaria* strains in order to obtain nutrients and energy. However, the signaling crosstalk between *G. elata* and *Armillaria* strains is still unclear. During our experiment, we found that the vegetative mycelium of *Armillaria gallica* 012m grew significantly better in the media containing gibberellic acid (GA3) than the blank control group (BK). In order to explore the response mechanism, we performed an RNA-sequencing experiment to profile the transcriptome changes of *A. gallica* 012m cultured in the medium with exogenous GA3. The transcriptome-guided differential expression genes (DEGs) analysis of GA3 and BK showed that a total of 1309 genes were differentially expressed, including 361 upregulated genes and 948 downregulated genes. Some of those DEGs correlated with biological process, including positive regulation of chromosome segregation, mitotic metaphase/anaphase transition, attachment of mitotic spindle microtubules to kinetochore, mitotic cytokinesis, and nuclear division. These analyses explained that GA3 actively promoted the growth of *A. gallica* to some extent.

Further analysis of protein domain features showed that deduced polypeptide contained 49 candidate genes of GA receptor, and 28 of them expressed in our samples. We speculate that GA receptors exist in *A. gallica* 012m. Comparative studies of proteins showed that the postulated GA receptor domains of *A. gallica* 012m have a higher homologous correlation with fungi than others based on cluster analysis.

1. Introduction

Gastrodia elata Blume is commonly called *Tian ma* in Chinese and is primarily distributed in mountainous areas of East Asia, Southeast Asia and Oceania (Ahn, Jeon, Lim, Jung, & Park, 2007; Chen, Ji, & Zhu, 1999; Liu et al., 2015; Tang & Eisenbrand, 1992; Zhan et al., 2016). Modern pharmacological experiments have demonstrated that extracts of *G. elata* or its active compounds possess wide-reaching biological activities, including antitumor, anti-virus, memory-improving, antioxidation and anti-aging actions (Heo et al., 2007; Hu & Shen, 2014; Huang & Wang, 1985). As a perennial, non-photosynthetic orchid, *G. elata* needs to establish a symbiotic relationship with *Armillaria* strains at late stages to obtain nutrients and energy (Chen et al., 2020; Lan & Li, 1994; Wang, Yang, Zeng, & Hu, 2007; Zou, Bai, Lv, Xu, & Sun, 2010). The species of genus *Armillaria* were well known as phytopathogens that caused serious root rot disease on natural forests, tree plantations for timber production, orchards, vineyards, and gardens (Heinzelmann et al., 2019; Koch, Wilson, Sense, Henkel, & Aime, 2017; Labbé et al., 2017; Onsando, Wargo, & Waud, 1997). However, several species of *Armillaria* had been identified to be able to form symbiont with *G. elata* (Cha & Igarashi, 1995; Cha & Igarashi, 1996; Guo, Wang, Xue, Zhao, & Yang, 2016; Sekizaki et al., 2008), and affect both the yields and the quality of the tubers of *Tian ma* (Guo et al., 2016).

It has been known for many years that phytohormones and their signaling network play a central role on the regulation of plant growth and development during plant life cycle, recent research revealed that these signaling pathways are probably present in nonplant species (Bari & Jones, 2009; Grant & Jones, 2009). Various microbes can produce or affect the phytohormones levels of their hosts, furthermore, auxins,

cytokinins(CKs), GAs, abscisic acid (ABA) and ethylene (ET) had been isolated from microbial fermentation (Chanclud & Morel, 2016; R. Eichmann et al., 2021; Ludwig-Müller, 2015). On the other side, many works disclosed that host hormones played an important role in plant-microbe interaction. IAA had been proved to be able to induced production of invasive filaments in *Saccharomyces cerevisiae* (Prusty et al., 2004), and increased the antimicrobial activity of *Streptomycetaceae* strains isolated from *Arabidopsis* roots (van der Meij et al., 2018). CKs improves the progression of fungal biotrophic pathogens and stimulate beneficial AMF interactions in pea (Chanclud & Morel, 2016). Exogenous ABA induced vast gene expression changes in the endophyte *Aspergillus nidulans* (Xu, Yang, Meng, & Wang, 2018). GAs inhibits the nodulation and AMF formation, while BRs seem to support it (Foo, Mcadam, Weller, & Reid, 2016; Foo, Ross, Jones, & Reid, 2013).

Historically, GA was primarily known as a prominent type of phytohormone that regulate many aspects of plant development and physiology (de Lucas et al., 2008; Olszewski, Sun, & Gubler, 2002). Although GAs biosynthesis by fungi has been documented and reviewed, few research focused on the signaling pathways of GAs in fungi (Hedden, 2020). Through transcriptome analysis and phytohormone quantification, Takeda et al. revealed the accumulation of GA in Japanese lotus root lines infected with AM fungi, indicating the importance of de novo synthesis of GA in arbuscular mycorrhizal development (Takeda et al., 2015). It was reported that exogenous GA3 significantly increased hyphopodium formation at the epidermis, thus leading to the promotion of fungal colonization and arbuscule formation in the root cortex (Tominaga et al., 2020). Furthermore, GAs could promote the germination of conidia and the growth of the mycelium of *Neurospora crassa* (Barker & Tagu, 2000). However, no specific studies have been carried out at the transcriptome level to clarify the responses of *Armillaria* starin to GA3 and how they relate to the symbiotic relationships.

Some research works have investigated the mechanisms of its symbiotic relationship with *G. elata* (Li, Guo, & Lee, 2020; Tsai et al., 2016). Yuan et al. observed that the strigolactone, as a phytohormone, could promote the branching and development of *Armillaria mellea* hyphae (Yuan et al., 2018). However, there is still limited knowledge on the symbiotic molecular mechanism between the *Armillaria* strain and *G. elata*. In previous works, we sequenced the genome of *A. gallica* 012m isolated from the tuber of *G. elata* collected from a plantation field, and investigated the symbiotic relationship with *G. elata* (Zhan et al., 2020). During the experiment, we found that the vegetative mycelium of *A. gallica* 012m grew significantly better in the media containing GA3 than the control. The experimental phenomenon implied us that GA3 might be an important signal molecule for *A. gallica* 012m. Here, we show the genome-guided analysis of differential expression between additional GA3 and BK. These studies provide a foundation to characterize GA3 signaling in *A. gallica* 012m.

2. Materials & Methods

2.1. Sampling of *A. gallica* 012m.

In previous study, we isolated *A. gallica* 012m from *G. elata* tubers in 2017 (Zhan et al., 2020). In this experiment, we activated the mycelium of *A. gallica* 012m with a modified Czapek-Dox medium with an addition of 2 mg/L GAs or not and incubated at 25 °C for 15 d in the dark (Zhan et al., 2020). The fresh mycelium was placed into RNA later, stored at room temperature for 24 h, then moved to -20 °C for storage until RNA isolation.

3 pellets from liquid cultivation were inoculated in liquid media of GAs and BK in each bottle. Each medium has 4 parallels and was cultured in a 160 r/min shaker at 25°C for 16 d. After the cultivation was completed, the pellets were collected and weighed by filtration through a Buchner funnel. Using IBM SPSS v23 (Tanner-Smith & Tipton, 2014) software, a paired-sample T-test was performed on the blank group, and the GAs groups were detected to the difference in hormone treatment on the growth of *A. gallica* 012m.

2.2. RNA extraction, library preparation and sequencing.

Total RNA was isolated from the fresh mycelium by using the RNeasy mini kit (Qiagen, Hiden, Germany) following the manufacturer's instructions. The cDNA library was sequenced using the Illumina HiSeq (Caporaso et al., 2012) with a double-end sequencing strategy in Novogene Bioinformatics Technology, Beijing, China. The original data was stored in the National Center for Biotechnology Information (NCBI) database with SRA research registration number PRJNA759758.

