J Plant Mol Breed (2021) 9(2): 35 - 42
DOI: 10.22058/JPMB.2022.562391.1263

RESEARCH ARTICLE

Molecular and morphological identification of endophytic fungi isolated from aerial parts of yew tree

Mahboubeh Ashnavar[1], Azim Ghasemnezhad[*1], Mostafa Khoshhal Sarmast[1], Kamran Rahnama[2]

[1] Department of Horticultural Sciences, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Iran.
[2] Department of Plant Protection, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Iran.

ABSTRACT: Plants are important parts of our diet; which, in addition to the primary metabolites, also produce a wide range of secondary metabolites. Endophytic fungi are among these microorganisms that not only do not cause infection and disease in the host plant but also play a role in the production or increase of some secondary metabolites and defense responses to biotic and abiotic stresses in the plant. Yew is one of the native trees of Iran and has medicinal value and has important fungal endophytes. Isolation and characterization of endophytic fungi in this plant can be important in order to discover new species with the potential to produce taxol. This study was carried out to isolate and identify molecularly and morphologically some fungal endophytes in the laboratory. In order to investigate the presence of ITS gene in fungi, ITS5 and ITS4 specific primers were used. 81 endophytic fungi were extracted from yew seedlings and eight samples were selected for molecular tests. The results of examining the presence of the ITS showed that all the extracted fungi gave a band in the desired region. Based on molecular and morphological studies, eight strains of identified endophytic fungi belonged to the genera *Purpureocillium*, *Akanthomyces*, *Fusarium*, *Phomopsis* and *Colletotrichum*. Therefore, the identification of more endophytic species can help to discover useful microorganisms in the production of secondary metabolites such as taxol.

KEYWORDS: Endophyte, *Fusarium*, Taxol, *Taxus baccata*

INTRODUCTION

Studying the history of world medicine has shown that in the past, many unknown diseases were treated by plants, and herbal medicines were the only available source for the treatment of pains and diseases for many centuries. With the progress of medical science, plants have found a special place in the treatment and medicine sectors, to the extent that the need to identify plants with valuable effective compounds and to know the methods of propagation and modification of these plants has become a necessity of agricultural sciences [27].

Yew (*Taxus baccata* L.) is a dioecious plant belonging to the Taxaceae family. This plant is evergreen and slow growing. All the organs of the plant except the fresh fruit, including the branch, leaf, bark and seeds of the plant contain a combination of alkaloids, diterpenoids, ligands, tannins and resins, which makes them highly toxic [17]. Since 1945, a lot of research has been done on yew and the researchers found out the existence of valuable active substances in the skin and some other organs of this plant and discovered their physiological and bioactive effects

*Corresponding author (✉): ghasemnezhad@gau.ac.ir
Received: 19 September 2022/ Revised: 17 October 2022
Accepted: 25 October 2022

on the body of living organisms [26]. For the first time in 1971, a diterpenoid named taxol was extracted from the bark of this tree, which was later noticed as a very important anticancer agent [37].

Researchers found that the yew tree coexists with microorganisms, some of which have the ability to produce paclitaxel [21]. Efforts in this field led to the discovery of paclitaxel-producing fungi [24]. Endophytic fungi with the production of secondary metabolites and making them available to their host, they provide many benefits to plants, which include increasing food availability, resistance to diseases, pests, and environmental stresses such as salinity, acidity, and drought [22]. A large number of yew endophytic fungi have been isolated from around the world [21]. There are many elicitor compounds in the cell wall of fungi, including proteins, oligosaccharides, glycoproteins, peptides, chitin, and chitosan, which play a role in inducing the production of secondary metabolites in host plants [32].

The ecological relationships between endophytic fungi and their host plants are complex. An endophytic fungus may remain in the plant by producing protective compounds (antifungal or antibacterial) against pathogens [36]. While the plant also provides nutrients for the fungus [5]. A large number of new natural products with different biological activities have been isolated from endophytes. Despite the biodiversity of endophytic microorganisms, only a few of them have been isolated and studied. Therefore, investigating endophytes as biological controllers against human pathogens can lead to the discovery of new bioactive molecules of natural origin [28]. In a study, 150 fungal isolates were isolated from *Taxus baccata* tree. The results showed that some fungi isolated from this plant have the ability to produce medicinal compounds such as taxol [6].

