

In-depth analysis of the genome sequence of the endophytic fungus *Serendipitia indica*

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INTRODUCTION

Due to the high environmental pollution resulting from chemical plant protection and the frequent occurrence of environmental changes, research on biological support for plant growth and protection has been undertaken. In recent years, the sector has focused heavily on the study of bio-compounds to be used as fertilizers (Pertile et al 2021) or as plant protection from pathogens (Dobrek M. et al 2024). Many times, the protagonists of these bio-compounds are organisms that help plants (Plant Growth Promoting Microorganisms (PGPM)). In the last 20 years, the endophytic fungus *Serendipita indica* (anamorphus phase *Piriformospora indica*; Weiß M. et al 2016) has been widely studied, which can bring many benefits to the host plant, including plant growth and yield, increased photosynthesis efficiency, supplying nutrients to plants and strengthening plant tolerance to biotic and abiotic stresses. For this reason, this endophytic fungus is considered an arbuscular mycorrhiza fungus (AMF) due to its behaviour in improving the host characteristics, even if physiologically it is classified as an endophytic fungus because it can be grown on normal culture medium. Thanks to the event of the latest sequencing methodologies, we have been able to identify numerous genes of AMF fungi that are involved in improving the life of the host, while for *S. indica* there is not much information yet about it, the first sequencing is thanks to the work of Zuccaro A. et al. (2011). For this reason, we wanted to perform a Whole Genome Sequencing (WGS) of *S. indica* grown on culture medium and then compare it with *Saccharomycetes cerevisiae* and AMF, as Zhou X. et al. (2021) observed that the 22, 21, and 12 orthologous proteins respectively part of the cAMP-PKA and SNF1, TOR (Nitrogen Sensing and Signaling), and PHO (Phosphate Sensing and Signaling) pathways identified in *S. cerevisiae* are highly conserved in AMF and *S. indica*.

MATERIAL & METHODS

To better understand the role of *S. indica*, we performed Whole Genome Sequencing after cultivation, of the endophyte fungus, on agar medium without the addition of antibiotics. For the sequencing of short reads, we used the Illumina Nextera Flex kit, while for the sequencing of long reads, we used the ONT Rapid Barcoding kit. Subsequently, the raw data obtained from the sequencing were processed by eliminating low-quality reads and chimeras, then they were assembled using SPAdes 3.15.5 and annotated using the *funannotate* package.

RESULTS & DISCUSSION

From the genomic analysis, we identified 2331 genes while 7107 correspond to hypothetical proteins (Hypothetical protein). According to the COG database, we divided all the reads obtained into 24 groups (Figure 1). As many as 1869 reads belong to the "Function unknown" group, while "Carbohydrate transport and metabolism", "Transcription", "Posttranslational modification, protein turnover, chaperones", "Signal transduction mechanisms", and "Intracellular trafficking, secretion, and vesicular transport" represented the largest groups. From this first analysis, we could observe that the endophyte analysed by us presented a large number of reads that have the function "Posttranslational modification, protein turnover, chaperones" which include those genes involved in the response to stress.

In the second phase we tried to divide only the identified genes into more specific groups obtaining very satisfactory results (Figure 2). Looking at the graph, we could see how this fungus had genes involved in the transport of substances and in the repair or that respond to DNA damage. Very importantly, we found many genes that respond to abiotic stresses such as heat, cold or non-specific stresses. As for our research, we were very interested in the genes involved in the response to abiotic stresses (such as drought), and for this reason we have identified many genes that participate in the following pathways cAMP-PKA and SNF1, TOR, and PHO. These pathways listed above, in AMF, are important as they are linked to the availability of different nutrients (such as carbon, nitrogen and phosphate) and consequently regulate the expression of genes involved in different pathways, including trehalose activity, resistance to different abiotic and biotic stresses, and spore germination (Zhou X. et al, 2021). Furthermore, Han Y. et al (2023) highlighted the importance of 14-3-3 protein in *Populus cathayana* in response to drought stress. They noted that under drought conditions this protein was induced in expression by the presence of AMF and the addition of phosphorus. Therefore, they concluded that AMF-induced 14-3-3 genes might be involved in osmotic regulation during drought. Searching the NCBI (National Center for Biotechnology Information) database, no sequence of this protein was deposited for *S. indica*. Aligning the sequence of the 14-3-3 protein from *Rhizophagus irregularis* (PKY52090.1) with the sequences obtained from *S. indica*, we observed 80% identity with a hypothetical protein, which was confirmed by the result obtained using the Uniprot database confirming 96.1% identity with the 14-3-3 domain-containing protein sequence from *S. vermifera*.