2.3. Reads assembly and transcriptomes annotation.

The high-quality clean data of samples were obtained by removing the sequence of adapters or filtering low-quality reads with Trimmomatic v.0.36 (Bolger, Lohse, & Usadel, 2014). Then, the quantification of clean reads was estimated by using FastQC v0.11.9 (Brown, Pirrung, & McCue, 2017). Next, Hisat2 v2.1.0 (Hical Indexing for Spliced Alignment of Transcripts), a sequence alignment software, was used to mapping the chain-specific transcriptome data sequence with *A. gallica* genome to obtain the SAM data. Gene expression levels were calculated using the Reads Per Kilobase per Million mapped reads (RPKM). In order to facilitate the analysis of differentially expressed genes, it was removed that the reads could be compared to multiple loci and had the same optimal comparison score. The read count matrix was obtained by using StringTie v2.1.0 (Pertea et al., 2015) for expression qualification.

2.4. Gene Ontology (GO) functional classification

The most detailed information on gene function was provided by enrichment analyses which facilitated us to screen for unique insights into GA3 responses. The read count matrix was imported into R v3.6.3. The edgeR of R package was used for differential gene analysis, with a FDR < 0.05 and $|\log_2FC| > 1$. Then, for functional annotation and classification, all transcripts and their corresponding genes were compared by eggNoG-mapper (Huerta-Cepas et al., 2017). The enriched GO terms ordered based on the three categories, including biological process (BP), molecular function (MF), and cellular component (CC).

2.5. Prediction and expression of GA3 receptors in *A. gallica* 012m.

To get complete GA receptor members in *A. gallica* 012m, we downloaded the existing GA receptor protein sequence from UniProt(<https://www.uniprot.org/>), compared it with the genome protein sequence of *A. gallica* 012m, selected the genome protein sequence with E-value $\leq 1e^{-5}$ and expressed in the sample, and detected their conserved domain on Pfam(<https://pfam.xfam.org/>).

2.6. Construction of receptor sequence evolutionary tree

We constructed an evolutionary tree including the screened sequences and the downloaded receptor protein sequences. All sequences were imported into MEGA7 (Kumar et al., 2016), and the ClustalW2 was used for multiple amino acid sequence alignment. The phylogenetic tree was constructed by NJ (neighbor-joining) method, and the number of calculations was 1000.

3. Results

3.1. Morphological characteristics of *A. gallica* grown under GA.

It was found that GA3 (2 mg/L) could promote the growth of *A. gallica* 012m in solid medium, as shown in the Fig. 1.

3.2. Effects of plant growth substances on the biomass of *A. gallica* 012m.

For further determination if *A. gallica* is growing and whether or not GA affected the biomass of *A. gallica* 012m. The result showed that the stimulation of GA significantly changed the biomass of *A. gallica* 012m (Fig. 2). In the three hormone-liquid medium, 2 mL/L GA made *Armillaria*'s largest biomass of 0.7741 ± 0.0470 pg/mL. According to the biological expression data of different concentrations and blank control group, we have found that GA3 could increase the biomass of *A. gallica* 012m by $56 \pm 9\%$. Using IBM SPSS v23 software, the result showed that the increase of its biomass was significant (Table S1).

3.3. Evaluation of transcriptome sequencing data.

We obtained the above 10.0 Gb raw data by sequencing on the Illumina HiSeq platform, which could be used for further analysis after quality control and comparative quantification. In addition, six transcriptome samples of GA3 and BK were sequenced by using Illumina HiSeq platform to obtain 27,631,384; 30,479,924; 25,566,599; 29,533,772; 30,365,200 and 26,125,609 pairs of PE reads (Table. 1).

After eliminating the reads with low-quality adapter sequences, an average of 21.916.601 to 28.961.759 clean reading pairs was chosen from GA3 and BK, respectively. Read mapped percentage of clean data from all samples were higher than 91.74%, and read mapped percentages of BK3 were the highest.

Additionally, 88.01%–91.48% of pure readings were successfully mapped to the reference genome of *A. gallica* 012m.

3.4. Enriched GO terms of upregulated and downregulated at DEGs.

In the GA3 vs. BK comparison, a total of 1309 genes were differentially expressed, including 361 up-regulated genes (Supplementary Table. S1) and 948 down-regulated genes (Fig. 3; Supplementary Table. S2). To better visualize the functions and pathways of DEGs obtained from RNA-Seq, we comparing sequences of *A. gallica* 012m with the GO database. The result of the annotation for the GO database was shown in Fig. 4.

In the up-regulated genes, a total of 361 DEGs were categorized into 37 functional groups under CC (9 functional groups), MF (8 functional groups) and BP (20 functional groups). In the CC categorized, the representative GO terms were 'cell' (30 DEGs), 'cell part' (30 DEGs) and 'organelle' (23 DEGs). In the MF categorized, the major terms included 'catalytic activity' (20 DEGs) and 'binding' (12 DEGs). In the BP categorized, the dominant of GO terms were 'cellular process' (26 DEGs), 'metabolic process' (22 DEGs) and 'response to stimulus' (16 DEGs). In the down-regulated genes of GA3 vs. BK comparison, a total of 948 DEGs were categorized into 44 functional groups under CC (12 functional groups), MF (10 functional groups) and BP (22 functional groups). In the CC categorized, the domain GO terms were 'cell part' (215 DEGs), 'organelle part' (144 DEGs) and 'protein-containing complex' (123 DEGs). In the MF categorized, the representative of GO terms were 'catalytic activity' (102 DEGs), 'binding' (122 DEGs) and 'structural molecule activity' (57 DEGs). In the BP categorized, 'metabolic process' (189 DEGs), 'cellular process' (203 DEGs) and 'cellular component organization or biogenesis' (108 DEGs) were the main parts of GO terms. For biological process, interestingly, 'cellular process' was the major part, but 'the biological process' was more important, and the result showed that GO terms of grown gene (GO:004007) has emerged from down-regulation.

3.5. Genes involved in growth of *A. gallica* 012m.

A total of 1309 differential genes were annotated in comparison of GA3 vs. BK, including 361 upregulated genes and 948 downregulated genes. To further explore the mechanism of stimulating the growth of *A. gallica* 012m, we compared the biological growth process of DEGs. The result showed that 68 differential genes were expressed in the upregulated genes, and 50 differential genes were expressed in the downregulated genes. In the upregulated genes, the genes controlling the growth of *A. gallica* 012m mainly involved cell cycle, signal transaction, and cellular protein metabolism, such as positive regulation of chromosome segregation (GO:0051984), positive regulation of mitotic metaphase /anaphase transition (GO:0045842), positive regulation of attachment of mitotic spindle microtubules to the kinetochore (GO:1902425) and so on. In the downregulated genes, the result showed that the significant function was cellular process such as negative regulation of cell cycle (GO:0045786), signaling such as negative regulation of signal transaction (GO:0009968) and development process such as negative

regulation of cell aging (GO:0090344). These phenomena indicated that GA3 could promote the growth of *Armillaria mellea* by stimulating the expression of biological process genes.

3.6. Prediction and expression of GA3 receptors in *A. gallica* 012m.

GAs are natural complex biomolecules initially identified as secondary metabolites in the fungus *Gibberella fujikuroi* with strong implications in plant physiology, and GAs receptors are a class of proteins that play a key role in the grown system. A total of 49 candidate genes containing GA receptor domain were identified, and 28 candidate genes containing GA receptor domain were expressed. Further elucidating gene expression, and analysis on differential expression of individual genes subset to the enriched GO terms can paved the way to observation of clusters of genes containing GA receptor domain which either been activated or attenuated during interaction with GAs. Fold change of gene expression in GA sample compared to control was applied to compute heat-map of selected significantly-expressed ($FDR < 0.05$; $|\log_2(\text{FoldChange})| > 1$) genes as shown in Fig. 5. DEGs in comparison group GA3 vs. BK, almost genes were significantly differential expression. Thus, Exogenous GA3 can stimulate the expression of candidate receptor genes in *A. gallica* 012m.