Due to the development and spread of drug-resistant pathogens, there is a need to search for new drugs and pharmacological agents, and infectious diseases, diabetes, rheumatoid arthritis, cardiovascular diseases, and neurological diseases remain a global problem [35]. In recent years, many valuable bioactive compounds with antimicrobial, cytotoxic, anticancer, antimalarial, antiviral, antituberculosis, etc. activities have been successfully discovered from endophyte mycoflora [1].

Currently, morphological studies and determination of phylogeny relationships using molecular tools are used to identify endophytic fungi. Identification based on morphological and physiological characteristics is very difficult for endophytic fungi because they include a large group of fungi. Molecular markers based on DNA analysis are a suitable method compared to classical methods to identify fungi. The use of molecular methods to study the ancestors of relatives to distinguish morphologically similar fungi has been very useful. In the study of kinship, the use of DNA sequences provides a lot of information for the studied organisms. The sequences of ITS, LSU, EF1-α and β-tubulin are the most commonly used markers to investigate molecular phylogeny [34]. By using the usual morphological methods, it is possible to determine the diversity between species, but these methods cannot express intraspecies diversity. The sequence of parts of DNA that encodes ribosomal RNA is one of the important parts of the genome of fungi in phylogenic and taxonomic studies. Although ribosomes show differences among living organisms, in general, they have considerable similarities in their basic structure and in the sequence of bases, even among distant organisms. But gradually, mutations happen on these genes and cause the bases to change. Over time, these changes have been accumulated and provide the possibility to compare different fungi and their evolutionary relationships according to their diversity. ITS and IGS regions have more diversity than other parts and are used to investigate the phylogenetic relationships of species of the same genus or within species. The diversity of fungal endophytes both within and between species of a host is relatively high [11].

Golestan province is a region rich in biodiversity and biological resources of medicinal plants. Considering the medicinal importance of the yew, the present study was conducted in order to isolate, isolate and identify some endophytic fungi by molecular and morphological method was done.

MATERIALS AND METHODS

In order to conduct the experiment, first, a number of one-year yew seedlings native to the region (*Taxus baccata*) and of the same size were grown in the month of March in the climatic conditions of Gorgan. After the establishment of the plants, samples were prepared from the stem and leaves of the yew seedlings.

Tissue culture of plant samples and isolation of endophytes

Yew plant samples were collected from the leaves and stems of seedlings grown in the field. Sampling of

disease-free organs was also done. After preparing five-centimeter samples, in order to free the plant samples from soil and other foreign particles, the samples were washed with running water for 30 min. The surface disinfection of the samples was done in a laminar hood using 70% alcohol for three minutes and 10% sodium hypochlorite for 15 min. Then the samples were washed three times with sterile distilled water. Sterilized plant samples were cultured in petri dishes containing PDA (Potato Dextrose Agar) medium. All cultures were kept in the dark and at a temperature of 25ºC.

The fungi that were removed from the plant organs two weeks after cultivation were considered as endophytic fungi. Grown fungi were transferred to PDA culture medium for purification and purification was done by hypha tip method [33]. In order to ensure the purity of the colonies, the purification process was repeated three times and all the isolates were numbered.

Molecular identification of selected endophytic fungi isolated from yew

Among 81 endophytic fungi isolated from yew, eight samples were selected for molecular identification tests. These samples were named as TB03, TR21, TR23, TF38, TR41, TP41, TP38 and TP03. Purified colonies were used to prepare suspension. To prepare a suspension of endophytic fungi, two circular pieces measuring 0.5 x 0.5 cm were separated from fungal isolates and transferred to 250 ml Erlenmeyer flasks containing 100 ml of BDP (Potato Dextrose Broth) liquid culture medium. The cultures were kept at a temperature of 25ºC and in the dark for 21 days on a shaker with a speed of 110 rpm. After this time, the mushrooms grown in PDB medium were separated from the liquid medium using three layers of sterile filter paper and washed twice with distilled sterile water to remove the rest of the medium from the surface of the mycelium. Then the dried mycelia were pounded with liquid nitrogen in a sterile mortar and stored at -20°C to be used for molecular experiments.