CONCLUSION

This first screening of the results obtained, makes us understand that the endophyte *Serendipita indica* has a very similar behaviour to AMF as it not only improves the living conditions of the plant (also at a nutritional level) in different abiotic stress conditions, but also at the genetic level we can observe the conservation of the main genes involved in the stress response pathways, as stated in the work of Zhou X. et al. (2021).

This underlines that *Serendipita indica* is more likely equal to AMF, the only difference is the easy reproduction of the mycelium without the need for the presence of the host for *S. indica*, indicating it as a good "tool" to understand more deeply and better the various mechanisms involved both at the physical and molecular level of the response of the fungus-plant consortium to the different climate changes to which crops around the world are recently exposed.

REFERENCE

- ✓ Dobrek M. et al 2024 *Microbial biostimulants affect the development of pathogenic microorganisms and the quality of fresh strawberries* (Fragaria ananassa Duch.) Scientia Horticulturae 327 doi: 10.1016/j.scienta.2023.112793
- ✓ Han Y. et al 2023 *Sugar metabolism and 14-3-3 protein genes expression induced by arbuscular mycorrhizal fungi and phosphorus addition to response drought stress in Populus cathayana* Journal of Plant Physiology doi: 10.1016/j.jplph.2023.154075
- ✓ Weiß M. et al 2016 *Sebacinale – one thousand and one interactions with land plants* New Phytologist 211 doi: 10.1111/nph.13977
- ✓ Zhou X. et al 2021 *Genome-wide analysis of nutrient signaling pathways conserved in arbuscular mycorrhizal fungi* Microorganisms 9 doi: 10.3390/microorganisms9081557
- ✓ Zuccaro A. et al 2011 *Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont Piriformospora indica* PLOS Pathogens 7 doi: 10.1371/journal.ppat.1002290

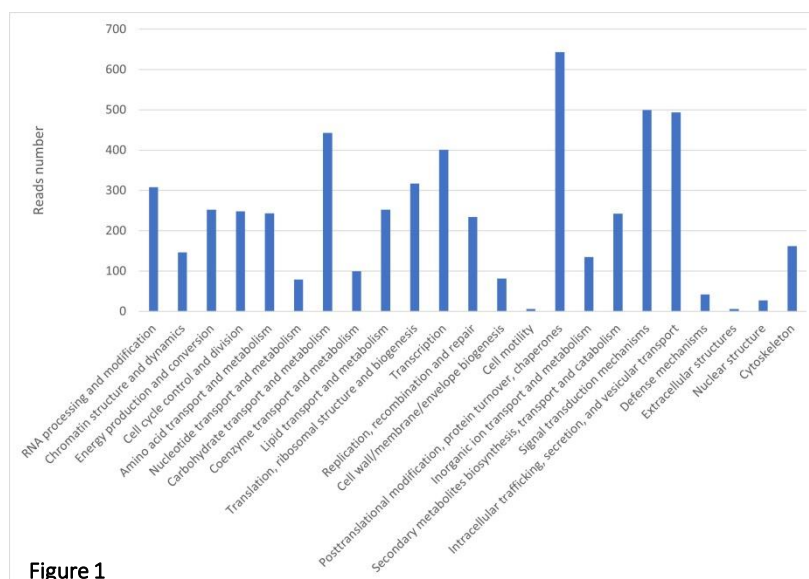


Figure 1

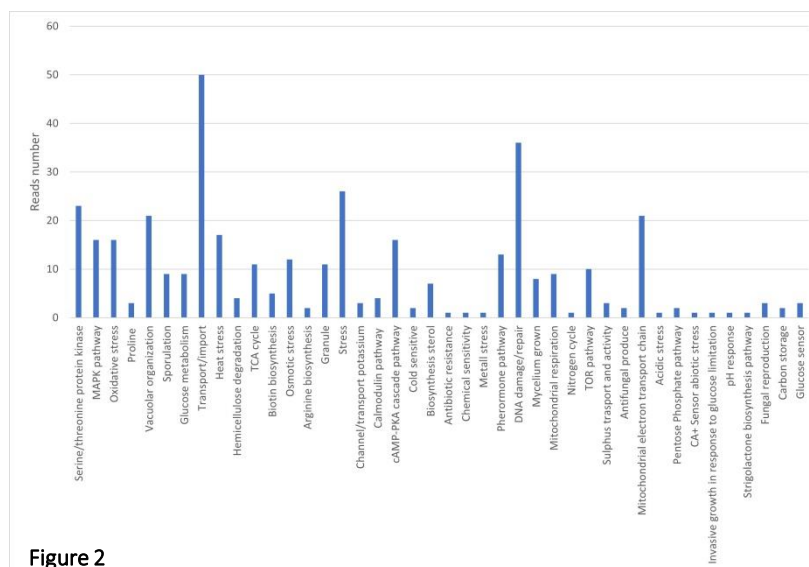


Figure 2