3.7. Genes containing GA receptor domain in *A. gallica* 012m.

We downloaded the sequence information of GA receptors in fungi, *peanut*, *soybean* and *rice* from GenBank (Supplementary Table. S3) with a total of 52 sequences. Finally, by comparing the 52 genes with GA receptor domain and *A. gallica* 012m, an analysis of the phylogenetic relationship is shown in Fig. 6. The result showed that *A. gallica* 012m (Armga012mGene20317) not only had homology with *Valsa mali* var. *pyri* (GenBank: KUI53266.1) but also had a high homology correlation with *Colletotrichum gloeosporioides* (GenBank: KAF3800814.1). The phylogenetic tree analysis inferred the results that *A. gallica* 012m might possess GA receptor domains.

4. Discussion

Plant-microbe interaction is essential to the balance in an ecosystem compared to the other microbial interaction (Sharma, Sudheer, Usmani, Rani, & Gupta, 2020). Plants and microorganisms have coexisted and coevolved for millions of years, during which time both plants and microorganisms have been exposed to many signaling molecules produced and released by each other (Eichmann, Richards, & Schafer, 2021). During our work on *A. gallica* culture, we attempted to investigate the responses of *A. gallica* to GA3. The results show that rhizomorph production of *A. gallica* was simulated significantly. In another coincidental research, Jacobs et al. had reported that the rhizomorph production of *A. gallica* had been seriously inhibited by paclobutrazol (PBZ) known as a GA inhibitor. Their experiments shown that only 4% of colonies growing in PBZ plates formed rhizomorphs compared to 100% of colonies in control

plates (Jacobs & Berg, 2000). Those results suggest that GA3 might influence the rhizomorph production of *A. gallica* as a signaling molecule.

The DEGs analysis of GA3 vs. BK showed that there were 361 up-regulated genes and 948 down-regulated genes. In addition, enrichment analyses of GO terms were performed for DEGs using EggNoG-mapper. One interesting finding that the genes regulating growth showed up-regulated expression in the GO analysis of differentially expressed genes, such as “regulation of biological process” “positive regulation of biological process” and “biological process”. These analyses explained that GA3 actively promotes the growth of *A. gallica* to some extent.

Increased analysis of signaling pathway revealed that the GA receptor members in *A. gallica* 012m were predicted in its genome and RNA-Seq based on BLASTp search with the full-length GA receptor protein sequence from UniProt. The results showed that candidate genes containing GA receptor domain of *A. gallica* 012m has better homologous correlation with those of fungi than plants. Interestingly, more and more phytohormones receptors, including bacteria, fungi, algae, animal and etc (Bodrato et al., 2009; Frebortova & Frebort, 2021; Galindo-Trigo, Gray, & Smith, 2016; Herivaux et al., 2017; Jiang & Guo, 2010; Ju et al., 2015; Lenasi, Bavec, & Zorko, 2002; Samanovic et al., 2018; Zwanenburg & Pospisil, 2013). had been predicted based on specific responses and gene sequences in non-plant species. Wang etc. had identified PcrK, a membrane-bound histidine kinase from the phytopathogenic bacterium *Xanthomonas Campestris*, as a bacterial receptor that specifically detects the plant cytokinin 2-isopentenyladenine(2iP) to promote adaptation on oxidative stress (Wang et al., 2017). In another case, SynEtr1 from *Synechocystis* PCC 6803 had been characterized as a functional ethylene receptor (Hernandez-Prieto et al., 2012; Klahn et al., 2015; Lacey & Binder, 2016; Narikawa et al., 2011; Ramakrishnan & Tabor, 2016; Song et al., 2011). Moreover, some CKs and ET receptor homologs were identified in zooxanthellae, diatoms, chromerids, free-living pseudofungus, filamentous marine protists, and brown algae (Bidon et al., 2020; Kabbara et al., 2019; Papon & Binder, 2019). In this point of view, phytohormones as inter-kingdom signaling molecules could influence several non-plant species and the receptors could exist in those species. Taken together, we speculate that GA could be a type of signaling molecules and there may be some receptors in *A. gallica* 012m.

5. Conclusions

In summary, GA3 improved the mycelium growth of *A. gallica* 012m significantly in both solid and liquid media. Several DEGs identified through RNA-seq analysis were related to biological process, including “regulation of biological process”, “positive regulation of biological process”, and “biological process”. These analyses explained that GA3 actively promote the growth of *A. gallica* to some extent. In this study, analysis of protein domain features showed that deduced polypeptide contained 49 candidate genes of GA receptor domain. Comparative studies of proteins showed that the postulated GA receptor domains of *A. gallica* 012m have a higher homologous correlation with fungi than others based on cluster analysis. We speculate that GA receptors exist in *A. gallica* 012m.

Declarations

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Competing Interests

The authors declare there are no competing interests, and the manuscript was approved by all authors for publication.

Data Availability

The following information was supplied regarding data availability:

Data is available at SRA: PRJNA759758.

Statement

We hereby certify that our research hasn't involved human participants and/or animal.

References

1. Ahn, E. K., Jeon, H. J., Lim, E. J., Jung, H. J., & Park, E. H. (2007). Anti-inflammatory and anti-angiogenic activities of *Gastrodia elata* Blume. *J Ethnopharmacol*, 110(3), 476-482. <https://doi.org/10.1016/j.jep.2006.10.006>
2. Bari, R., & Jones, J. D. (2009). Role of plant hormones in plant defence responses. *Plant Mol Biol*, 69(4), 473-488. <https://doi.org/10.1007/s11103-008-9435-0>
3. Barker, S. J., & Tagu, D. (2000). The Roles of Auxins and Cytokinins in Mycorrhizal Symbioses. *J Plant Growth Regul*, 19(2), 144-154. <https://doi.org/10.1007/s003440000021>
4. Bidon, B., Kabbara, S., Courdavault, V., Glevarec, G., Oudin, A., Hericourt, F., Carpin, S., Spichal, L., Binder, B. M., Cock, J. M., & Papon, N. (2020). Cytokinin and Ethylene Cell Signaling Pathways from Prokaryotes to Eukaryotes. *CELLS*, 9(11). <https://doi.org/10.3390/cells9112526>
5. Bodrato, N., Franco, L., Fresia, C., Guida, L., Usai, C., Salis, A., Moreschi, I., Ferraris, C., Verderio, C., Basile, G., Bruzzone, S., Scarfi, S., De Flora, A., & Zocchi, E. (2009). Absciscic Acid Activates the Murine