Genomic DNA extraction was done by CTAB method [23]. First, 0.5 mg of the mycelia that were beaten with nitrogen were placed in 2 ml vials and 700 µl of CTAB buffer was added to them and mixed thoroughly. Then, 1 microliter of β-mercaptoethanol was added to each of the vials and placed in a Bain-Marie at 65ºC for 30 min. After this time, in order to deproteinize, 700 µl of chloroform-isoamyl alcohol mixture at a ratio of 24:1 was added to each vial and centrifuged for 10 min at 12,000 revolutions. After centrifugation, three phases were formed. The lower phase contained chloroform and the upper phase contained DNA. Between these two liquid phases, a layer consisting of cell wall remains was placed. The upper phase was carefully transferred to new vials and the same volume of cold isopropanol was added to the contents of the vials and after turning the vials upside down ten times, they were placed at -20ºC half an hour. After this time, the vials were centrifuged for 10 min at 12000 rpm and finally, after the formation of DNA sediment, the liquid phase was drained and the resulting sediment was washed with 70% ethanol and centrifuged at 9000 rpm for 5 min. Afterwards, the ethanol was discarded and the vials were placed at room temperature for 30 min to dry the DNA plate. After drying the DNA precipitate, 50 µl of sterile distilled water was added to each vial, and after 60 min of storage in the refrigerator, it was transferred to a -20 freezer.

In order to investigate the presence of ITS gene in fungi, ITS5 and ITS4 specific primers were used [38] (Table 1). After dilution, the desired primer was placed together with master mix Red amplicon (Ampliqon Company, Denmark) and genomic DNA and distilled water in the PCR machine in a final volume of 25 microliters [30]. The PCR mixture was incubated for five minutes at 94°C, followed by 35 cycles of 94°C for 30 sec, 59°C for 30 sec, 72°C for 30 sec, and one cycle of 72°C for 15 min. PCR product analysis was done in 1% agarose gel and electrophoresis machine (Bio Rad) in TBE buffer with 85 voltage [40].

The products obtained from PCR were sent to Pioneer Biotechnology Company for sequencing, purified and then sequenced. The sequences sent by the company were blasted with other sequences in the NCBI database in the Nucleotide BLAST section and the degree of similarity with other sequences in the World Bank was checked. Determining sequences and removing possible errors was done with Chromas software.

The obtained data were analyzed using SAS V.9.1and the mean data were compared with the Least Significant Difference (LSD) test at the 5% probability level.

Table 1. Primers used for the presence of ITS gene

Gene	Primer	sequence (5´ to 3´)
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG
ITS	ITS4	TCCTCCGCTTATTGATATGC

RESULTS

Investigating the presence of ITS gene in fungi isolated from yew

In general, 81 endophytic fungi were extracted from yew seedlings and eight samples were selected for molecular tests. After DNA extraction, PCR of fungus samples was performed. The results of examining the presence of the ITS gene (internal transcription spacer region for identification of true fungi) showed that all the extracted fungi gave a band in the desired region (Fig. 1). Therefore, these fungi were used as endophytic fungi for sequencing and subsequent experiments.

Molecular identification of selected endophytic fungi isolated from yew

Sequencing analysis of the ITS primer PCR product in endophytic fungi revealed that out of the eight fungal isolates investigated, two isolates belong to the endophytic fungus *Akanthomyces muscarius*, one isolate belongs to the endophytic fungus *Purpureocillium lilacinum*, three isolates belong to the genus *Fusarium* and two isolates belong to were *Phomopsis* and *Colletotrichum* genera (Table 2).

Morphology of isolated endophytic fungi

Purpureocillium lilacinum (Thom):

The colony of this endophytic fungus was 12 mm after 7 days at 25°C. The surface of the colony was pink with a lighter border. This fungus forms a dense mycelium that gives rise to conidiophores. The aerial mycelium was white at first, which changed to pink over time. Vegetative hyphae also have smooth walls (Fig. 2a). This species previously belonged to *Paecilomyces* genus and *P. lilacinus* species, which was later transferred to this genus [19]. Research has shown that this species has anticancer properties [18].