- Microglial Cell Line N9 through the Second Messenger Cyclic ADP-ribose. *JOURNAL OF BIOLOGICAL CHEMISTRY*, 284(22), 14777-14787. <https://doi.org/10.1074/jbc.M802604200>
6. Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114-2120. <https://doi.org/10.1093/bioinformatics/btu170>
 7. Brown, J., Pirrung, M., & McCue, L. A. (2017). FQC Dashboard: integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. *Bioinformatics*, 33(19), 3137-3139. <https://doi.org/10.1093/bioinformatics/btx373>
 8. Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J*, 6(8), 1621-1624. <https://doi.org/10.1038/ismej.2012.8>
 9. Cha, J. Y., & Igarashi, T. (1995). Armillaria species associated with *Gastrodia elata* in Japan [<https://doi.org/10.1111/j.1439-0329.1995.tb01347.x>]. *European Journal of Forest Pathology*, 25(6-7), 319-326. <https://doi.org/https://doi.org/10.1111/j.1439-0329.1995.tb01347.x>
 10. Cha, J. Y., & Igarashi, T. (1996). *Armillaria jezoensis*, a new symbiont of *Galeola septentrionalis* (Orchidaceae) in Hokkaido. *Mycoscience*, 37(1), 21-24. <https://doi.org/https://doi.org/10.1007/BF02461451>
 11. Chanclud, E., & Morel, J. B. (2016). Plant hormones: a fungal point of view. *Mol Plant Pathol*, 17(8), 1289-1297. <https://doi.org/10.1111/mpp.12393>
 12. Chen, S., Wang, X., Wang, Y., Zhang, G., Song, W., Dong, X., Arnold, M. L., Wang, W., Miao, J., Chen, W., & Dong, Y. (2020). Improved de novo Assembly of the Achlorophyllous Orchid *Gastrodia elata*. *Front Genet*, 11, 580568. <https://doi.org/10.3389/fgene.2020.580568>
 13. Chen, X., Ji, Z., & Zhu, G. (1999). Notes on some subtribes of the Orchidaceae. *Acta Phytotaxonomica Sinica*, 37(2), 113-116. <http://europepmc.org/abstract/CBA/326380>
 14. de Lucas, M., Daviere, J. M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J. M., Lorrain, S., Fankhauser, C., Blazquez, M. A., Titarenko, E., & Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. *Nature*, 451(7177), 480-484. <https://doi.org/10.1038/nature06520>
 15. Eichmann, R., Richards, L., & Schaefer, P. (2021). Hormones as go-betweens in plant microbiome assembly. *PLANT JOURNAL*, 105(2), 518-541. <https://doi.org/10.1111/tpj.15135>
 16. Foo, E., McAdam, E. L., Weller, J. L., & Reid, J. B. (2016). Interactions between ethylene, gibberellins, and brassinosteroids in the development of rhizobial and mycorrhizal symbioses of pea. *J Exp Bot*, 67(8), 2413-2424. <https://doi.org/10.1093/jxb/erw047>
 17. Foo, E., Ross, J. J., Jones, W. T., & Reid, J. B. (2013). Plant hormones in arbuscular mycorrhizal symbioses: an emerging role for gibberellins. *Ann Bot*, 111(5), 769-779. <https://doi.org/10.1093/aob/mct041>
 18. Frebortova, J., & Frebort, I. (2021). Biochemical and Structural Aspects of Cytokinin Biosynthesis and Degradation in Bacteria. *Microorganisms*, 9(6). <https://doi.org/10.3390/microorganisms9061314>

19. Galindo-Trigo, S., Gray, J. E., & Smith, L. M. (2016). Conserved Roles of CrRLK1L Receptor-Like Kinases in Cell Expansion and Reproduction from Algae to Angiosperms. *Front Plant Sci*, 7. <https://doi.org/10.3389/fpls.2015.01260>
20. Grant, M. R., & Jones, J. D. (2009). Hormone (dis)harmony moulds plant health and disease. *Science*, 324(5928), 750-752. <https://doi.org/10.1126/science.1173771>
21. Guo, T., Wang, H. C., Xue, W. Q., Zhao, J., & Yang, Z. L. (2016). Phylogenetic Analyses of Armillaria Reveal at Least 15 Phylogenetic Lineages in China, Seven of Which Are Associated with Cultivated *Gastrodia elata*. *PLoS One*, 11(5), e0154794. <https://doi.org/10.1371/journal.pone.0154794>
22. Hedden, P. (2020). The Current Status of Research on Gibberellin Biosynthesis. *Plant Cell Physiol*, 61(11), 1832-1849. <https://doi.org/10.1093/pcp/pcaa092>
23. Heinzelmann, R., Dutech, C., Tsykun, T., Labbé, F., Soularue, J.-P., & Prospero, S. (2019). Latest advances and future perspectives in Armillaria research. *Canadian Journal of Plant Pathology*, 41(1), 1-23. <https://doi.org/10.1080/07060661.2018.1558284>
24. Heo, J. C., Woo, S. U., Son, M., Park, J. Y., Choi, W. S., Chang, K. T., Kim, S. U., Yoon, E. K., Kim, Y. H., Shin, H. M., & Lee, S. H. (2007). Anti-tumor activity of *Gastrodia elata* Blume is closely associated with a GTP-Ras-dependent pathway. *Oncol Rep*, 18(4), 849-853.
25. Herivaux, A., de Bernonville, T. D., Roux, C., Clastre, M., Courdavault, V., Gastebois, A., Bouchara, J. P., James, T. Y., Latge, J. P., Martin, F., & Papon, N. (2017). The Identification of Phytohormone Receptor Homologs in Early Diverging Fungi Suggests a Role for Plant Sensing in Land Colonization by Fungi. *mBio*, 8(1). <https://doi.org/10.1128/mBio.01739-16>
26. Hernandez-Prieto, M. A., Schon, V., Georg, J., Barreira, L., Varela, J., Hess, W. R., & Futschik, M. E. (2012). Iron Deprivation in Synechocystis: Inference of Pathways, Non-coding RNAs, and Regulatory Elements from Comprehensive Expression Profiling. *G3-GENES GENOMES GENETICS*, 2(12), 1475-1495. <https://doi.org/10.1534/g3.112.003863>
27. Hu, Y., Li, C., & Shen, W. (2014). Gastrodin alleviates memory deficits and reduces neuropathology in a mouse model of Alzheimer's disease. *Neuropathology*, 34(4), 370-377. <https://doi.org/10.1111/neup.12115>
28. Huang, J. H., & Wang, G. L. (1985). [Studies on some pharmacological effects of gastrodia injection and gastrodin]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*, 7(5), 399-402.
29. Huerta-Cepas, J., Forslund, K., Coelho, L. P., Szklarczyk, D., Jensen, L. J., von Mering, C., & Bork, P. (2017). Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. *Molecular Biology and Evolution*, 34(8), 2115-2122. <https://doi.org/10.1093/molbev/msx148>
30. Jacobs, K. A., & Berg, L. C. (2000). Inhibition of fungal pathogens of woody plants by the plant growth regulator paclobutrazol [[https://doi.org/10.1002/\(SICI\)1526-4998\(200005\)56:5<407::AID-PS139>3.0.CO;2-T](https://doi.org/10.1002/(SICI)1526-4998(200005)56:5<407::AID-PS139>3.0.CO;2-T)]. *Pest Management Science*, 56(5), 407-412. [https://doi.org/https://doi.org/10.1002/\(SICI\)1526-4998\(200005\)56:5<407::AID-PS139>3.0.CO;2-T](https://doi.org/https://doi.org/10.1002/(SICI)1526-4998(200005)56:5<407::AID-PS139>3.0.CO;2-T)

31. Jiang, Z. Q., & Guo, H. W. (2010). A comparative genomic analysis of plant hormone related genes in different species. *Journal of Genetics and Genomics*, 37(4), 219-230. [https://doi.org/10.1016/S1673-8527\(09\)60040-0](https://doi.org/10.1016/S1673-8527(09)60040-0)
32. Ju, C. L., Van de Poel, B., Cooper, E. D., Thierer, J. H., Gibbons, T. R., Delwiche, C. F., & Chang, C. R. (2015). Conservation of ethylene as a plant hormone over 450 million years of evolution. *NATURE PLANTS*, 1(1). <https://doi.org/10.1038/NPLANTS.2014.4>
33. Kabbara, S., Herivaux, A., de Bernonville, T. D., Courdavault, V., Clastre, M., Gastebois, A., Osman, M., Hamze, M., Cock, J. M., Schaap, P., & Papon, N. (2019). Diversity and Evolution of Sensor Histidine Kinases in Eukaryotes. *GENOME BIOLOGY AND EVOLUTION*, 11(1), 86-108. <https://doi.org/10.1093/gbe/evy213>
34. Klahn, S., Orf, I., Schwarz, D., Matthiessen, J. K. F., Kopka, J., Hess, W. R., & Hagemann, M. (2015). Integrated Transcriptomic and Metabolomic Characterization of the Low-Carbon Response Using an *ndhR* Mutant of *Synechocystis* sp PCC 6803. *PLANT PHYSIOLOGY*, 169(3), 1540-1556. <https://doi.org/10.1104/pp.114.254045>
35. Koch, R. A., Wilson, A. W., Sene, O., Henkel, T. W., & Aime, M. C. (2017). Resolved phylogeny and biogeography of the root pathogen *Armillaria* and its gasteroid relative, *Guyanagaster*. *BMC Evol Biol*, 17(1), 33. <https://doi.org/10.1186/s12862-017-0877-3>
36. Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7), 1870-1874. <https://doi.org/10.1093/molbev/msw054>
37. Labbé, F., Lung-Escarmant, B., Fievet, V., Soularue, J.-P., Laurent, C., Robin, C., & Dutech, C. (2017). Variation in traits associated with parasitism and saprotrophism in a fungal root-rot pathogen invading intensive pine plantations. *Fungal Ecology*, 26, 99-108. <https://doi.org/https://doi.org/10.1016/j.funeco.2017.01.001>
38. Lacey, R. F., & Binder, B. M. (2016). Ethylene Regulates the Physiology of the Cyanobacterium *Synechocystis* sp PCC 6803 via an Ethylene Receptor. *PLANT PHYSIOLOGY*, 171(4), 2798-2809. <https://doi.org/10.1104/pp.16.00602>
39. Lan, J., Xu, J., & Li, J. (1994). Study on symbiotic relation between *Gastrodia elata* and *Armillariella mellea* by autoradiography. *Acta Mycologica Sinica*, 13(3), 219-222. <http://europepmc.org/abstract/CBA/266003>
40. Lenasi, H., Bavec, A., & Zorko, M. (2002). Membrane-bound progesterone receptors coupled to G proteins in the fungus *Rhizopus nigricans*. *FEMS MICROBIOLOGY LETTERS*, 213(1), 97-101. [https://doi.org/10.1016/S0378-1097\(02\)00790-5](https://doi.org/10.1016/S0378-1097(02)00790-5)
41. Li, Y. Y., Guo, S. X., & Lee, Y. I. (2020). Ultrastructural changes during the symbiotic seed germination of *Gastrodia elata* with fungi, with emphasis on the fungal colonization region. *BOTANICAL STUDIES*, 61(1). <https://doi.org/10.1186/s40529-019-0280-z>
42. Liu, T., Li, C. M., Han, Y. L., Chiang, T. Y., Chiang, Y. C., & Sung, H. M. (2015). Highly diversified fungi are associated with the achlorophyllous orchid *Gastrodia flavilabella*. *BMC Genomics*, 16, 185.

<https://doi.org/10.1186/s12864-015-1422-7>

43. Ludwig-Müller, J. (2015). Bacteria and fungi controlling plant growth by manipulating auxin: balance between development and defense. *J Plant Physiol*, 172, 4-12.
<https://doi.org/10.1016/j.jplph.2014.01.002>
44. Narikawa, R., Suzuki, F., Yoshihara, S., Higashi, S., Watanabe, M., & Ikeuchi, M. (2011). Novel Photosensory Two-Component System (PixA-NixB-NixC) Involved in the Regulation of Positive and Negative Phototaxis of Cyanobacterium *Synechocystis* sp PCC 6803. *Plant and Cell Physiology*, 52(12), 2214-2224. <https://doi.org/10.1093/pcp/pcr155>
45. Olszewski, N., Sun, T. P., & Gubler, F. (2002). Gibberellin signaling: biosynthesis, catabolism, and response pathways. *Plant Cell*, 14 Suppl, S61-80. <https://doi.org/10.1105/tpc.010476>
46. Onsando, J. M., Wargo, P. M., & Waud, S. W. (1997). Distribution, Severity, and Spread of *Armillaria* Root Disease in Kenya Tea Plantations. *Plant Dis*, 81(2), 133-137.
<https://doi.org/10.1094/PDIS.1997.81.2.133>
47. Papon, N., & Binder, B. M. (2019). An Evolutionary Perspective on Ethylene Sensing in Microorganisms. *TRENDS IN MICROBIOLOGY*, 27(3), 193-196.
<https://doi.org/10.1016/j.tim.2018.12.002>
48. Perte, M., Perte, G. M., Antonescu, C. M., Chang, T. C., Mendell, J. T., & Salzberg, S. L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol*, 33(3), 290-295. <https://doi.org/10.1038/nbt.3122>
49. Prusty, R., Grisafi, P., & Fink, G. R. (2004). The plant hormone indoleacetic acid induces invasive growth in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*, 101(12), 4153-4157.
<https://doi.org/10.1073/pnas.0400659101>
50. Ramakrishnan, P., & Tabor, J. J. (2016). Repurposing *Synechocystis* PCC6803 UirS-UirR as a UV-Violet/Green Photoreversible Transcriptional Regulatory Tool in *E. coli*. *ACS SYNTHETIC BIOLOGY*, 5(7), 733-740. <https://doi.org/10.1021/acssynbio.6b00068>
51. Samanovic, M. I., Hsu, H. C., Jones, M. B., Jones, V., McNeil, M. R., Becker, S. H., Jordan, A. T., Strnad, M., Xu, C. C., Jackson, M., Li, H. L., & Darwin, K. H. (2018). Cytokinin Signaling in *Mycobacterium tuberculosis*. *mBio*, 9(3). <https://doi.org/10.1128/mBio.00989-18>
52. Sekizaki, H., Kuninaga, S., Yamamoto, M., Asazu, S. N., Sawa, S., Kojima, M., Yokosawa, R., & Yoshida, N. (2008). Identification of *Armillaria nabsnona* in *Gastrodia* Tubers. *Biological and Pharmaceutical Bulletin*, 31(7), 1410-1414. <https://doi.org/10.1248/bpb.31.1410>
53. Sharma, M., Sudheer, S., Usmani, Z., Rani, R., & Gupta, P. (2020). Deciphering the Omics of Plant-Microbe Interaction: Perspectives and New Insights. *Curr Genomics*, 21(5), 343-362.
<https://doi.org/10.2174/1389202921999200515140420>
54. Song, J. Y., Cho, H. S., Cho, J. I., Jeon, J. S., Lagarias, J. C., & Park, Y. I. (2011). Near-UV cyanobacteriochrome signaling system elicits negative phototaxis in the cyanobacterium *Synechocystis* sp PCC 6803. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA*, 108(26), 10780-10785. <https://doi.org/10.1073/pnas.1104242108>