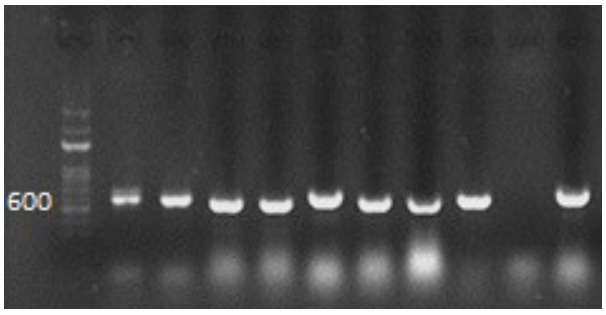

Figure 1. Presence of ITS gene in endophytic fungi isolated from yew, negative control and positive control, respectively.

Akanthomyces muscarius (Petch):

This fungus, belonging to the order Hypocreales and family Cordycipitaceae, had a white mycelium with very slow growth, cottony or hairy, so that after 14 days of cultivation, the diameter of the colony was about 1 cm (Fig 2b).

Fusarium solani (Mart):

The colony growth rate on PDA culture medium at 25ºCelsius after three days was 2.6 cm. In this endophytic fungus, myceliums were white to cream colored and

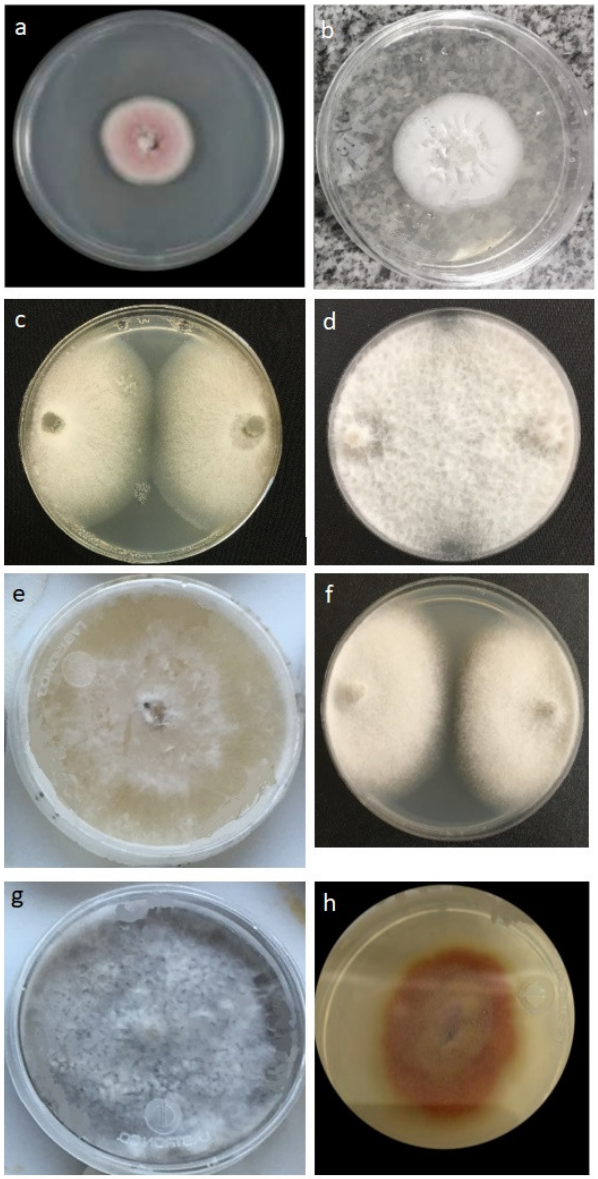

Figure 2. Morphology of endophytic fungi isolates on PDB medium. a) *Purpureocillium lilacinum*; b) *Akanthomyces muscarius*; c) *Fusarium solani*; d) *Fusarium brachygibbosum*; e) *Phomopsis* sp.; f) *Colletotrichum gloeosporioides*; g) *C. gloeosporioides* after 21 days; h) *Fusarium proliferatum.*

Table 2. Molecular identification of endophytic fungi isolated from yew

NCBI registered code	Percentage of similarity	Fungi species	Isolated name	Plant code
MK174973	95.98	*Purpureocillium lilacinum*	TP41	41
MN960455	96.99	*Akanthomyces muscarius* (Leaf)	TP38	38
MN960455	96.99	*Akanthomyces muscarius* (Stem)	TP03	03
MT446060	100	*Fusarium solani*	TR41	41
MN452141	100	*Fusarium brachygibbosum*	TF38	38
AB640842	98.5	*Phomopsis* sp.	TB03	03
MH168336	98.9	*Colletotrichum gloeosporioides*	TR21	21
MT560216	100	*Fusarium proliferatum*	TR23	23

scattered. Over time, they become greenish-brown or bluish-green in color. Chlamydospores are abundant and have uneven walls. Conidiophores are simple to branched and walled with a simple to branched phialide and long conidiophores (Fig. 2c).