55. Takeda, N., Handa, Y., Tsuzuki, S., Kojima, M., Sakakibara, H., & Kawaguchi, M. (2015). Gibberellins interfere with symbiosis signaling and gene expression and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. *Plant Physiol*, 167(2), 545-557.
<https://doi.org/10.1104/pp.114.247700>
56. Tang, W., & Eisenbrand, G. (1992). *Gastrodia elata* Bl. In W. Tang & G. Eisenbrand (Eds.), *Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine* (pp. 545-548). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-73739-8_71
57. Tanner-Smith, E. E., & Tipton, E. (2014). Robust variance estimation with dependent effect sizes: practical considerations including a software tutorial in Stata and SPSS. *RESEARCH SYNTHESIS METHODS*, 5(1), 13-30. <https://doi.org/10.1002/jrsm.1091>
58. Tominaga, T., Miura, C., Takeda, N., Kanno, Y., Takemura, Y., Seo, M., Yamato, M., & Kaminaka, H. (2020). Gibberellin Promotes Fungal Entry and Colonization during Paris-Type Arbuscular Mycorrhizal Symbiosis in *Eustoma grandiflorum*. *Plant Cell Physiol*, 61(3), 565-575.
<https://doi.org/10.1093/pcp/pcz222>
59. Tsai, C. C., Wu, K. M., Chiang, T. Y., Huang, C. Y., Chou, C. H., Li, S. J., & Chiang, Y. C. (2016). Comparative transcriptome analysis of *Gastrodia elata* (Orchidaceae) in response to fungus symbiosis to identify gastrodin biosynthesis-related genes. *BMC GENOMICS*, 17.
<https://doi.org/10.1186/s12864-016-2508-6>
60. van der Meij, A., Willemse, J., Schneijderberg, M. A., Geurts, R., Raaijmakers, J. M., & van Wezel, G. P. (2018). Inter- and intracellular colonization of *Arabidopsis* roots by endophytic actinobacteria and the impact of plant hormones on their antimicrobial activity. *Antonie Van Leeuwenhoek*, 111(5), 679-690. <https://doi.org/10.1007/s10482-018-1014-z>
61. Wang, F. F., Cheng, S. T., Wu, Y., Ren, B. Z., & Qian, W. (2017). A Bacterial Receptor PcrK Senses the Plant Hormone Cytokinin to Promote Adaptation to Oxidative Stress. *CELL REPORTS*, 21(10), 2940-2951. <https://doi.org/10.1016/j.celrep.2017.11.017>
62. Wang, H. X., Yang, T., Zeng, Y., & Hu, Z. (2007). Expression analysis of the gastrodianin gene ga4B in an achlorophyllous plant *Gastrodia elata* Bl. *Plant Cell Rep*, 26(3), 253-259.
<https://doi.org/10.1007/s00299-006-0226-2>
63. Xu, G., Yang, S., Meng, L., & Wang, B. G. (2018). The plant hormone abscisic acid regulates the growth and metabolism of endophytic fungus *Aspergillus nidulans*. *Sci Rep*, 8(1), 6504.
<https://doi.org/10.1038/s41598-018-24770-9>
64. Yuan, Y., Jin, X., Liu, J., Zhao, X., Zhou, J., Wang, X., Wang, D., Lai, C., Xu, W., Huang, J., Zha, L., Liu, D., Ma, X., Wang, L., Zhou, M., Jiang, Z., Meng, H., Peng, H., Liang, Y., Li, R., Jiang, C., Zhao, Y., Nan, T., Jin, Y., Zhan, Z., Yang, J., Jiang, W., & Huang, L. (2018). The *Gastrodia elata* genome provides insights into plant adaptation to heterotrophy. *Nat Commun*, 9(1), 1615. <https://doi.org/10.1038/s41467-018-03423-5>
65. Zhan, H. D., Zhou, H. Y., Sui, Y. P., Du, X. L., Wang, W. H., Dai, L., Sui, F., Huo, H. R., & Jiang, T. L. (2016). The rhizome of *Gastrodia elata* Blume - An ethnopharmacological review. *J Ethnopharmacol*, 189,

361-385. <https://doi.org/10.1016/j.jep.2016.06.057>

66. Zhan, M., Tian, M., Wang, W., Li, G., Lu, X., Cai, G., Yang, H., Du, G., & Huang, L. (2020). Draft genomic sequence of *Armillaria gallica* 012m: insights into its symbiotic relationship with *Gastrodia elata*. *Braz J Microbiol*, 51(4), 1539-1552. <https://doi.org/10.1007/s42770-020-00317-x>
67. Zou, N., Bai, X., Lv, J., Yang, J., Xu, G., & Sun, D. (2010). Study on symbiotic mechanism between *Gastrodia elata* Blume and *Armillaria mellea* in tissue culture system. *Medicinal Plant*, 1(10), 91-95.
68. Zwanenburg, B., & Pospisil, T. (2013). Structure and Activity of Strigolactones: New Plant Hormones with a Rich Future. *Mol Plant*, 6(1), 38-62. <https://doi.org/10.1093/mp/sss141>

Tables

Table 1 Summary of sequencing data for 6 samples of GA and BK

<i>Sample_name</i>	Raw_reads	Clean_reads	Read mapped(%)	Total mapped	Multiple mapped	Uniquely mapped
<i>GA1</i>	27,631,384	26,185,401	92.96	25,011,006	860,533	24,150,473
<i>GA2</i>	30,479,924	28,961,759	92.88	27,448,632	1,019,680	26,428,952
<i>GA3</i>	25,566,599	23,467,973	91.74	22,045,007	727,854	21,317,153
<i>BK1</i>	29,533,772	27,395,065	93.06	25,970,049	871,066	25,098,983
<i>BK2</i>	30,365,200	28,784,179	93.24	27,459,809	780,605	26,679,204
<i>BK3</i>	26,125,609	24,916,601	93.27	23,812,273	645,411	23,166,862

Figures

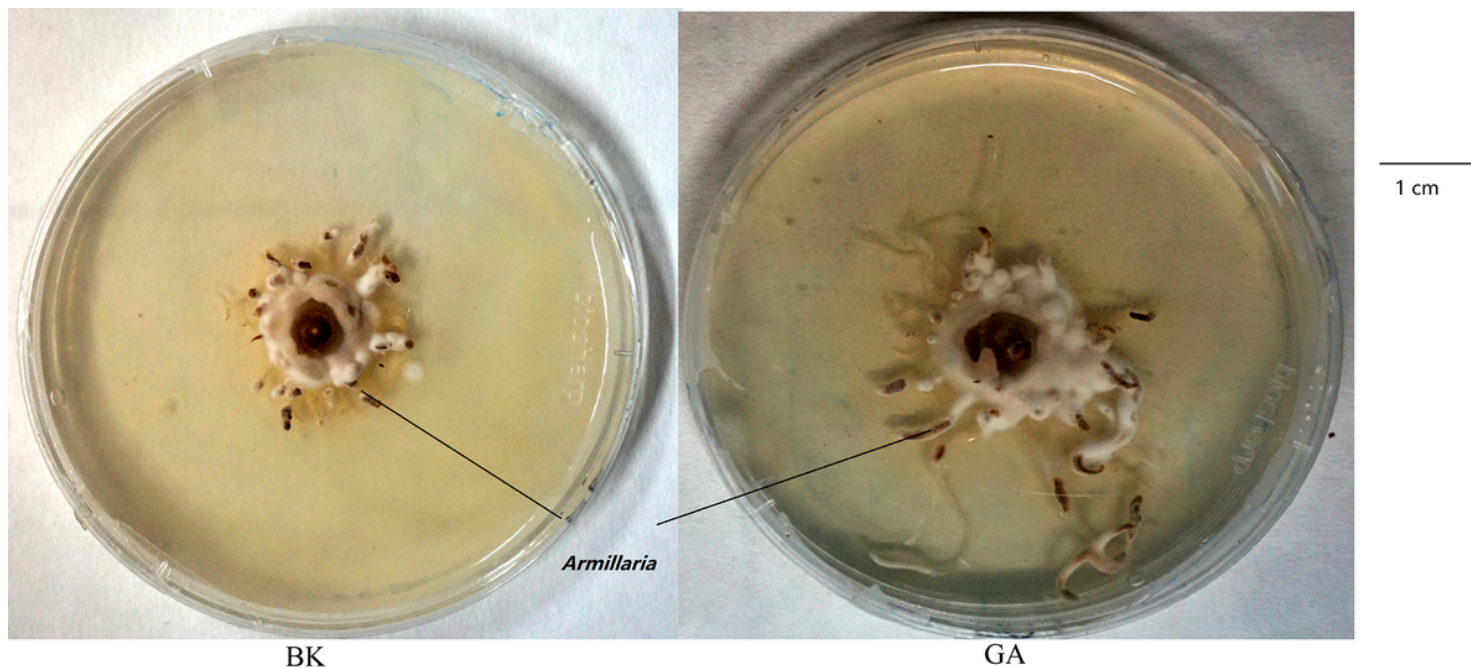


Figure 1

Growth status of *A. gallica* 012m in GA and BK

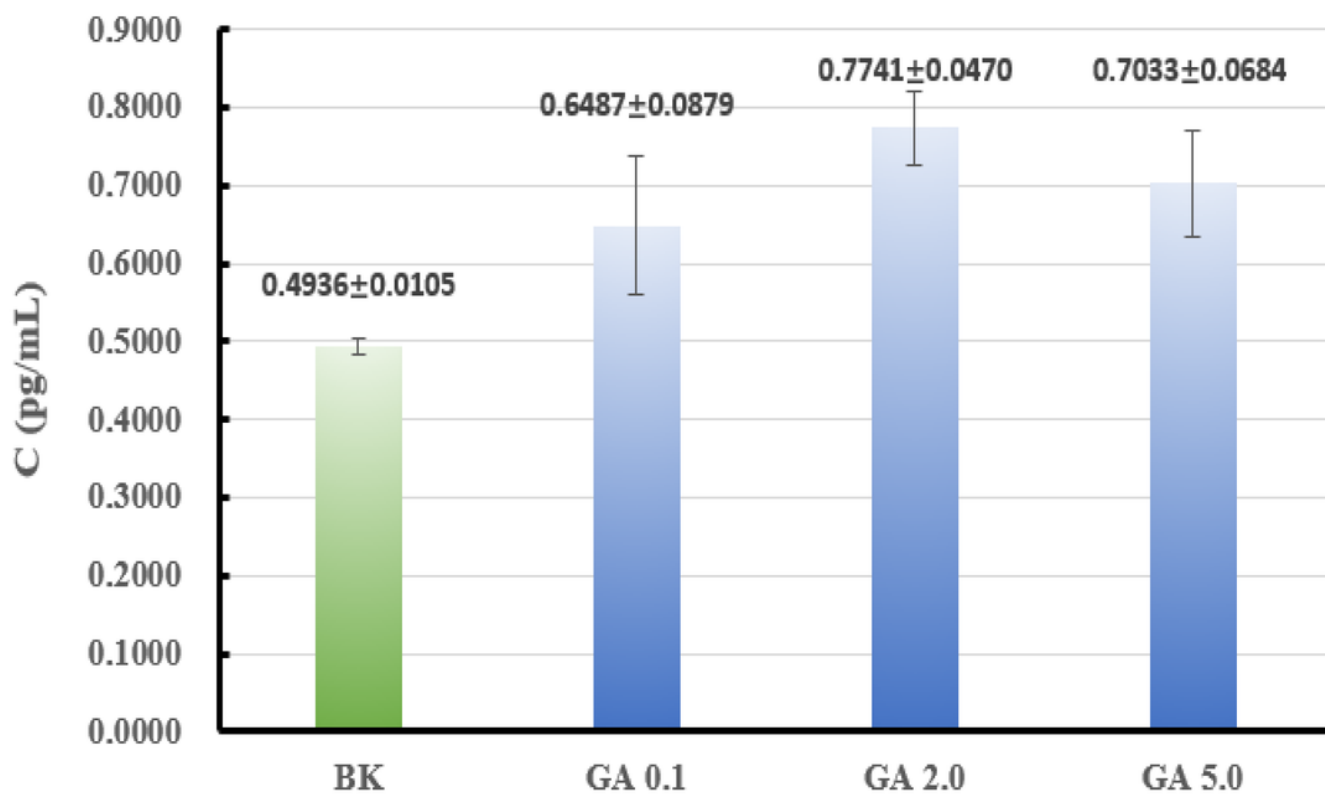


Figure 2

Effects of GAs on biomass of *A. gallica* 012m. Independent sample T for sample data by IBM SPSS

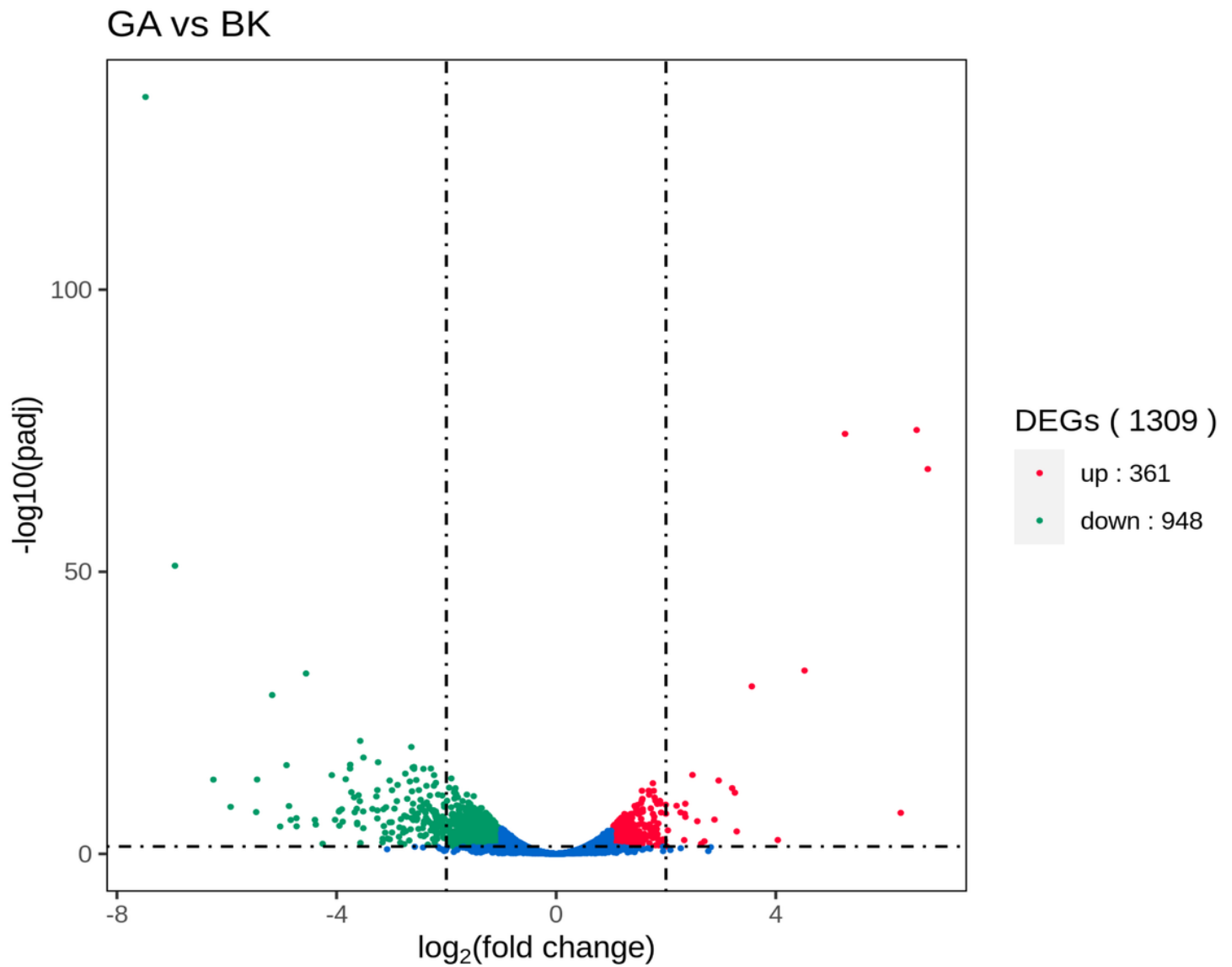


Figure 3

Volcano map of differentially expressed genes. Differentially expressed genes (DEGs) were defined by edgeR with a $\text{FDR} < 0.05$ and $|\log_2\text{FC}| > 1$ and corrected p value (padj) < 0.05