Fusarium brachygibbosum (Padwick):
This fungus has white aerial mycelium and had a high growth rate. After four days at 25ºCelsius, the fungus colony had a diameter of 2.5 cm. The conidia were colorless and oval. Chlamydospores are abundant, some researchers introduced this fungus as a symbiotic endophyte of licorice plant (Fig. 2d) [4].

***Phomopsis* sp.** (Sacc):
This fungus had aerial mycelium and slow growth, so that after seven days, the diameter of the colony was about 1.5 cm. In this endophytic fungus, myceliums were white to cream colored and scattered. Also, the color of the culture medium was seen as cream to pale yellow. Conidia were filamentous, straight or slightly curved (Fig. 2e). This fungus was also observed as a symbiotic endophyte in mangrove plants [8].

Colletotrichum gloeosporioides (Penz):
After seven days at 25ºC, the mushroom colony had a diameter of two centimeters. After 21 days, the entire petri dish was filled with fungal mycelium. Also, due to the production of pigment in the culture medium, the color of the colony could be seen from the back of the petri dish in dark gray to black color (Fig. 2f and 2g).

Fusarium proliferatum (Matsushi):
The colony on PDA culture medium at 25ºC and exposure to light conditions was white to orange in color with abundant aerial mycelia. Due to the production of pigment in the culture medium, the color of the colony from the back of the petri dish was orange to brown and its diameter reached 45 mm after 14 days (Fig. 2h).

DISCUSSION

Among endophytic microorganisms, endophytic fungi have attracted much research attention because they have not only provided new sources of cytotoxic compounds such as anticancer molecules [2], but also biostimulants for essential oil biosynthesis [9]. They may increase nutrient dissolution in the plant rhizosphere [20], promote plant growth, act as biological control agents [29] or increase systemic plant resistance to stresses, increase biotic and abiotic factors [7]. The diversity of fungal endophytes is high both within and among species of a host. So far, some of the plant microorganisms have been investigated and endophytes may include a significant part of undiscovered fungi [3]. Identification based on morphological and physiological characteristics for endophytic fungi is very difficult, because these fungi include a wide group of fungi. Molecular markers based on DNA analysis are a suitable method compared to classical methods to identify different fungi [34].

Based on the research, the endophytic fungus *Colletotrichum gloesporioides* was isolated from Mexican yew (*Taxus globosa*) [31] and Knock out Rose [13]. 81 endophytic fungi were isolated from *Taxus media* and were divided into eight genera based on morphological and molecular identification. *Guignardia* and *Colletotrichum* were the dominant genera in this plant [39]. *Akanthomyces muscarius* was also isolated from Leontopodium nivale [25].

In general, the most common endophytes isolated from plants belonged to the genera *Colletotrichum*, *Cladosporium*, *Fusarium* and *Xylaria* [14]. *Fusarium solani* as an endophytic fungus has been isolated from *Taxus celebica*, *Taxus brevifolia* [16], *Cajanus cajan* [41] and the rhizome and leaves of *Hedychium acumin* [12].

Colletotrichum genus is one of many fungi as endophytes in plants. This genus was previously isolated from this plant in a study on *T. bacaata* [10]. It was also seen as an endophyte in *T. media* [39] and *Hedychium acuminatum* [12], and the production of taxol by this fungus has also been proven in *T. media*. Studies showed that *Fusarium proliferatum* was extracted from the stem bark of *Dysoxylum binectariferum* on PDB culture medium [15]. Taxol production by this fungus has been proven in *Taxus media* [39].

CONCLUSION

In general, 81 endophytic fungi were isolated from yew plant. The results of examining the presence of the ITS showed that all the extracted fungi gave a band in the desired region. Among which eight isolates were identified, belonging to the genera *Akanthomyces*, *Purpureocillium*, *Fusarium*, *Phomopsis* and *Colletotrichum*. Therefore, it is suggested to carry out more studies in this field, especially in selecting the type of organ and growth environment of the host plant, and identifying and discovering more endophytic fungi.

REFERENCES

[1] Aharwal RP., Kumar S, Sandhu SS. 2014. Isolation and antibacterial property of endophytic fungi isolated from Indian medicinal plant *Calotropis procera* (Linn.) R.Br. World J Pharm Pharm Sci, 3: 678-691.

[2] Alam B, Li J, Ge Q, Alam Khan M, Gong J, Mehmood Sh, Yuan Y, Gong W. 2021. Endophytic Fungi: From Symbiosis to Secondary Metabolite Communications or Vice Versa? Front Plant Sci, 12: 791033.

[3] Arnold AE, Maynard A, Gilbert GS, Coley PD, Kursar TA. 2000. Are tropical fungal endophytes hyperdiverse? Ecol Lett, 3: 267-274.

[4] Arora P, Wani ZA, Ahmad T, Sultan P, Gupta S, Riyaz-Ul-Hassan S. 2019. Community structure, spatial distribution, diversity and functional characterization of culturable endophytic fungi associated with *Glycyrrhiza glabra* L. Fungal Biol, 123(5): 373-383.

[5] Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ahmed N, Ashraf M, Zhang L. 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci, 10:1068.

[6] Caruso M, Colombo AL, Fedeli L, Pavesi A, Quaroni S, Saracchi M. 2000. Isolation of endophytic fungi and actinomycetes taxane producers. Ann Microbiol, 50(1): 3-13.

[7] Cui R, Lu X, Chen X, Malik WA, Wang D, Wang J, Wang Sh, Guo L, Chen C, Wang X, Wang X, Dai M, Ye W. 2021. A novel raffinose biological pathway is observed by symbionts of cotton= *Verticillium dahliae* to improve salt tolerance genetically on cotton. J Agron Crop Sci, 207: 956-969.

[8] Ding B, Yuan J, Huang X, Wen W, Zhu X, Liu Y, Li H, Lu Y, He L, Tan H, She Z. 2013. New dimeric members of the phomoxanthone family: phomolactonexanthones A, B and deacetylphomoxanthone C isolated from the fungus *Phomopsis* sp. Mar Drugs, 11(12): 4961-4972.

[9] El Enshasy HA, Hanapi SZ, Malek RA, Abdelgalil SA, Leng OM. 2019. Endophytic fungi: the desired biostimulants for essential oil production. In: Fungal Biology. Advances in Endophytic Fungal Research. Pp: 211-232.

[10] El-Bialy HA, El-Bastawisy HS. 2020. Elicitors stimulate paclitaxel production by endophytic fungi isolated from ecologically altered *Taxus baccata*. J Radiat Res Appl Sci J, 13: 79-87.

[11] Gamboa MA, Bayman P. 2001. Communities of endophytic fungi in leaves of a tropical timber tree (*Guarea guidonia*: Meliaceae). Biotropical, 33(2): 352-360.

[12] Hastuti US, Al Asna PM, Rahmawati D. 2018. Histologic Observation, Identification, and Secondary Metabolites Analysis of Endophytic Fungi Isolated from a Medicinal Plant, *Hedychium acuminatum* Roscoe. AIP Conf Proc, 2002: 020070.

[13] Heinz KM, Harding PA, Ek-Ramos MJ, Hernandez H, Krauter PC, Sword GA. 2018. Fungal endophytes in Knock out Rose and performance effects of entomopathogens on marigold and zinnia. Hortic Sci, 53 (12): 1791-1798.

[14] Köhl J, Kolnaar R, Ravensberg WJ. 2019. Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. Front Plant Sci, 10: 845.

[15] Kumara PM, Zuehlke S, Priti V, Ramesha B, Shweta S, Ravikanth G, Vasudeva R, Santhoshkumar TR, Spiteller M, Shaanker R. 2012. *Fusarium proliferatum*, an endophytic fungus from *Dysoxylum binectariferum* Hook.f, produces rohitukine, a chromane alkaloid possessing anti-cancer activity. Antonie van Leeuwenhoek, 101: 323-329.