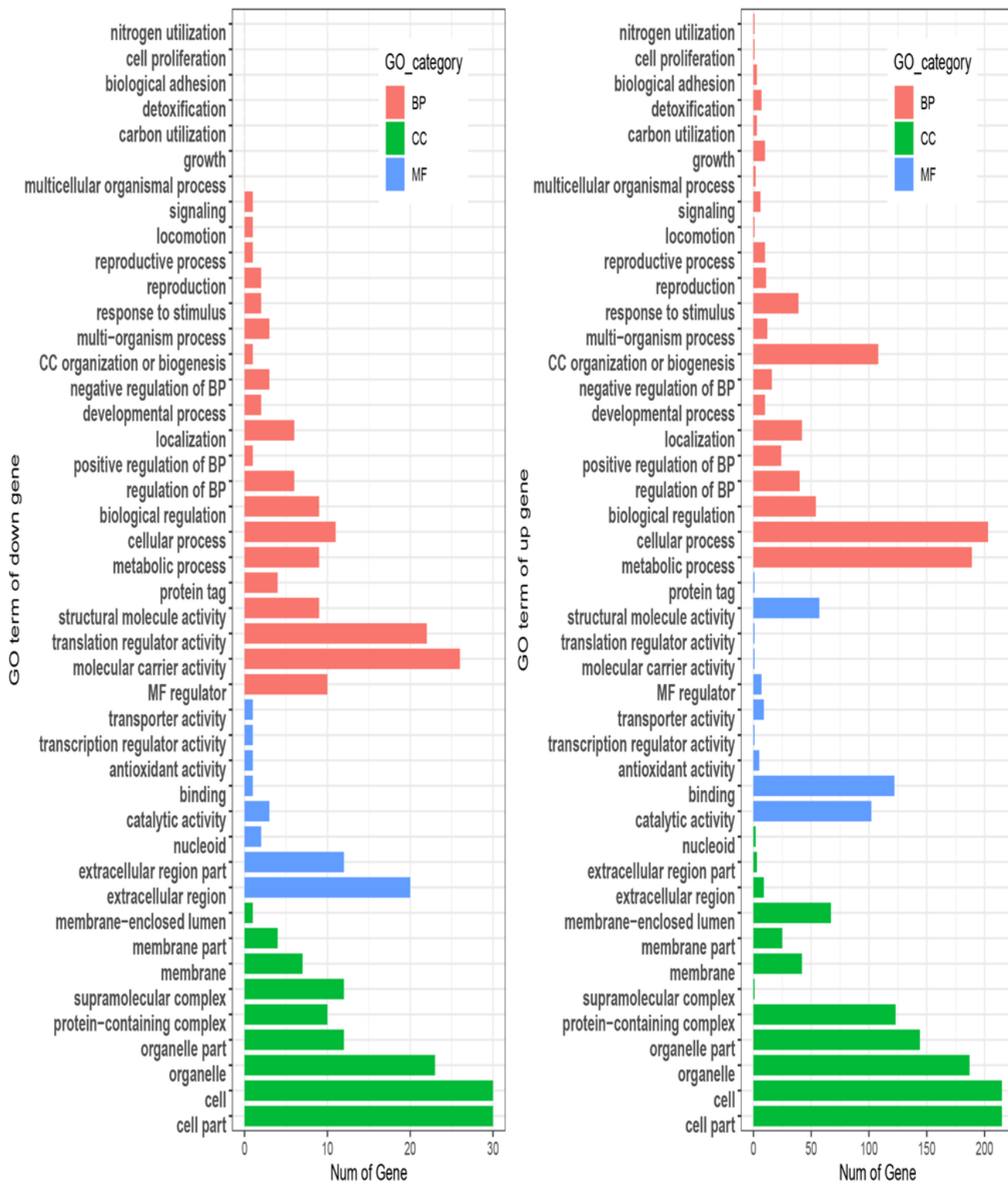


Figure 4

Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs)

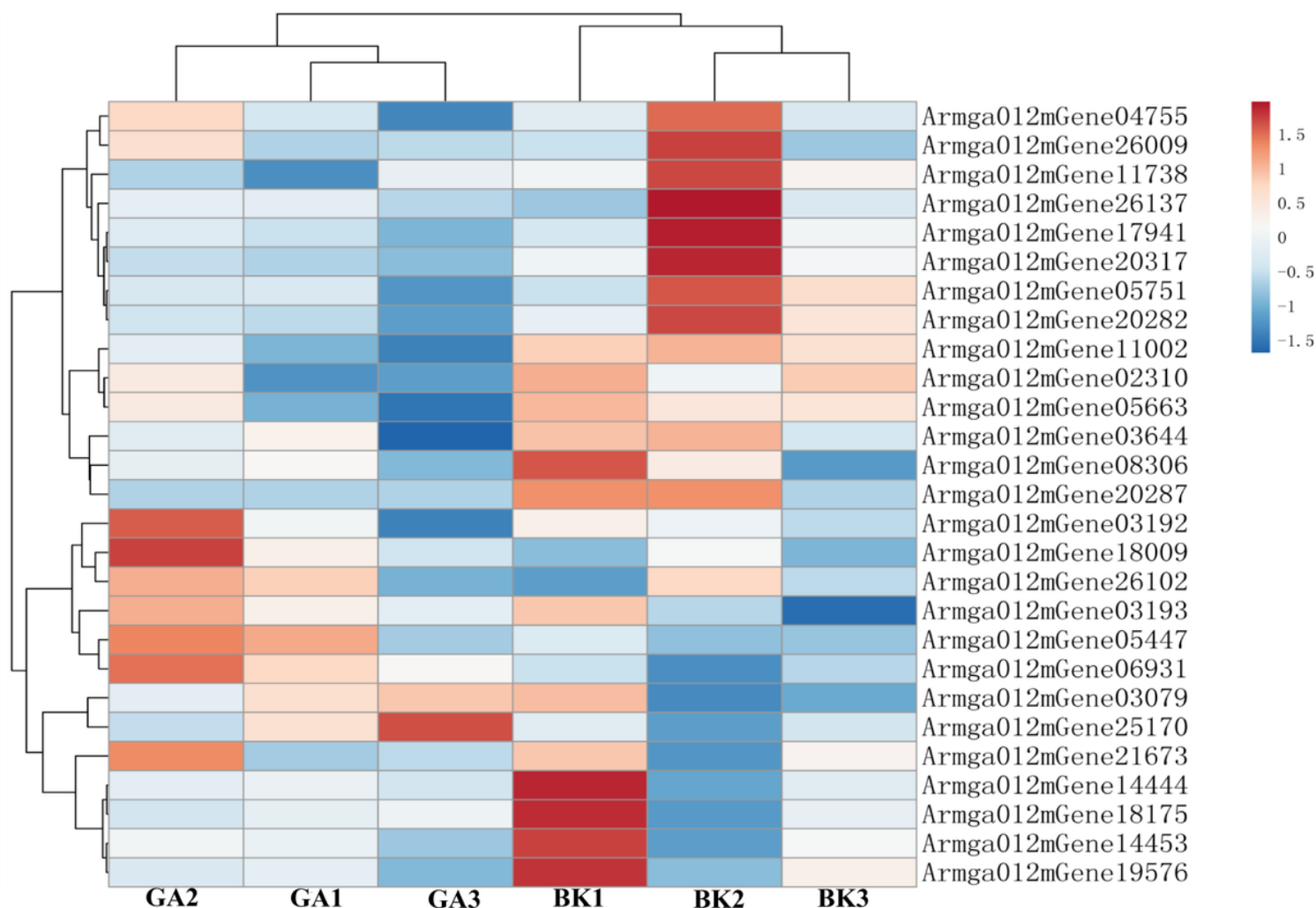


Figure 5

Expression pattern of selected clusters of genes containing GA receptor domain. The color intensity of each gene is based on Log2[fold change] values of genes containing GA receptor domain in BK samples and GA samples