[16] Kuriakose GC, Arathi BP, Lakshmanan MD, Jiby MV, Gudde RS, Jayabhaskaran C. 2020. Sub-acute Toxicity Assessment of Taxol Isolated from *Fusarium Solani*, an Endophytic Fungus of *Taxus Brevifolia*, in Wistar Rats and Analyzing Its Cytotoxicity and Apoptotic Potential in Lung Cancer Cells. Origin Res, 10: 538865.

[17] Labossiere AW, Thompson DF. 2018. Clinical Toxicology of Yew Poisoning. Ann Pharmacother, 52(6): 591-599.

[18] Lenta BN, Ngatchou J, Frese M, Ladoh-Yemeda F, Voundi S, Nardella F, Michalek C, Wibberg D, Ngouela S, Tsamo E, Kaiser M, Kalinowski J, Sewald N. 2016. Purpureone, an antileishmanial ergochrome from the endophytic fungus *Purpureocillium lilacinum*. Z Naturforsch, 71(11) b: 1159-1167.

[19] Luangsa-Ard J, Houbraken J, van Doorn T, Hong SB, Borman AM, Hywel-Jones NL, Samson RA. 2011. *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. FEMS Microbiol Lett, 321(2): 141-149.

[20] Mehta P, Sharma R, Putatunda C, Walia A. 2019. Endophytic fungi: role in phosphate solubilization. In Advances in Endophytic Fungal Research: Present Status and Future Challenges, ed. Singh, B.P. pp: 183-209.

[21] Morales-Sánchez V, Fe Andrés M, Díaz CE, González-Coloma A. 2020. Factors affecting the metabolite productions in endophytes: biotechnological approaches for production of metabolites. Curr Med Chem, 27(11): 1855-1873.

[22] Mosaddegh A. 2001. Yew tree. Ecol, 27(28): 73-74. (In Persian)

[23] Murray M, Thompson W. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res, 8(19): 4321-4325.

[24] Naik BS. 2019. Developments in taxol production through endophytic fungal biotechnology: a review. Orient Pharm Exp Med, 19(1): 1-13.

[25] Oberhofer M, Wackerlig J, Zehl M, Buyuk H, Jian Cao J, Prado-Roller A, Urban E, Zotchev SB. 2021. *Endophytic Akanthomyces* sp. LN303 from Edelweiss Produces Emestrin and Two New 2- Hydroxy-4-Pyridone Alkaloids. ACS Omega, 6: 2184-2191.

[26] Omidbaigi R. 2010. Production and Processing of Medicinal Plants. Astan Qods Razavi Publications. 423 pp. (In Persian)

[27] Pandey KB, Rizvi SI. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev, 2(5): 270-278.

[28] Patil RH, Patil MP, Maheshwar V. 2016. Bioactive secondary metabolites from endophytic fungi: A review of biotechnological production and their potential applications. Stud Nat Prod Chem, 49: 189-205.

[29] Poveda J, Eugui D, Abril-Urías P, Velasco P. 2021. Endophytic fungi as direct plant growth promoters for sustainable agricultural production. Symbiosis, 85: 1-19.

[30] Raja A, Miller N, Pearce J, Oberlies H. 2017. Fungal identification using molecular tools: A primer for the natural products research community. Nat Prod J, 80: 756-770.

[31] Rivera-Orduna FN, Suarez-Sanchez RA, Flores-Bustamante ZR, Gracida-Rodriquez JN, Flores-Cotera LB. 2011. Diversity of endophytic fungi of *Taxus globosa* (Mexican yew). Fungal Divers, 47: 65-74.

[32] Salehi M, Moieni A, Safaie N, Farhadi S. 2020. Whole fungal elicitors boost paclitaxel biosynthesis induction in *Corylus avellana* cell culture. PloS One, 15(7): e0236191.

[33] Schulz B, Wanke U, Draeger S. 1993. Endophytes from herbaceous and shrubs: effectiveness of surface sterilization method. Mycol Res, 97: 1447-1450.

[34] Tazik Z, Rahnama K, Iranshahi M, White JF, Soltanloo H. 2020. A new species of Pithoascus and first report of this genus as endophyte associated with Ferula ovina. Mycoscience, 61: 145-150.

[35] Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 39: 44-84.

[36] Wang CJ, Wang YZ, Chu ZH, Wang PS, Liu BY, Li BY, Yu XL, Luan BH. 2020. Endophytic *Bacillus amyloliquefaciens* YTB1407 elicits resistance against two fungal pathogens in sweet potato (*Ipomoea batatas* (L.) Lam.). J Plant Physiol, 253: 153260.

[37] Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. 1971. Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. Journal of the J Am Chem Soc, 93(9): 2325-2327.

[38] White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In book: PCR - Protocols and Applications - A Laboratory Manual. Pp: 315-322.

[39] Xiong ZQ, Yang YY, Zhao N, Wang Y. 2013. Diversity of endophytic fungi and screening of fungal paclitaxel producer from Anglojap yew, *Taxus × media*. BMC Microbiol, 13: 71.

[40] Zhang P, Zhou PP, Yu LJ. 2009. An Endophytic taxol-producing fungus from *Taxus media*, *Cladosporium cladosporioides* MD2. Curr Microbiol, 59(3): 227-232.

[41] Zhao J, Fu Y, Luo M, Zu Y, Wang W, Zhao C, Gu C. 2012. Endophytic fungi from pigeon pea (*Cajanus cajan* (L.) Mill sp.) produce antioxidant cajaninstilbene acid. J Agric Food Chem, 60: 4314-4319.

.

شناسایی مولکولی و مورفولوژیکی قارچ‌های اندوفیت جداشده از اندام‌های هوایی درخت سرخدار

محبوبه آشناور[1]، عظیم قاسم‌نژاد*[1]، مصطفی خوشحال سرمست[1]، کامران رهنما [2]

[1] گروه علوم باغبانی، دانشکده تولید گیاهی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان

[2] گروه گیاهپزشکی، دانشکده تولید گیاهی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان

*نویسنده مسئول: ghasemnezhad@gau.ac.ir

چکیده

گیاهان بخش مهمی از رژیم غذایی ما را تشکیل می‌دهند که علاوه بر متابولیت‌های اولیه، طیف وسیعی از متابولیت‌های ثانویه را نیز تولید می‌کنند. قارچ‌های اندوفیت از جمله میکروارگانیسم‌های همزیست در گیاهان هستند که نه تنها سبب ایجاد آلودگی و بیماری در گیاه میزبان نمی‌شوند، بلکه در تولید و یا افزایش برخی متابولیت‌های ثانویه و نیز ایجاد پاسخ‌های دفاعی در برابر تنش‌های زنده و غیرزنده در گیاه نیز نقش دارند. گیاه سرخدار از جمله درختان بومی ایران و باارزش دارویی و دارای اندوفیت‌های قارچی مهمی می‌باشد. جداسازی و شناسایی قارچ‌های اندوفیت در این گیاه می‌تواند در جهت کشف گونه‌های جدیدی با پتانسیل تولید تاکسول، مهم باشد. به همین منظور، پژوهشی جهت جداسازی و شناسایی مولکولی و مورفولوژیکی برخی اندوفیت‌های قارچی سرخدار در شرایط آزمایشگاه انجام شد. به منظور بررسی حضور ژن ITS در قارچ‌ها از پرایمرهای اختصاصی ITS5 و ITS4 استفاده شد. ۸۱ قارچ اندوفیت از نهال سرخدار استخراج و ۸ نمونه برای آزمایش‌های مولکولی انتخاب شدند. نتایج بررسی حضور ITS نشان داد که تمامی قارچ‌های استخراج شده در ناحیه موردنظر، باند ایجاد کردند. بر اساس بررسی‌های مولکولی و مورفولوژیکی، ۸ سویه از قارچ‌های اندوفیت شناسایی شده متعلق به جنس‌های *Purpureocillium*، *Akanthomyces*، *Fusarium*، *Phomopsis* و *Colletotrichum* بودند. بنابراین، شناسایی گونه‌های اندوفیت بیشتر می‌تواند به کشف میکروارگانیسم‌های مفید در تولید متابولیت‌های ثانویه مانند تاکسول کمک کند.

کلمات کلیدی: اندوفیت، فوزاریوم، تاکسول، *Taxus baccata*.